Attenuated cardiac baroreflex in men with presyncope evoked by lower body negative pressure

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ABSTRACT

Mechanisms responsible for presyncope during lower body negative pressure (LBNP) in otherwise healthy subjects are poorly understood. Muscle sympathetic nerve activity (MSNA), blood pressure, heart rate (HR), HR power spectra, central venous pressure (CVP) and stroke volume were determined in 14 healthy men subjected to incremental LBNP. Of these, seven experienced presyncope at LBNP of −15 mmHg. Subjects who tolerated LBNP > −15 mmHg had significantly lower CVP (2 ± 6 versus 7 ± 1 mmHg; mean ± S.E.M., P < 0.02), HR (59 ± 2 versus 66 ± 3 beats/min, P < 0.05) and MSNA burst frequency (29 ± 2.4 versus 39 ± 3.5 bursts/min, P < 0.05) during supine rest. LBNP at −15 mmHg had no effect on blood pressure, but caused similar and significant reductions in stroke volume and cardiac output in both groups. Subjects who tolerated LBNP had significant reflex increases in HR, MSNA burst frequency and burst amplitude with LBNP of −15 mmHg. These responses were absent in those who experienced presyncope. The gain of the cardiac baroreflex regulation of MSNA was markedly attenuated in pre-syncopal subjects (1 ± 2.6 versus 8 ± 1.4 bursts/100 heart beats per mmHg; P < 0.001). Healthy subjects who experience presyncope in response to LBNP appear more dependent, when supine, upon MSNA to maintain preload, and less able to increase sympathetic vasoconstrictor discharge to skeletal muscle reflexively in response to orthostatic stimuli.

INTRODUCTION

The cause of vasodepressor syncope in otherwise healthy adults remains obscure. Much attention has been directed at neural events, often sudden, occurring at the time blood pressure begins to fall [1–4] and at physical or pharmacological manoeuvres that might unmask any underlying predisposition to faint [5,6]. A common theme in such experiments has been the selection of patients with a prior history of recurrent syncope, presumably due to a neurally mediated mechanism.

During the course of our investigation of sympathetic nervous system responses to lower body negative pressure (LBNP) in healthy, middle-aged men, symptoms or signs of presyncope at levels greater than −15 mmHg LBNP resulted in premature termination of this stimulus in 50% of these subjects. Because none of these individuals had a history of prior syncope, and several were engaged in occupations requiring a high degree of alertness, this finding was unanticipated. Our purpose in conducting the present analysis was to compare the haemodynamic and sympathoneural characteristics of those who became pre-syncopal, as opposed to those who did not, under two conditions: at rest, prior to the application of LBNP; and in response to LBNP at −15 mmHg, which was the stimulus that all subjects were able to tolerate without developing symptoms. Differences between these two groups, both at rest and in

Key words: baroreceptor reflex, central venous pressure, heart rate variability, microneurography, sympathetic nervous system.
Abbreviations: LBNP, lower body negative pressure; MSNA, muscle sympathetic nerve activity; CVP, central venous pressure; HR, heart rate.
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response to this simulated orthostatic stimulus, raise hypotheses concerning neural aspects of circulatory regulation of individuals at risk of presyncope, which merit prospective testing in future investigations.

**METHODS**

**Subjects**

Healthy, active, middle-aged men (n = 14, 44.5 ± 1.7 years; mean ± S.E.M.) with no prior history of syncope were studied. Their mean weight was 81.4 ± 3.5 kg and their height 180.4 ± 1.4 cm. None were taking any medication. All subjects provided informed written consent as approved by our Institutional Human Subjects Review Committee, in accordance with the Declaration of Helsinki.

**Procedures**

Subjects emptied their bladder, then were placed supine into a chamber custom-built for recording muscle sympathetic nerve activity (MSNA) from the right peroneal nerve during LBNP [7]. An airtight seal was obtained by a neoprene kayak skirt fitted at the iliac crests. The right leg was held in position by form-fitting supports at the thigh, knee and ankle, and caudal displacement of the subject was prevented by bilateral foot plates. Before any additional recording instruments were applied, a brief trial of LBNP was performed so as to familiarize subjects with the sensation of lower body suction and thereby minimize any inadvertent contraction of leg muscles during the protocol.

A volume-clamp cuff (Finapres model 2300; Ohmeda, Rexdale, Canada) was placed on the left-middle finger for continuous non-invasive beat-by-beat recording of blood pressure. After local anaesthesia, a central venous catheter was introduced into an antecubital vein of the right arm [7]. An airtight seal was obtained by a neoprene kayak skirt fitted at the iliac crests. The right leg was held in position by form-fitting supports at the thigh, knee and ankle, and caudal displacement of the subject was prevented by bilateral foot plates. Before any additional recording instruments were applied, a brief trial of LBNP was performed so as to familiarize subjects with the sensation of lower body suction and thereby minimize any inadvertent contraction of leg muscles during the protocol.

**Protocol**

Supine bed rest (20 min) was followed by a 10 min baseline period. LBNP was then applied at −5, −15 −30 and −40 mmHg. If tolerated, each level of LBNP was sustained for 10 min. Blood pressure, HR, CVP and MSNA were recorded continuously. Stroke volume was derived over the last 2 min of baseline, each level of LBNP and the last 2 min of the recovery period.

Subjects were monitored for any sensation or indication of presyncope, such as pallor, tachypnea, yawning, a drop in systolic blood pressure > 30 mmHg, or heart rate > 20 beats/min. If these arose, LBNP was stopped and subjects were instructed to begin mild leg muscle contractions to facilitate venous return.

**Data analysis**

**Muscle sympathetic nerve activity**

Pulse synchronous bursts of MSNA were identified by inspection of the mean voltage neurogram [7] by two trained, but blinded, assessors. Any disagreement 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 units of integrated nerve activity (the product of burst frequency and mean burst amplitude in mm). The latter serves as a quantitative representation of the strength of each burst of post-ganglionic discharge.

**Spectral analysis of heart rate variability**

A total of 7 min of the ECG (from the second to the eighth minute of each level of LBNP) for each experimental condition was analysed to determine HR variability, using coarse-graining spectral analysis. The specific details of this technique have been reported previously [10–12]. Total spectral power (P_T) was divided into its fractal power (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_H) comprising the sum of P_L and P_H. The ratio P_H/P_T was used to estimate the contribution of parasympathetic modulation of HR to total spectral power [13–16], and ratio P_T/P_H was applied to estimate the contribution of sympathetic nervous system modulation of HR, or the ‘sympathovagal balance’, to power spectral density [8,13,15–20]. The non-harmonic component of spectral power was characterized further by plotting the log of spectral power as a function of the log of frequency (a 1/[f^β] plot), with β the slope of this linear regression [10,11,21].

**Statistical analysis**

Mean values ± S.E.M. are reported throughout. Subjects were divided on the basis of their response to LBNP into pre-syncopal and non-pre-syncopal groups. The principal comparison in this analysis was between baseline measurements and responses to −15 mmHg LBNP, which was the highest level tolerated by all subjects. Within-group responses were assessed, applying Stu-
dent’s paired t test to normally distributed data and the Wilcoxon rank sum test for non-parametric data, whereas Student’s unpaired t test and the Mann Whitney rank sum test were used for between-group comparisons. Statistical significance was accepted if P < 0.05.

RESULTS

All subjects completed the −5 and −15 mmHg LBNP segments of the protocol, but three developed presyncope at −30 mmHg and four at −40 mmHg. Characteristics, at baseline, of subjects who completed the full protocol and subjects who developed presyncope appear in Table 1. A continuous measure of CVP was obtained in 12 subjects, six in each group.

There was no significant difference in mean age, blood pressure, stroke volume or cardiac output between subjects who completed the full protocol and those who experienced presyncope. However, the latter group was characterized by significantly higher sympathetic burst frequency, heart rate and CVP (Table 1). A comparison of baseline power spectral analysis data did not detect any difference in the initial indices for heart rate variability in these two groups (Table 2). Five of these subjects had participated in previous research studies and were familiar with these procedures. Of these, three developed presyncope and two did not.

Responses to −15 mmHg LBNP

This level of LBNP did not significantly affect systolic or diastolic blood pressure in either group. As anticipated, there were significant reductions in CVP, stroke volume and cardiac output in both groups. LBNP at −15 mmHg

Table 1 Haemodynamics and muscle sympathetic nerve activity in subjects without and with presyncope

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without presyncope</th>
<th>With presyncope</th>
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<tbody>
<tr>
<td></td>
<td>0 mmHg</td>
<td>−15 mmHg</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 6</td>
<td>121 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 ± 4</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>45 ± 3</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>CVP (mmHg)§</td>
<td>2.6 ± 1.0</td>
<td>0.3 ± 0.2†</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>71 ± 6</td>
<td>60 ± 4†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>59 ± 2</td>
<td>64 ± 3†</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>4.1 ± 0.4</td>
<td>3.8 ± 0.3†</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>29.0 ± 2.4</td>
<td>41.5 ± 1.1†</td>
</tr>
<tr>
<td>MSNA (bursts/100 heart beats)</td>
<td>47.7 ± 3.2</td>
<td>62.3 ± 3.4§</td>
</tr>
<tr>
<td>MSNA burst amplitude (mm)</td>
<td>6.8 ± 0.4</td>
<td>10.5 ± 1.4†</td>
</tr>
<tr>
<td>MSNA (units/min)</td>
<td>201 ± 19</td>
<td>441 ± 70†</td>
</tr>
<tr>
<td>MSNA (units/100 heart beats)</td>
<td>328 ± 36</td>
<td>651 ± 89†</td>
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Table 2 R–R interval and heart rate variability in subjects without and with presyncope

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without presyncope</th>
<th>With presyncope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mmHg</td>
<td>−15 mmHg</td>
</tr>
<tr>
<td>R–R interval (ms)</td>
<td>987 ± 67</td>
<td>912 ± 51*</td>
</tr>
<tr>
<td>P₁ (ms²)</td>
<td>1493 ± 459</td>
<td>1200 ± 180</td>
</tr>
<tr>
<td>P₂ (ms²)</td>
<td>828 ± 243</td>
<td>870 ± 151</td>
</tr>
<tr>
<td>P₁/P₂</td>
<td>0.62 ± 0.07</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td>P₄ (ms²)</td>
<td>654 ± 278</td>
<td>337 ± 57</td>
</tr>
<tr>
<td>P₆ (ms²)</td>
<td>241 ± 121</td>
<td>72 ± 28</td>
</tr>
<tr>
<td>P₈/P₁</td>
<td>0.11 ± 0.03</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>P₂ (ms²)</td>
<td>423 ± 160</td>
<td>266 ± 42</td>
</tr>
<tr>
<td>P₈/P₄</td>
<td>3.8 ± 1.3</td>
<td>18.7 ± 8.0</td>
</tr>
<tr>
<td>†</td>
<td>1.15 ± 0.10</td>
<td>1.46 ± 0.10**</td>
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Because LBNP at $-15$ mmHg did not affect harmonic power spectra in either group. However, there was a significant increase in the slope of the log power–log frequency relationship in the non-presyncopal group (Table 2).

**Cardiac reflex control of MSNA**

Because LBNP at $-15$ mmHg did not have any significant effect on arterial blood pressure, or on $P_{at}/P_{ac}$, the principal representation of parasympathetic withdrawal in response to arterial baroreceptor unloading, the primary analysis of reflex control of MSNA focused on the gain of the cardiac reflex. To estimate this, a ratio relating changes in MSNA to changes in CVP was constructed. Regardless of the representation of MSNA selected, there was a marked attenuation in the calculated gain of this reflex in those subjects who experienced presyncope at higher levels of LBNP (Figure 1). In the group who tolerated LBNP without presyncope, there was an $8.8 \pm 1.4$ unit increase in burst incidence for every mmHg decrease in CVP and a $248 \pm 87$ unit incidence increase for every mmHg decrease in CVP. In contrast, in the pre-syncopal group, there was a $1.2 \pm 0.6$ burst/100 heart beat increase in MSNA for every 1 mmHg decrease in CVP and a $17.0 \pm 19.2$ units/100 heart beat increase in MSNA for each mmHg decrease in CVP ($P < 0.001$ and $P = 0.027$ respectively).

**DISCUSSION**

Our objective in this analysis was to compare the haemodynamic and sympathoneural characteristics of healthy, middle-aged subjects, with no prior history of unexplained syncope, who did or did not experience presyncope in response to graded LBNP. The two groups differed significantly in the following three respects. Subjects who became pre-syncopal: (1) had higher initial CVP, heart rate and MSNA burst frequency, prior to the application of LBNP; (2) did not increase heart rate, MSNA burst frequency, amplitude, or integrated MSNA in response to $-15$ mmHg LBNP; and (3) displayed marked attenuation of the gain of the cardiac baroreflex control of MSNA in response to this stimulus. For similar levels of CVP, stroke volume was substantially lower in subjects who subsequently experienced presyncope.

These observations suggest that subjects with a predisposition to presyncope in response to an orthostatic stimulus are more dependent on central sympathetic outflow to skeletal muscle, to maintain preload, and on sympathetic nerve traffic to the sino-atrial node to maintain cardiac output. Venous pooling and withdrawal of preload by graded LBNP would therefore predispose these subjects to syncope unless countered by reflex sympathetic activation. However, these individuals were unable to mount the appropriate additional increase in sympathetic vasoconstrictor discharge in response to this stimulus, presumably due to impairment of their cardiac baroreflex control of MSNA. Right atrial pressure remained higher in the pre-syncopal group throughout LBNP, and there was no abrupt cut-out of MSNA during the course of the experiment, which argues against the ‘collapse-firing’ hypothesis of neurogenic syncope.

Young subjects with borderline or mild essential hypertension also have increased MSNA burst frequency and incidence, often in the setting of higher central blood volume, heart rate, cardiac output and decreased venous distensibility [22–25]. However, in contrast to the present findings, their reflex forearm vasoconstrictor and MSNA responses to non-hypotensive LBNP are either augmented, or unchanged, when compared with age-matched normotensive counterparts [26,27].

Because all were healthy, active individuals with no prior history of fainting, the pre-syncopal response to hypotensive LBNP was unanticipated. This may be a feature unique to this specific protocol, in which, initially, there was greater unloading of low pressure than high pressure mechanoreceptors. In contrast, standing or tilt
unloads simultaneously both low pressure cardiopulmonary and high pressure aortic baroreceptor afferent nerve endings. This redundancy, which provides two independent pathways for reflex increases in efferent sympathetic vasoconstrictor discharge, may be sufficient to protect healthy individuals from presyncope during standing, but not during the stimulus of graded LBNP. For several decades incremental LBNP has been used to isolate reflexes arising from the atri, pulmonary veins and the left ventricle (which sense changes in filling pressures and chamber volumes and elicit reflex changes in sympathetic discharge to skeletal muscle) from reflexes arising from afferent nerve endings situated in the aortic arch and carotid sinus (which sense changes in blood pressure and stroke volume) [28–30]. Both the haemodynamic changes observed in the present protocol, and the absence of any significant drop in parasympathetic heart rate modulation, imply that unloading of these low pressure receptors was the predominant stimulus to sympathetic activation \( \leq -15 \text{ mmHg}\) LBNP.

In a retrospective comparison of healthy subjects, Jacobs et al. [31] reported a significant increase in forearm noradrenaline spillover, in response to \(-15\) mmHg in subjects who tolerated \(-40\) mmHg LBNP, whereas there was a non-significant trend to lower forearm noradrenaline spillover in those who experienced presyncope during that stimulus. There were no differences between these two groups, at baseline, in blood pressure, heart rate, plasma beta-endorphin concentrations, arterial catecholamines, total body noradrenaline spillover or forearm noradrenaline spillover into plasma. These authors did not record CVP, and therefore could not quantify cardiac reflex gain.

Mosqueda-Garcia et al. [4] performed a detailed study of responses to incremental tilt in three groups of subjects: controls, healthy subjects with no prior history of syncope, but who consistently fainted during tilt, and subjects under investigation for recurrent neurally mediated syncope. They described a progressive reduction, across these three groups, in the gain of the relationship between the absolute change in CVP and the relative (\%) increase in MSNA induced by progressive tilting. Reductions in CVP and systemic blood pressure with this stimulus also differed in their time course and magnitude. In those with recurrent neurogenic syncope, they observed attenuation of the MSNA response at low tilt angles, followed by a gradual decline and then an abrupt cut-off. (However, in our experience, it is difficult to discriminate between abrupt reflex cessation of central sympathetic outflow and displacement of the micro-electrode by leg movement during negative pressure.) The greatest fall in CVP was observed in the otherwise healthy subjects in whom repeated tilt-testing evoked syncope, i.e. analogous to the response observed in our pre-syncopal subjects. Although, in contrast to the present findings, MSNA increased significantly, in response to tilt, in this group. However, no statistical comparison between these three calculated gains was performed; relative, rather than absolute increases in MSNA were plotted on the ordinate; and the tilt stimulus applied was not specific to cardiac mechanoreceptor unloading.

Morillo et al. [2] also examined responses to tilt in subjects with a history of orthostatic syncope. Approx. 40% of these developed recurrent syncope during this experiment. At baseline, heart rate, blood pressure and MSNA were similar in pre-syncopal and non-syncopal patients, but parasympathetic responses to nitroprusside-induced reductions in blood pressure were attenuated. The arterial baroreflex control of MSNA was not different between these two groups. Since CVP was not determined, these authors could not comment on cardiac reflex gain.

Our demonstration of markedly lower cardiac reflex control of MSNA in subjects who experienced presyncope would therefore appear to be a novel finding. This may reflect long-standing differences in the discharge characteristics of the afferent nerve endings, perhaps due to altered compliance of the cardiac chambers or large vessels which house these mechanoreceptors, or differences in the distribution of blood volume [32] between these two groups. If so, this could explain why the significantly higher CVP did not appear to reflexively suppress MSNA under resting conditions. It is also possible that LBNP stimulated the release of sympathetic inhibitory peptides, such as vasopressin, atrial natriuretic peptide and endogenous endorphins. Plasma concentrations of these peptides increase markedly prior to syncope, or when orthostatic manoeuvres elicit nausea, light-headedness and related symptoms of impending faint [3,31,33]. An inhibitory interaction between such peptides and the sympathetic nervous system, centrally, or at the ganglionic level [7,34–36], could reset the cardiac baroreflex acutely, causing a gradual or abrupt loss of sympathetic vasoconstrictor discharge to skeletal muscle. If these peptidergic responses differed within subjects, from one test stimulus to the next, such variability could explain why subjects differ in their tolerance to repeated orthostatic stress.

In a previous report [37], subjects with unexplained syncope who developed recurrent symptoms during tilt-testing had a decrease in their \(P_f / P_{II}\) ratio, whereas this ratio increased in those without symptoms. We therefore explored frequency domain characteristics of heart rate variability. There was no difference in baseline values for this ratio between the two groups, and \(-15\) mmHg LBNP had no effect in either group, supporting our previous conclusion that this proposed power spectral estimate of efferent sympathetic outflow to the sinoatrial node is insensitive to significant between-group differences in the magnitude of sympathetic nervous system activation [8].
There are several limitations to the present analysis. First, this was a post hoc comparison of subjects who did or did not develop presyncope in response to a protocol of LBNP applied for another purpose, rather than a prospective study of subjects predisposed to recurrent syncope. Second, it is not known whether these observations would be reproducible within subjects if this protocol were to be repeated. Thirdly, our observations do not permit us to determine the mechanisms responsible for these baseline differences between groups, their contrasting sympathoneural responses to LBNP and the attenuated cardiac reflex control of MSNA. These may reflect long-standing differences in neurogenic cardiovascular control in such individuals. Alternatively, they may arise from day-to-day variations in sodium balance, and therefore cardiopulmonary and total intravascular blood volume, or reflect an alerting response to the laboratory environment. These differences may also be due to variations between subjects, in the corelease of sympatho-inhibitory peptides and neurotransmitters, or in their actions. Thus the present report should be considered as hypothesis-generating. Future studies of this issue should address questions of reproducibility, predictive value and mechanism. Fourthly, the two groups were well matched with respect to age, height, blood pressure and several other baseline characteristics, but differed with respect to their initial CVPs. However, the higher initial CVP of the pre-syncopal group would suggest that they were operating at the steeper portion of the sigmoid CVP–MSNA stimulus–response relationship, and therefore might be expected to display an augmented, rather than a diminished, sympatho-neural response to the fall in CVP elicited by non-hypotensive LBNP. Fifthly, we did not study concurrently blood volume, or plasma markers of other vasoactive systems involved in salt and water balance. It is possible that these pre-syncopal subjects maintain increased blood volume or redistribute their blood volume centrally as a result of a generalized activation of several neurohormonal mechanisms. Finally, we cannot comment on whether women predisposed to presyncope would display the same baseline differences or attenuated reflex responses to LBNP.

In summary, this analysis would suggest that the predisposition to presyncope in response to graded LBNP is associated with greater dependence on vasoconstriction and venoconstriction in skeletal muscle, to maintain preload under conditions of supine rest, and attenuation of the reflex vasoconstrictor response to preload reduction, subserved by the low pressure cardiopulmonary receptors. This diminished cardiac reflex control of muscle sympathetic nerve activity would then result in an inability to maintain stable blood pressure and cerebral blood flow in the face of sustained displacement of blood volume from the cardiopulmonary circulation.

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