Persistence of muscle sympathetic nerve activity during vasovagal syncope

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Aims
To determine whether sympathetic nerve firing to skeletal muscle vasculature is withdrawn during vasovagal syncope (VVS) precipitated by passive graded head-up tilt (HUT) table testing.

Methods and results
We performed passive graded HUT table testing in 18 patients with a history of recurrent postural VVS whom we evaluated during the syncopal event. All patients developed typical VVS during testing. Blood pressure was measured continuously via intra-brachial arterial line. Muscle sympathetic nerve activity (MSNA) was measured using an electrode in the peroneal nerve. Passive graded HUT was then applied. No pharmacological agents were used to provoke syncope. The recording site was maintained through the syncopal event in 10 of 18 patients and we were able to demonstrate persistence of MSNA during syncope in 9. The predominant haemodynamic pattern of syncope in this cohort was mixed—hypotension and bradycardia, with heart rate not falling <40 b.p.m. (n = 10).

Conclusion
Our data challenge the established view that the final trigger for human orthostatic vasovagal reactions is sympathetic nervous system inhibition. Efferent sympathetic nerve traffic to the skeletal muscle vasculature was nearly always maintained through the faint. This finding supports an alternative viewpoint, that vasodilator mechanisms underlie the blood pressure fall in VVS.

Keywords
Syncope • Vasovagal • Sympathetic nervous system • Autonomic

Introduction
Vasovagal syncope (VVS) is the commonest cause of postural syncope with a lifetime cumulative incidence of 35%.1 Syncope is a significant cause of morbidity2 and accounts for 0.9–1.2% of hospital emergency department visits.3,4 Nearly all cases of syncope in young adults are due to VVS.3 Despite numerous investigations, the mechanism of postural syncope remains controversial and treatment remains unsatisfactory.5

Pathophysiology of vasovagal syncope
Fainting has been observed throughout human history; Sir John Hunter described it during phlebotomy sessions in the eighteenth century. The observation of bradycardia preceding syncope led to therapeutic attempts at preserving heart rate (HR). Intervention with permanent cardiac pacing has shown disparate results; however, two randomized controlled trials in which pacemakers were present in both treatment arms [VPS II (Second Vasovagal Pacemaker Study)]6 and SYNPACE (Vasovagal Syncope and Pacing Trial)7 did not show benefit. The failure of ‘bradycardia prevention’ to stop syncope supports the idea that alternative mechanisms may be operative in VVS.

The assumption of upright posture results in reduced venous return to the heart due to gravity-mediated venous pooling of blood in the pelvis and lower limbs. Many investigators believe that this results in acute central hypovolaemia and subsequent vigorous myocardial contraction and inappropriate C-fibre activation from the ventricular myocardium. The usual signals generated from C-fibres in the left ventricle are in response to stretch rather than hypovolaemia.8 Affertent signals are sent to the brainstem, mimicking neural traffic seen during arterial pressure rises and thus results in paradoxical sympathetic inhibition providing the substrate for hypotension and syncope.8–10 Abrupt cessation of sympathetic nerve activity to skeletal muscle vasculature, representing near-total sympathetic withdrawal,11–14 has been considered a critical step leading to hypotension. During routine clinical autonomic testing, we observed persistent muscle sympathetic nerve activity (MSNA) during VVS, an observation that was entirely unexpected.

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We therefore hypothesized that cessation of MSNA is not the predominant mechanism that precedes the onset of haemodynamic collapse in patients with a history of recurrent postural VVS.

Methods

Subjects

The research protocol complies with the Declaration of Helsinki and was approved by The Alfred Hospital Human Research and Ethics Committee. Eighteen patients (16 female and 2 male, 27 ± 3 years old) with a history consistent with recurrent VVS participated in the study after giving written informed consent. These 18 patients were selected on the basis of clinical history and a positive tilt table test for typical VVS. All patients were referred to the Alfred Hospital Syncope Service (tertiary referral centre), having suffered two or more syncopal events in the preceding 6 months. They underwent comprehensive medical assessment, other causes of syncope being excluded in a manner consistent with current best practice. All patients underwent ‘invasive’ (arterial line) passive graded head-up tilt (HUT) testing, and the blood pressure and HR responses were consistent and clinically safe prior to tilt back to allow for maximum possible survival. Patients were maintained upright for as long as it was deemed safe and appropriate.

Experimental protocol

Participants were placed supine on a motorized tilt table and were instrumented for continuous BP monitoring and microneurography recordings. Caffeine and alcohol intake was excluded from 7 p.m. on the evening prior to the study. The brachial artery was cannulated percutaneously (3 F, 5 cm, Cook Medical), and an intravenous cannula was placed in an antecubital vein. A lead III electrocardiograph was recorded and respiration measurements were determined with a transducer incorporating a piezo-electric device (ADInstruments, Castle Hill, NSW, Australia). Following complete instrumentation, patients were allowed to rest in a darkened room for 30 min. During the last 20 min of supine rest, baseline haemodynamic and MSNA data were obtained. Patients then underwent graded HUT at angles of 20°, 30°, 40°, and 60° for 10 min at a time. Blood pressure, the ECG, and MSNA were digitized with a sampling frequency of 1000 Hz (PowerLab recording system, model ML785/8SP, ADI Instruments) and monitored continuously.

Once pre-syncpe (symptomatic haemodynamic collapse) developed, patients were maintained upright for as long as it was deemed safe and appropriate. The time taken to return to supine (tilt back) using our motorized tilt table was 15 s from 60° tilt and 12 s from 40° tilt.

Sympathetic nerve recording

Multunit sympathetic nerve firing rates in post-ganglionic fibres distributed to the skeletal muscle vasculature were recorded by clinical microneurography as described previously. The common peroneal nerve was located near the head of the fibula, initially by palpation, then with electrical stimulation via a surface probe. A tungsten microelectrode (FHC, Bowdoinham, ME, USA) was inserted percutaneously and adjusted until a satisfactory spontaneous MSNA signal was observed in accordance with previously described criteria. After an acceptable nerve-recording site was obtained, MSNA was recorded during the last 20 min of supine rest. Muscle sympathetic nerve activity was expressed as multiunit nerve burst firing frequency (bursts min⁻¹) and normalized for HR, burst incidence (bursts 100 heart beats⁻¹).

Cardiac baroreflex sensitivity

Baroreflex sensitivity was assessed by the sequence method, using BaroCor software (AtCor Medical, West Ryde, NSW, Australia). This procedure identifies ‘spontaneous’ sequences of three or more consecutive beats in which systolic BP (SBP) progressively rises (by at least 1 mmHg) and cardiac interval lengthens, or SBP progressively decreases (by at least 1 mmHg) and cardiac interval progressively shortens, with a lag of one beat. For each sequence, the linear correlation coefficient between cardiac interval and SBP was computed, and the sequence was validated when r = 0.80. The slope between cardiac interval and SBP was calculated for each validated sequence, and an average slope was calculated for each recording.

Statistics

Data were analysed using Sigmasat Version 3.5. Two-way ANOVA was employed for repeated measures. Pairwise multiple comparison procedures were used when appropriate. Testing was two-sided and results are reported as mean ± SEM for repeated measures and mean ± SD for non-repeated measures (age and body mass index). Statistically significant differences are reported for P < 0.05.

Results

Baseline measurements

Baseline values for patients with VVS are summarized in Table 1. There was no difference in MSNA at rest (17 ± 2 bursts min⁻¹) compared with historical control data (17 ± 2 bursts min⁻¹). Cardiac baroreflex sensitivity was significantly lower than historical control data (11.4 ± 2 vs. 17.5 ± 1.7 ms/mmHg; P < 0.01) and consistent with prior findings. No subjects fainted during instrumentation.

Tilt test responses

All patients developed haemodynamic compromise during tilt testing, and the blood pressure and HR responses were consistent with recognized patterns seen in VVS.

Haemodynamics

Haemodynamic responses at stable time points prior to the development of syncope are summarized in Table 2. Progressive HUT

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline average values (supine rest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>18</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>2/16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27 ± 7a</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25 ± 2a</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Heart rate (b.p.m.)</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Respiration frequency (breaths per min)</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Muscle sympathetic nerve activity (bursts min⁻¹)</td>
<td>17 ± 2a</td>
</tr>
<tr>
<td>Muscle sympathetic nerve activity (bursts 100 heart beats⁻¹)</td>
<td>25 ± 3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM except age and body mass index. Mean ± SD.
increased HR (4 b.p.m. at 20°, 14 b.p.m. at 30°, P < 0.01; 23 b.p.m. at 40°, P < 0.001; 32 b.p.m. at 60°, P < 0.01). There was a trend to lower SBP which achieved significance only at 40° tilt (P = 0.01); diastolic BP (DBP) and respiration remained unaltered during HUT until the development of a vasovagal event. Haemodynamic responses seen during syncope can be classified using the modified VASIS (Vasovagal Syncope International Study) classification.17

Type 1 mixed: Heart rate falls at the time of syncope, but the ventricular rate does not fall below 40 b.p.m. or does so for <10 s with or without asystole of <3 s. Blood pressure falls before the HR falls.

Type 2A—cardioinhibition without asystole: Heart rate falls to <40 b.p.m. for >10 s but asystole of >3 s does not occur. Blood pressure falls before the HR falls.

Type 2B—cardioinhibition with asystole: Asystole occurs for >3 s and HR falls coincide with or precede the fall in blood pressure.

Type 3 vasodepressor: Heart rate does not fall >10% from its peak prior to syncope.

Adopting this classification, haemodynamic responses were VASIS 1 in 10 patients, VASIS 2A in 4 patients, and VASIS 3 in 4 patients.

### Discussion

The finding of persistent MSNA during syncope came as a great surprise to us and is at odds with published data.11–14 We investigated a cohort of relatively young patients referred to a tertiary centre for the assessment of recurrent postural VVS. An MSNA recording site was maintained through to the development of syncope in 10 of 18 subjects; of these, only 1 had ‘cessation’ of MSNA. It is particularly perplexing as we have previously shown that cardiac and renal spillover of noradrenaline (NA) is reduced during VVS occurring during cardiac catheterization, implicating sympathetic withdrawal in the pathogenesis.18 This study challenges the notion that near-total sympathetic withdrawal as defined by cessation of MSNA is the ‘final common trigger’ that results in hypotension during VVS.

The assumption of upright posture results in gravity-mediated displacement of blood into the veins of the pelvis and lower limbs, reducing venous return to the heart. In healthy people, this leads to a reflex increase in sympathetic nervous system (SNS) activity, increasing peripheral vascular resistance and HR such that arterial pressure is maintained.19 As the SNS is central to the neurocirculatory response to posture, it has been assumed that these neural mechanisms fail in postural VVS. The neural pathophysiology,
however, remains poorly understood and the importance of SNS dysfunction in causing the faint is disputed.

**Differences between previous studies identifying muscle sympathetic nerve activity cessation and the present study**

Human research supporting sympathetic withdrawal defined by cessation of MSNA was first demonstrated by Wallin and Sundlof\(^{13}\) in two subjects with no history of syncope. Subject 1 developed syncope after standing, whereas Subject 2 (hypertensive female) developed syncope while recumbent, following an infusion of sodium nitroprusside. Muscle sympathetic nerve activity increased progressively prior to syncope, probably reflecting a baroreflex response to reduced arterial pressure, particularly in the subject receiving sodium nitroprusside. Muscle sympathetic nerve activity cessation occurred at the onset of syncope in Subject 1, but no nerve recording trace is shown as the recording site was lost at syncope. Muscle sympathetic nerve activity ceased at the time of loss of consciousness in Subject 2; however, 20 s prior to interruption in sympathetic outflow, there was a rapid decline in blood pressure associated with high levels of MSNA. This implies that the vasovagal event had begun, heralded by a precipitous fall in blood pressure. Muscle sympathetic nerve activity did not cease at the onset of haemodynamic collapse, rather, it did so when loss of consciousness (syncope) occurred. The timing of neurocirculatory events during VVS is crucial in making an inference about cause and effect. An accurate temporal relationship between blood pressure changes, the occurrence of symptoms, and changes in MSNA must be established. Syncope occurs due to cerebral hypoperfusion and it is likely that, when this occurs, neural traffic from the CNS will be reduced in some individuals and therefore cessation of MSNA may be seen. This does not implicate withdrawal of MSNA in the development of syncope.

Fifteen years later, Morillo et al.\(^{12}\) measured MSNA in seven patients with a history of recurrent syncope and described abrupt cessation of nerve firing immediately prior to the onset of haemodynamic collapse. The tilt test protocol was different to that used in our study and involved abrupt tilting to 60°. Isoproterenol, a sympathomimetic β-agonist, was used to provoke syncope in all but two microneurography (MSNA) patients.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Simultaneous recordings of muscle sympathetic nerve activity (MSNA), heart rate (HR), and intra-arterial blood pressure (BP) are illustrated during vasovagal syncope in seven patients who underwent graded head-up tilting. The interruption of the intra-arterial BP trace in Patients 2 and 6 was due to an arterial blood sample being drawn from the line. The vertical line represents the onset of pre-syncope, and the vertical arrows represent syncope and subsequent tilt back. Patient 1 demonstrates cessation of muscle sympathetic nerve activity (our only example) and serves as a counterpoint to the common finding of persistent muscle sympathetic nerve activity seen in the remaining six patients. Patients 2–7 demonstrate clear persistence of muscle sympathetic nerve activity during haemodynamic collapse in three common haemodynamic variants of vasovagal syncope.
Pharmacological provocation could conceivably alter sympathetic neural responses and contrasts with our study, which utilized passive graded HUT with no drugs to provoke syncope.

In Morillo et al.’s study, MSNA increased normally during the early phases of tilt. There was a gradual decline in blood pressure and a non-significant fall in MSNA (P = 0.19) 100 s prior to presyncope. At the onset of pre-syncope, there was a precipitous fall in blood pressure that was ‘ushered in by abrupt cessation of sympathetic nerve traffic to skeletal muscle’. This paper does not provide any raw MSNA traces to illustrate ‘cessation’. The temporal relationship between blood pressure and MSNA is shown using a sequential histogram of 5 s averages of MSNA. The onset of pre-syncope, which is characterized by a precipitous decline in blood pressure, is associated with a decline in MSNA based on the histogram but it does not appear to show the total disappearance of nerve firing that is purported to herald haemodynamic collapse.

Mosqueda-Garcia et al., measured MSNA in 14 patients during syncope and described a progressive decrease in nerve firing until total disappearance of the signal and syncope. Passive graded HUT was applied, and no drugs were used to provoke syncope. The patients were quite ‘sick’ with an average of five syncopal events per month, which is more severe than in our cohort. Interestingly, blood pressure showed a progressive decline, even at low levels of tilt until the onset of syncope typically at 60° or 75°. Our patients, by comparison, had stable blood pressure until 40° tilt. Surprisingly, these patients had a marked blunting of the MSNA response to tilt, the normal response to hypotension being an increase in MSNA. In contrast, we have shown that MSNA increases normally compared with historic control data, a finding consistent with Morillo et al.’s paper. The haemodynamic pattern characterized by progressive hypotension from low levels of tilt suggests a different form of orthostatic intolerance, perhaps a dysautonomia, which may explain the unusual blunted MSNA response. Once again the temporal relationship between MSNA cessation and haemodynamic collapse is difficult to establish. These data differ from Morillo et al.’s study, which suggested that pre-syncope as opposed to syncope is heralded by cessation of MSNA. Only a single patients’ MSNA recording, purported to show cessation of nerve firing, is shown, with motion artefact in the trace confounding interpretation.

Jardine et al., measured MSNA during HUT at 60° in 15 patients with a history of VVS who were instrumented with a pulmonary artery catheter for the determination of cardiac output (CO). This older cohort, mean age 58 years, had co-morbidities such as hypertension and coronary artery disease. Muscle sympathetic nerve activity, not surprisingly, was much higher at rest (35 ± 4 bursts min⁻¹) compared with our young and otherwise healthy patients (17 ± 2 bursts min⁻¹). Muscle sympathetic nerve activity rose to a peak of 48 ± 4 bursts min⁻¹ and then declined to a nadir of 11 ± 2 bursts min⁻¹ at syncope. These data suggest that MSNA is preserved, although at a much reduced level during haemodynamic collapse, and differs from findings reported previously. We analysed MSNA at 1 and 5 min prior to syncope and found no difference in burst incidence. Vaso-vagal syncope follows a bi-modal age distribution with two peaks of incidence (second/third decade and fifth/sixth decade); the disparate findings in this study may in part be explained by age-related changes in the pathophysiology of VVS.

Significance of persistent muscle sympathetic nerve activity during syncope

It is tempting to use the finding of MSNA cessation to support the idea that inappropriate C-fibre activation from a vigorously contracting heart sends afferent impulses to the CNS, resulting in sympathetic withdrawal manifesting as cessation of MSNA. Many investigators have expressed reservations about this theory, and there is significant experimental data supporting alternative mechanisms such as active vasodilatation. For example, paradoxical vasodilatation is observed in the forearm during syncope and may be non-neural as it is not inhibited by total pharmacological autonomic blockade. Although this phenomenon may be explained by local metabolite trapping due to reduced tissue perfusion, analysis of the haemodynamic response to exercise shows paradoxical vasodilatation in non-exercising muscle beds in patients with a history of recurrent VVS. We can thus speculate that a currently unspecified active vasodilator mechanism may be operative during VVS.

An alternative hypothesis is that CO falls as the predominant physiological event that determines hypotension. In a recent study of 56 patients presenting with suspected VVS who underwent HUT, stroke volume was computed from pressure pulsation data. There was a 50% reduction in calculated CO while systemic vascular resistance was maintained until pre-syncope (tilt back). There was no material difference between VVS that was provoked by sublingual glyceryl trinitrate and drug-free patients. This is supported by an invasive measure of CO which suggested a fall to 2.0 L min⁻¹ just prior to syncope. Low CO may play an important role in the development of some episodes of VVS, but its position as the prime mover in this condition remains controversial.

Our finding of persistent nerve firing during the syncopal event in all but 1 of 10 patients with a maintained MSNA recording is supported by Cooke et al., who applied lower body negative pressure (LBNP) to simulate haemorrhage (n = 12) and noted that MSNA was maintained throughout cardiovascular collapse. Goldstein et al., measured cardiac release of NA using radioisotope dilution and demonstrated reduced NA release in patients with VVS during recumbency. This suggests a ‘mismatch’ between the neural signal to skeletal muscle vasculature and the release or availability of NA, which our preliminary data suggest is reduced during HUT. The pathogenesis of VVS may lie at the level of sympathetic nerve proteins which regulate neurotransmitter availability accompanying the electrical neural signal. The noradrenaline transporter (NET), one such protein, is responsible for clearing NA from the sympathetic nerve sub-synaptic space, thus terminating the neural signal. Increased activity of NET would clear NA more rapidly, reducing neural compensatory vasoconstriction, and so predisposing to postural hypotension. This proposition is supported by increased tolerance of tilting in healthy subjects given the NET-antagonist reboxetine.

Limitations

The physiology of a postural syncopal event is difficult to study in humans because of its unpredictable nature and the rapid onset of
haemodynamic collapse in some patients. Clinical microneurography is technically challenging and it is common to lose a recording site, particularly when a patient loses consciousness and has to be emergently returned to the supine position. We were unable to maintain the recording site during syncope in 8 of 18 patients, a proportion of our cohort that is similar to that achieved in comparable studies.\textsuperscript{12,14} We cannot exclude cessation of MSNA in these subjects. None of our patients demonstrated prolonged asystole (VASIS 2B); we cannot exclude that MSNA cessation might occur in this uncommon VVS variant. The fainting reflex is present in all humans and is triggered by many stimuli such as emotional stress and the sight of blood. Arousal stimuli in the form of electrical stimulation has been shown to produce exaggerated inhibition of MSNA in subjects with a history of blood/needle phobia-induced syncope.\textsuperscript{28} These ‘situational’ fays may have a different physiology to postural VVS, although we do not know of any studies investigating MSNA responses during syncope in this subject group.

Muscle sympathetic nerve activity measured in the peroneal nerve reflects adrenergic neural drive to skeletal muscle vasculature. Although our study does not provide a direct measure of adrenergic drive to other regions such as the heart, MSNA correlates well with sympathetic activation. We have demonstrated that MSNA parallels cardiac NA release in hypertensive patients.\textsuperscript{29} Recently, we applied radio-tracer techniques and MSNA measurements in patients who underwent radio-frequency ablation of renal sympathetic nerves for the treatment of uncontrolled hypertension.\textsuperscript{30} Successful ablation and improvement in blood pressure control correlate with reductions in MSNA. Whole-body and renal NA silpoover also decline, which are precise biochemical markers of SNS activation.\textsuperscript{31}

Conclusions

Syncope is a common medical disorder for which effective treatment is usually lacking. This stems in part from an incomplete knowledge of the mechanism of faint. Our study refutes the current position that abrupt cessation of MSNA is the usual final step resulting in hypotension and syncope. We have been able to demonstrate that MSNA persists in the most common haemodynamic variants of VVS and even in the VASIS 3 (vasodepressor) variety in which one might expect MSNA silence to occur. This observation provides key indirect, supporting evidence that alternative mechanisms must play an important role in the pathogenesis of this common and disabling disease.

Funding

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Conflict of interest: None of the authors had any relationships with industry.

References

An 11-month-old boy presented with a grade 2/6 continuous murmur in the second left intercostal space. History revealed no clinical symptoms. Doppler echocardiography demonstrated a high-velocity systolic jet (6 m/s) into the right atrium (Panels A and B). Selective angiography confirmed a large coronary artery fistula from the left coronary sinus, which drained into the right atrium (Panel C). The calculated pulmonary to systemic flow ratio was 1.3. The fistula was closed with an 8 mm Amplatzer vascular plug, via a 4 F delivery catheter (Panel D). Follow-up by echocardiography 24 h later confirmed complete closure (Panel E).

In more than 90% of the cases, coronary fistulas drain to the right side of the heart. Complications include myocardial steal, atrial fibrillation, and endocarditis. Congestive heart failure may occasionally manifest in infancy, and spontaneous rupture of the aneurysmal fistula has been reported. In selected patients, transcatheter closure can be safely undertaken.

(Panel A) Colour Doppler echocardiography demonstrating a broad jet entering the floor of the right atrium. (Panel B) Continuous-wave Doppler signal demonstrating a high velocity of flow, suggesting communication between the right atrium and a systemic artery. (Panel C) Ascending aortogram in the frontal projection, demonstrating the fistula. The left coronary artery appears to arise as a side branch of the fistula. (Panel D) Lateral angiographic projection demonstrating the location of the vascular plug used to close the fistula. (Panel E) Surface echocardiogram 24 h later, demonstrating the vascular plug in situ. (Panel F) Colour flow Doppler interrogation confirming complete absence of flow through the fistula.