Recurrent Postural Vasovagal Syncope: Sympathetic Nervous System Phenotypes

Running title: Vaddadi et al.; Sympathetic function in Vasovagal Syncope

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Journal Subject Codes: [5] Arrhythmias, clinical electrophysiology, drugs; [85] Autonomic, reflex, and neurohumoral control of circulation; [97] Other Vascular biology
Abstract:

**Background** - The pathophysiology of vasovagal syncope (VVS) is poorly understood and the treatment usually ineffective. Our clinical experience is that VVS patients fall into two groups, based on their supine systolic blood pressure, which is either normal (>100mmHg) or low (70-100mmHg). We investigated neural circulatory control in these two phenotypes.

**Methods and Results** - Sympathetic nervous testing was at three levels: electrical, measuring sympathetic nerve firing (microneurography (MSNA)); neurochemical, quantifying norepinephrine spillover to plasma; cellular, with Western blot analysis of sympathetic nerve proteins. Testing was done during head-up tilt (HUT), simulating the gravitational stress of standing, in 18 healthy controls and 36 VVS patients, 15 with the low blood pressure phenotype (LBPS) and 21 with normal BP (NBPS). MSNA and norepinephrine spillover increased significantly during HUT in healthy subjects. The MSNA response during HUT was normal in NBPS, and accentuated in LBPS (P=0.05). NE spillover response was paradoxically subnormal during HUT in both patient groups (P=0.001), who thus exhibited disjunction between nerve firing and neurotransmitter release, this lowered norepinephrine availability impairing the neural circulatory response. Subnormal norepinephrine spillover in LBPS was linked to low tyrosine hydroxylase (43.7 % normal, P=0.001), rate-limiting in norepinephrine synthesis, and in NBPS to increased levels of the norepinephrine transporter (135 % normal, P=0.019), augmenting transmitter reuptake.

**Conclusions** - Patients with recurrent VVS, when phenotyped into two clinical groups based on their supine blood pressure, show unique sympathetic nervous system abnormalities. It is predicted that future therapy targeting the specific mechanisms identified here should translate into more effective treatment.

**Key words:** syncope, vasovagal, low blood pressure (hypotension), norepinephrine, sympathetic nervous system
Introduction

Syncope is a transient loss of consciousness, consequential to reduced cerebral blood flow, which is associated with a loss of postural tone, collapse and spontaneous recovery\(^1\). Vasovagal syncope (VVS), characterised by abrupt hypotension variably accompanied by bradycardia, is the commonest cause of postural syncope, accounting overall for 21% of patients with syncope\(^2\). Syncope is a significant cause of morbidity\(^1\), responsible for 1 – 2% of hospital emergency department visits\(^3,4\) and costs the United States health care system $2.4 billion annually for syncope related hospitalizations\(^5\). Quality of life scores suggest an impact similar to that of other major chronic diseases, such as epilepsy\(^6\). Despite numerous research studies, the mechanism of recurrent postural VVS remains controversial and treatment is unsatisfactory.

Our clinical experience, in the running of a tertiary referral syncope clinic, is that patients with recurrent VVS fall into 2 clinical phenotypes: normal supine systolic blood pressure (>100 mmHg) and low supine systolic blood pressure (70 mmHg – 100 mmHg). Our identifying of patients with low BP as a distinct VVS phenotype is supported by Mathias, who recognised the low supine BP variant in a landmark observational study\(^4\). Of 641 patients who were referred for the assessment of recurrent syncope, 18 (17 female, 1 male) had “a persistently low basal level of blood pressure which led to a diagnosis of chronic systemic hypotension, of unknown cause”. This distinction is important for a number of reasons, foremost being our clinical experience that those patients with low blood pressure VVS (LBPS) respond more favourably to treatments such as water drinking, high salt diet and fludrocortisone than patients with normal blood pressure VVS (NBPS).

All humans have the propensity to faint if sufficiently challenged. The “trigger” which results in VVS occurring will vary from person to person. The sympathetic nervous system (SNS) is critical in protecting against postural VVS. We hypothesize that differing abnormalities in the
SNS regulation of BP may act as the predisposing factor underlying recurrent VVS in both the NBPS and LBPS phenotypes.

The aim of the present study was to comprehensively evaluate SNS function in patients with VVS, stratified into the 2 phenotypic groups based on clinic BP measurements. This was undertaken at three levels: electrical (muscle sympathetic nerve activity- MSNA), neurochemical (norepinephrine (NE) release), and cellular, with analysis of sympathetic nerve proteins which influence NE synthesis, storage, release and reuptake.

Methods

Subjects

The research conformed to National Health and Medical Research Council of Australia guidelines and was approved by The Alfred Human Research and Ethics Committee. Eighteen healthy subjects (11 female, 7 male, 22±3 yrs) and 36 patients (34 female and 2 male, 28±9 yrs) with a history of recurrent VVS participated in the study after giving written informed consent. Of the 36 patients with recurrent VVS, 15 had LBPS (14 female, 1 male, 28±9 yrs) and 21 had NBPS (20 female, 1 male, 28±9 yrs). Patients were deemed to have LBPS if 2 or more clinic supine cuff systolic BP (SBP) measurements were ≤100 mmHg (the range being 70-100 mm Hg). No patient was taking medication that could lower BP. All patients had been referred to our Syncope Management Service, having suffered 2 or more syncopal events in the preceding twelve months. They underwent comprehensive medical assessment to exclude other causes of syncope. Patients with the postural tachycardia syndrome were excluded.

Experimental protocol

All participants underwent progressive head-up tilt (HUT) on a motorized table with a footboard, to simulate the gravitational stress of standing (no drugs were used to provoke syncope). They were instrumented (brachial artery and forearm venous cannulation) for continuous BP
monitoring, measurement of plasma NE spillover and MSNA. Caffeine and alcohol intake was excluded from 7pm on the evening prior to the study. A lead III electrocardiograph was recorded. Following instrumentation, patients were rested in a darkened room for 30 minutes. During the last 20 minutes of rest, baseline hemodynamic and MSNA data were gathered, and arterial blood samples were obtained. Patients then underwent graded HUT at angles of 20°, 30°, 40° and 60°, each for 10 minutes. Arterial blood samples were taken at the end of each tilt angle. BP, ECG and MSNA were digitized with a sampling frequency of 1000 Hz (PowerLab recording system, model ML785/8SP, ADI Instruments) and monitored continuously. If pre-syncope developed (symptomatic pending hemodynamic collapse), patients were tilted back supine.

**Sympathetic nerve recording**

MSNA in postganglionic fibres distributed to the skeletal muscle vasculature was recorded using a tungsten microelectrode (FHC, Bowdoinham, ME, USA) inserted percutaneously into the common peroneal nerve near the head of the fibula. MSNA was expressed as multiunit nerve burst firing frequency (bursts.min⁻¹), and also normalised for heart rate (bursts.100 heart beats⁻¹).

**Measurement of whole body norepinephrine spillover**

The rate of spillover of NE from sympathetic nerves to plasma was determined by isotope dilution during an intravenous infusion of tritiated NE ([³H]NE), a technique developed by us. Arterial blood was collected into chilled tubes containing reduced glutathione and EGTA. Plasma was separated by refrigerated centrifugation (4°C at 3000g) and stored at -80°C for assay of NE and [³H]NE. The rate of total body spillover of NE to plasma was determined according to the formula:

\[
\text{Total NE Spillover Rate} = \frac{[³H] \text{NE Infusion Rate} \text{ (dpm/min)}}{\text{Neural input}}
\]
Plasma NE Specific Radioactivity (dpm/pmol)

Quantification of sympathetic nerve proteins: Tyrosine hydroxylase (TH), norepinephrine transporter (NET), vesicular monoamine transporter 2 (VMAT2) and Dynamin I

Sympathetic nerve proteins (figure 1) were extracted from subcutaneous veins, which have a dense sympathetic innervation\textsuperscript{10,11}. Seven patients with LBPS, 6 with NBPS and 7 healthy controls underwent the procedure. A skin incision was performed on the dorsum of the forearm and 10mm length of vein was removed (2-3mm diameter) and placed in liquid nitrogen, then stored at – 80°C until assay.

\textit{TH, VMAT2, Dynamin I and NET protein immunoblotting procedure}

The SNPs analysed were TH, VMAT2, Dynamin I and NET, proteins important in the synthesis, storage, release and reuptake of NE. The tissue samples were homogenized in PRO-Prep Protein extraction solution (17081, INtRON Biotechnology). Proteins were separated by electrophoresis on a 10% acrylamide minigel, transferred to PVDF membranes (NEF1002, PerkinElmer Life sciences, Inc), and detected by incubation with specific primary antibodies followed by further incubation with a peroxidase-conjugated anti-IgG and then with enhanced chemiluminescence reagents (NEL104, PerkinElmer LAS, Inc). Antibodies used include anti-hNET (NET17-1, Abcam), anti-TH (AB152, Chemicon), anti-Dynamin I (3G4B6) (sc-53877, Santa Cruz biotechnology, Inc), anti-VMAT2 (AB1767, Chemicon), anti-GAPDH (sc-32233, Santa Cruz biotechnology, Inc). Scanned signals were analysed with BIO-RAD Quantity One software.

\textbf{Statistics}

Data was analysed using Sigmastat Version 3.5 and STATA 10. One-way ANOVA was used to analyse baseline data and two-way repeated measures ANOVA was employed to investigate changes in haemodynamics, NE spillover and MSNA. Multiple comparison procedures (Holm-Sidak) were applied when the overall significance between groups was
P<0.05. Mixed effects model (XTmixed- STATA) was also employed to test for significant differences in NE spillover and MSNA between groups during HUT and to determine the influence of variables such as gender and age. Testing was 2 sided and results are reported as mean±SEM for repeated measures and mean±SD for non-repeated measures (age, body mass index, office blood pressure and heart rate). Statistically significant differences are reported for P<0.05.

Results

A. Baseline measurements

Baseline values for patients with LBPS, NBPS and controls are summarized in table 1. There was no significant difference in age or BMI. Intra-arterial systolic BP was significantly lower in LBPS patients (SBP, 120±2mmHg versus 132±2mereg in NBPS subjects and 129±4mmHg in controls; p=0.005). SBP was substantially higher in patients with LBPS on the day of the invasive HUT compared with clinic cuff pressures (70-100mmHg), representing a response to the invasive procedure.

NE plasma spillover during supine rest was significantly lower in LBPS subjects (201±23ng/min) compared to controls (441±26ng/min (P=0.003) and NBPS patients (370±48ng.min (P=0.02 ) (Table 1). NBPS subjects had non-significant trend to lower NE spillover at rest when compared to controls P=0.25). There was no significant difference in MSNA at rest between the 3 groups (P=0.18). Age, gender and BMI had no independent effect on the resting data. No subjects fainted during instrumentation.

B. Tilt test outcomes

Of the control subjects, 1 developed VVS at tilt 30º and 2 at tilt 40º. In the LBPS group, syncope occurred at 20º in 1 subject, 30º in 2 subjects, 40º in 1 subject, and 60º in 5 subjects, leaving 6 of
15 subjects (40%) free of syncope during tilting. NBPS patients showed a higher rate of attrition, with 14% only avoiding syncope. Syncope developed in 1 subject at 20º, 2 subjects at 30º, 10 subjects at 40º, and 5 subjects at 60º.

C. Hemodynamics

Hemodynamic responses, NE spillover and MSNA (beats.100 heart beats⁻¹) at stable time points prior to the development of syncope are summarised in table 2. Progressive HUT increased heart rate (HR) significantly in all 3 groups. At tilt 40º LBPS and NBPS subjects had a mean heart rate of 90±4bpm and 94±2bpm respectively; significantly higher than controls (81±2bpm, P<0.001). SBP was significantly lower in LBPS patients compared to both controls and NBPS at all tilt angles (P=0.01). The most common hemodynamic pattern seen during syncope was mixed hypotension and bradycardia (no asystole). Age, BMI and gender had no independent effect on the HUT data.

D. Sympathetic Nerve Firing

MSNA in LBPS subjects increased more during tilting than in the other groups, at 20º and 30º tilt. For 30º tilt, in LBPS patients compared with NBPS patients and controls, MSNA increased by +22 bursts.100 heart beats⁻¹ versus +11 and +9 bursts.100 heart beats⁻¹; p=0.05 (figure 2 and 3). At tilt 40º in LBPS subjects, the difference lost significance (P=0.14) due to the low number of patients who both tolerated the tilt angle and maintained an intact microneurography recording site.

E. Norepinephrine Release

Whole body NE spillover to plasma was measured at all tolerated tilt angles. NE spillover increased significantly during HUT in healthy subjects (+152 ng.min⁻¹ at tilt 40º; P<0.001)
(Figure 4). In contrast, NBPS patients had a severely blunted NE spillover response to HUT, with no increase in NE spillover during HUT (P=0-.32, Figure 4), indicating a failure of SNS response to postural change.

NE spillover was significantly lower at all tilt angles in LBPS subjects compared to controls and NBPS (figure 3; P=0.001). The finding of low NE spillover in LBPS subjects contrasts with their marked increase in MSNA (Figure 2) indicating a “mismatch” between nerve firing and NE release. A similar mismatch was present in NBPS patients, who had a normal increase in MSNA during HUT, but no increase in NE spillover.

**F. Sympathetic Nerve Tyrosine hydroxylase, VMAT2, Dynamin I and NET proteins**

Figure 5 illustrates representative Western blots of 7 control subjects, 7 LBPS and 6 NBPS patients. Analysis of TH protein revealed reduced intensity of the 50-kDa band in LBPS compared with controls and NBPS subjects. Quantification of TH protein against a reference loading protein, GAPDH, indicated markedly decreased abundance of TH (mean 43.6 % normal) in the LBPS group compared to controls (P=0.001). As TH is the rate-limiting enzyme in NE synthesis, the reduced abundance in LBPS patients is noteworthy, given their low NE spillover in the face of high sympathetic nerve firing rates during HUT.

Western blot analysis of NET protein in controls revealed the 80kDa band, which is the glycosylated, active and membrane bound form of human NET. NET was significantly reduced (mean 50.0 %) in the LBPS patients (P<0.001) compared to controls (Figure 5). In contrast, NBPS subjects demonstrated normal TH and increased abundance of NET (mean 135%) compared to controls (P=0.019). VMAT 2 and dynamin 1 quantification was similar in the 3 groups (not shown in figure).

**Comment**
Recurring postural VVS is common, frequently poses a diagnostic challenge and lacks effective evidence based treatment. A framework for this research was our clinical experience indicating that VVS patients fall into two distinct clinical groups based on their supine SBP, normal (>100mmHg) or low (70-100mmHg). Recognition of low BP as a specific clinical phenotype of VVS has not received emphasis before, only Mathias having identified this variant\textsuperscript{4}. Our expectation is that better understanding of the neural pathophysiology of VVS variants will lead to more successful treatment.

The SNS is the principal acute regulator of BP, and provides the pivotal reflex neural circulatory adjustments stabilising BP during standing\textsuperscript{12}. Standing results in gravity-mediated displacement of blood into the veins of the pelvis and lower limbs. Arterial baroreceptors detect the change in central blood volume and arterial pressure, sending afferent signals to the brain. This leads to a reflex increase in sympathetic activity, increasing peripheral vascular resistance and heart rate such that BP is maintained\textsuperscript{12}. We postulated that abnormalities in these reflex sympathetic nervous responses might underlie the tendency to VVS in both the NBPS and LBPS phenotypes.

In the present study we have applied sophisticated methodology to the investigation of SNS function in patients with recurrent VVS at three key levels:

(i) \textit{Electrical - Sympathetic nerve firing}: We applied the established but challenging technique of sympathetic microneurography to determine the rates of sympathetic nerve firing to the lower limb skeletal muscle vascular bed during the gravity challenge with HUT.

(ii) \textit{Neurochemical - Neurotransmitter release}: We measured the overflow of NE from sympathetic nerves to arterial plasma (NE spillover) using radiotracer techniques we developed\textsuperscript{13}. This is a well-validated measure of sympathetic activity\textsuperscript{14} and is superior to measuring the concentration of NE in plasma because it is immune to the confounding influence of reduced clearance of NE from plasma during HUT\textsuperscript{15}. 
(iii) **Cellular - Sympathetic nerve proteins:** A previous limitation to investigation of the human SNS has been the lack of access to sympathetic nervous tissue. In this study, we used biopsied forearm subcutaneous veins as a source of SNPs to overcome this deficiency\(^\text{10}\). Forearm subcutaneous veins are densely innervated, and as a source of SNPs proved superior to biopsies of skeletal muscle and adipose tissue (unpublished analysis).

In the present study we utilised HUT to simulate the gravity mediated orthostatic stress occurring during prolonged standing. It has been shown in healthy people that the reflex sympathetic activation maintaining BP during HUT is evident in a progressive escalation in MSNA\(^\text{16}\) and NE release to plasma\(^\text{10}\); findings replicated by us. Some studies have suggested differences in MSNA responses in patients with VVS during low level lower body negative pressure (LBNP) and early tilt table testing\(^\text{16-18}\). Béchir et al measured MSNA during LBNP in 8 healthy controls and 10 patients with VVS\(^\text{17}\). There was no difference in systolic blood pressure between the 2 groups, however MSNA was markedly higher in the VVS group at rest (42.4±7.3 vs 26.5±10.1 bursts.min\(^{-1}\)). This resting MSNA level is significantly higher in both controls and VVS subject when compared to our data. There is no obvious explanation for this marked difference based on patient selection and other variables when compared to the present study. Our resting data compares favorably with other studies\(^\text{16, 18, 19}\). Mosqueda-Garcia et al.\(^\text{16}\) 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 pressures until the development of syncope. 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 in patients with VVS\textsuperscript{20}. 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. Wasmund applied LBNP to healthy subjects with no history of syncope and found attenuated MSNA responses in the group that developed pre-syncope\textsuperscript{18}. Tilting differs from LBNP in that in addition to reduction in filling pressures, it activates the vestibular system and is more likely to cause abdominal and lower extremity skeletal muscle contractions, which could significantly alter MSNA limiting any direct comparisons with our results. Furthermore, the healthy subjects had a mean age approaching 40 years, significantly older than our cohort. It is fair to say that measurements of reflex autonomic changes during an orthostatic challenge in subjects who exhibit VVS physiology have produced conflicting results. Our data supports the notion that MSNA increases progressively during orthostatic stress in patients with VVS, the degree of this escalation being exaggerated in patients with the LBPS phenotype, which we have identified. In both phenotypes of VVS, however, there was a failure of the normal neural circulatory response to orthostatic stress. We anticipated that MSNA might be low during HUT in the LBPS patients, as a basis for their low BP and predisposition to syncope; surprisingly, MSNA increased at twice the normal rate during HUT. If the linkage of nerve firing to neurotransmitter release was normal in LBPS patients we would expect to see a substantial increase in NE during HUT, but this was not the case. LBPS patients, despite their high sympathetic nerve firing rates, actually had markedly reduced rates of NE release at all angles of tilting compared with controls. This implies a mismatch between the electrical and neurotransmitter components of the sympathetic neural response. The NBPS patients exhibited a different pathophysiology. In them, MSNA increased normally during HUT, but NE spillover failed to increase at all. Thus despite differences between the two VVS variants, in both, there is a “mismatch” between nerve firing and neurotransmitter release.
We sought to explain this “mismatch” with analysis of sympathetic nerve proteins involved in NE synthesis, storage, release and reuptake. We quantified the key regulatory proteins (figure 1).

- TH- rate limiting enzyme in NE synthesis.
- VMAT2- responsible for incorporation of cytoplasmic NE into storage vesicles\textsuperscript{21, 22}.
- Dynamin I- vesicle formation and recycling\textsuperscript{23}.
- NET- clears 60-95% of NE released into the synaptic cleft\textsuperscript{24}.

Analysis of sympathetic nerve proteins in LBPS patients revealed low levels of TH. In them, measured NE spillover was low, presumably due to reduced NE synthesis. Their high rates of MSNA may be compensatory, in the face of reduced NE availability. VMAT 2 and dynamin 1 were normal, while NET was significantly reduced. Impaired reuptake of NE due to decreased NET would increase NE spillover, not what we found in LBPS. Reduced NET expression in LBPS is presumably an adaptive response to lowered NE synthesis. We note parallels between LBPS patients and those with dopamine-\emph{\beta}-hydroxylase (DBH) deficiency, a rare genetic disorder which also impairs NE synthesis\textsuperscript{25}. Patients with DBH deficiency typically have low-normal BP, postural syncope and near-zero NE spillover. Nerve firing is high\textsuperscript{25} but dysfunctional, much as it is in LBPS patients, because of absence of NE.

These findings in LBPS patients contrast with those seen in NBPS, who exhibit normal TH expression but elevated NET levels. Each pulse of the sympathetic neural signal is terminated primarily by rapid reuptake of the released NE (60 – 95\%)\textsuperscript{24} into the sympathetic varicosity via NET\textsuperscript{22}. Increased levels of NET could reduce the concentration of NE in the synaptic space, blunting the NE response to upright posture, even in the presence of normal increases in MSNA. This is the probable mechanism of the blunted NE spillover response to HUT in NBPS patients.

We have previously shown that MSNA persists during the onset of hemodynamic collapse in patients with VVS suggesting that an alternative mechanism to silencing of sympathetic nerves, of the types we describe here involving low NE availability, may predispose patients to VVS\textsuperscript{20}. 
The importance of excessive action of NET as a driver for postural VVS has been intimated in a prior study, where antagonism of NET with the selective NET inhibitor Reboxetine, was shown to protect against syncope during prolonged HUT\textsuperscript{26}.

The origins of low TH levels in LBPS patients, and increased NET in NBPS patients remains unknown. Low TH could, perhaps, be due reduced sympathetic nerve tissue density but VMAT2, which is also specific for sympathetic nerves, is expressed normally, arguing against this. To this point it has not been possible to definitively test for a genomic or epigenetic mechanism lowering TH. A genomic basis for low TH is possible, but TH gene expression could not be evaluated directly because the yield of sympathetic nerve mRNA is very low in biopsy material, not surprising given that the distance of the forearm vein biopsy site from the cell bodies in the sympathetic ganglion is nearly one metre. The origin of the increased abundance of NET protein in NBPS is equally obscure.

Postural syncope has been challenging to diagnose and treat, in part a consequence of the pathophysiology being poorly understood. We believe that the identification of discrete VVS variants, with unique disease mechanisms, will facilitate the treatment of patients. Drinking more fluids, increasing dietary salt intake, and physical counter-pressure maneuvers\textsuperscript{1} should still be universally applied, with administration where indicated of medication, such as fludrocortisone, to expand plasma volume\textsuperscript{27} and pressor agents such as midodrine\textsuperscript{28}. But more specific, targeted drug treatment, directed at elucidated mechanisms of syncope, should now follow.

The overarching philosophy to this research was our conviction that successful treatment of postural syncopal disorders would not be achieved without delineation of the neural circulatory pathophysiology. NET now emerges as a potential therapeutic target in patients with the NBPS variant of VVS, and clinical trials using a NET antagonist such as Reboxetine should be conducted. In patients with LBPS, who have low TH expression, NE synthesis needs to be augmented. This might be achieved with L-DOPS (l-threo-3,4-dihydroxyphenylserine), a NE
pro-drug, which is converted to NE by DOPA-decarboxylase, bypassing TH\textsuperscript{29}. L-DOPS has been used to advantage in patients with dopamine-\(\beta\)-hydroxylase deficiency\textsuperscript{25}, who have total failure of NE synthesis.

**Limitations**

Our study was confined to younger patients in keeping with the bimodal age distribution of VVS. The illness also occurs in older people, in whom the pathophysiology may perhaps be different. The LBPS group was almost entirely female (14 of 15) which we believe reflects the natural distribution of this condition and is consistent with the observations by Mathias\textsuperscript{4}. Our data does not allow us to comment on LBPS in males, other than the fact that it is probably a particularly rare phenotype. It is uncertain whether the abundance of sympathetic nerve proteins from forearm veins is representative of expression in other sites. We have, however, compared the abundance of NET in subcutaneous forearm veins with that in the heart in individual cadaver donors and find the values in the two sites to be approximately in parallel\textsuperscript{10}. Hemodynamic patterns seen during VVS vary widely. None of our patients exhibited the asystolic variant which might have a differing underlying pathophysiology\textsuperscript{30}.

**Conclusions**

Patients with recurrent VVS can be phenotyped into two clinical groups based on their supine SBP in the clinic- low or normal. Both these patient groups exhibit disjunction between sympathetic nerve firing and neurotransmitter release to plasma, with lowered NE availability impairing the neural circulatory response and predisposing them to postural syncope. Subnormal NE spillover in the LBPS variant is most likely attributable to low levels of the NE synthesizing enzyme, TH, and in the NBPS variant, to increased levels of the NET protein augmenting
transmitter reuptake after release. These molecular mechanisms we identify may ultimately be translated into effective, targeted therapy.

**Funding Sources:** National Health and Medical Research Council of Australia and National Heart Foundation (Australia)

**Conflict of Interest Disclosures:** None

**References:**

27. Raj SR, Rose S, Ritchie D, Sheldon RS. The second prevention of syncope trial (post ii)--a randomized clinical trial of fludrocortisone for the prevention of neurally mediated syncope: Rationale and study design. Am Heart J. 2006;151:1118 e1111-1187
Table 1. Baseline values in healthy controls and syncope groups: Supine rest

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=18)</th>
<th>LBPS (n=15)</th>
<th>NBPS (n=21)</th>
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<tr>
<td>Age (years)</td>
<td>22±4</td>
<td>29±9</td>
<td>27±9</td>
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<td>Body Mass Index (BMI)</td>
<td>24±4</td>
<td>24±3</td>
<td>25±4</td>
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<td>Office systolic blood pressure, mmHg* (P=0.008)</td>
<td>118±3</td>
<td>98±3</td>
<td>122±3</td>
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<td>Office diastolic blood pressure, mmHg* (P=0.007)</td>
<td>70±1</td>
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<td>70±1</td>
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<td>Office heart rate, bpm</td>
<td>64±1</td>
<td>67±2</td>
<td>66±2</td>
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<tr>
<td>Intra-arterial systolic blood pressure, mmHg* (P=0.006)</td>
<td>129±4</td>
<td>120±2</td>
<td>132±2</td>
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<td>Intra-arterial diastolic blood pressure, mmHg* (P=0.01)</td>
<td>72±2</td>
<td>67±2</td>
<td>71±1</td>
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<td>Heart rate, bpm at the time of invasive tilt table testing</td>
<td>67±1</td>
<td>69±3</td>
<td>68±2</td>
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<td>Respiration frequency, breaths per min</td>
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<td>MSNA, bursts per min</td>
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<td>Plasma NE spillover, ng/min* (P=0.003)</td>
<td>441±26</td>
<td>201±23</td>
<td>370±48</td>
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MSNA: Muscle Sympathetic Nerve Activity  NE: Norepinephrine

* indicates where an overall significant difference between groups exists (P<0.05)
Table 2. Responses to head-up tilting

<table>
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<tr>
<th>Diagnosis and number of subjects*</th>
<th>Tilt Angle (degrees)</th>
<th>Heart Rate</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MSNA b.100 heart beats</th>
<th>NE spillover</th>
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<td>Healthy (n=18)</td>
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<td>201±23</td>
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<td>265±27</td>
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<td>24±3 (n=11)</td>
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<td>35±4 (n=5)</td>
<td>450±53</td>
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</table>

*number of subjects refers to the number of patents who tolerated the given tilt angle sufficiently to provide stable haemodynamic data prior to syncope.

**n= refers to the number of subjects with available microneurography data.
Figure Legends:

Figure 1: Sympathetic Nerve Varicosity. Tyrosine hydroxylase (TH) catalyzes the rate limiting step in norepinephrine (NE) synthesis. NE is stored in vesicles within the sympathetic varicosity and released to effector sites such as arterioles and venules in response to sympathetic nerve firing (MSNA). Norepinephrine transporter (NET) recaptures 60 - 95% of released NE and, of this, 70 – 90% is returned to intraneuronal vesicular storage\textsuperscript{22, 24}. Vesicular monoamine transporter (VMAT2) is responsible for translocating NE from the cytoplasm into storage vesicles and is specific for sympathetic nerves. Dynamin\textregistered{}s are ubiquitous GTPases that support vesicular budding and fusion.

Figure 2: Mean change in muscle sympathetic nerve activity (burst 100 heart beats\textsuperscript{-1}) from baseline during head-up tilting. MSNA is shown as the mean change (±SEM) from baseline. This is representative of the sympathetic neural response during HUT. LBPS patients showed a striking mean increase in MSNA from rest, indicative of an exaggerated sympathetic neural response to postural change compared to both NBPS and control subjects; compared with control subjects, at 20° and 30° P=0.05. At 40° in LBPS patients statistical significance was lost, due to the low number of patients who both tolerated the tilt angle and maintained an intact microneurography site. NBPS subjects showed no significant difference from controls in the mean increase in MSNA. *error bars = SEM

Figure 3: Sample traces of MSNA and associated hemodynamics in a patient with NBPS (A) and LBPS (B). Panel A illustrates a sample trace of heart rate, BP and MSNA of a patient with NBPS. Panel B is a sample trace of a patient with LBPS clearly demonstrating higher
MSNA firing rates at a lower tilt angle (tilt 30° was also chosen because the quality of the MSNA trace was better for publication purposes)

**Figure 4:** NE spillover during head-up tilt in control subjects, LBPS and NBPS subjects

Norepinephrine spillover (ng.min⁻¹) increased progressively during HUT in controls. LBPS patients demonstrated significantly lower NE spillover throughout HUT (P=0.001), on average 39-52% lower than controls. NBPS patients had a higher mean NE spillover compared to LBPS but lower than controls (P=0.126). The striking finding in NBPS was the absence of an increase in NE spillover during HUT (P=0.32), indicating a failure of the normal neurocirculatory transmitter response to postural change. *error bars= SEM

**Figure 5:** Western Blots of TH and NET proteins in patients with recurrent vasovagal syncope.

The norepinephrine transporter (NET) is significantly increased in patients with NBPS (+135%, *P=0.019) compared to controls. Tyrosine hydroxylase (TH) is markedly reduced in LBPS subjects (43.6% of normal, **P=0.001) when compared to both NBPS and controls. LBPS subjects also demonstrate a marked reduction in NET expression compared to controls and NBPS (50% of normal, ***P<0.001). (error bars represents standard deviation)
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Circ Arrhythm Electrophysiol. published online August 15, 2011;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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