Recurrent Postural Vasovagal Syncope
Sympathetic Nervous System Phenotypes

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Background—The pathophysiology of vasovagal syncope is poorly understood, and the treatment usually ineffective. Our clinical experience is that patients with vasovagal syncope fall into 2 groups, based on their supine systolic blood pressure, which is either normal (>100 mm Hg) or low (70–100 mm Hg). We investigated neural circulatory control in these 2 phenotypes.

Methods and Results—Sympathetic nervous testing was at 3 levels: electric, measuring sympathetic nerve firing (microneurography); neurochemical, quantifying norepinephrine spillover to plasma; and 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 control subjects and 36 patients with vasovagal syncope, 15 with the low blood pressure phenotype and 21 with normal blood pressure. Microneurography and norepinephrine spillover increased significantly during HUT in healthy subjects. The microneurography response during HUT was normal in normal blood pressure and accentuated in low blood pressure phenotype (P=0.05). Norepinephrine 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 low blood pressure phenotype was linked to low tyrosine hydroxylase (43.7% normal, P=0.001), rate-limiting in norepinephrine synthesis, and in normal blood pressure to increased levels of the norepinephrine transporter (135% normal, P=0.019), augmenting transmitter reuptake.

Conclusions—Patients with recurrent vasovagal syncope, when phenotyped into 2 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 in the present report should translate into more effective treatment.

Key Words: syncope ▪ vasovagal ▪ low blood pressure (hypotension) ▪ norepinephrine ▪ sympathetic nervous system

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), characterized by abrupt hypotension variably accompanied by bradycardia, is the most common cause of postural syncope, accounting overall for 21% of patients with syncope.2 Syncope is a significant cause of morbidity,1 responsible for 1% to 2% of hospital emergency department visits3,4 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.

Clinical Perspective on p 718

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 mm Hg) and low supine systolic blood pressure (70–100 mm Hg). Our identifying of patients with low blood pressure (BP) as a distinct VVS phenotype is supported by Mathias, who recognized 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

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female, 1 male) had “a persistently low basal level of blood pressure that led to a diagnosis of chronic systemic hypertension, of unknown cause.” This distinction is important for a number of reasons, foremost being our clinical experience that those patients with low BP VVS (LBPS) respond more favorably 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” that 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 3 levels: electric (muscle sympathetic nerve activity, MSNA), neurochemical (norepinephrine [NE] release), and cellular, with analysis of sympathetic nerve proteins (SNPs) that 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; age, 22 ± 3 years) and 36 patients (34 female and 2 male; age, 28 ± 9 years) 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; age, 28 ± 9 years) and 21 had NBPS (20 female, 1 male; age, 28 ± 9 years). Patients were deemed to have LBPS if 2 or more clinic supine cuff systolic BP (SBP) measurements were ≤100 mm Hg (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 had 2 or more syncopal events in the preceding 12 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 foot-board, 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 7 PM on the evening before the study. A lead III ECG was recorded. After instrumentation, patients were rested in a darkened room for 30 minutes. During the last 20 minutes of rest, baseline hemodynamic and MSNA data were obtained, 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 presyncope developed (symptomatic pending hemodynamic collapse), patients were tilted back supine.

Sympathetic Nerve Recording

MSNA in postganglionic fibers distributed to the skeletal muscle vasculature was recorded using a tungsten microelectrode (FHC, Bowdoinham, ME) inserted percutaneously into the common peroneal nerve near the head of the fibula.7 MSNA was expressed as multiunit nerve burst firing frequency (bursts/min) and normalized for heart rate (bursts/100 heart beats).

Measurement of Whole-Body NE Spillover

The rate of spillover of NE from sympathetic nerves to plasma was determined by isotope dilution during an intravenous infusion of tritiated NE ([1H]NE), a technique developed by us.8,9 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 [1H]NE.8 The rate of total body spillover of NE to plasma was determined according to the formula: Total NE Spillover Rate = ([1H]NE) Plasmad activity (dpm/min)/Plasma NE specific radioactivity (dpm/mmol).

Quantification of SNPs: Tyrosine Hydroxylase, NE Transporter, Vesicular Monoamine Transporter 2, and Dynamin 1

SNPs (Figure 1) were extracted from subcutaneous veins, which have a dense sympathetic innervation.10,11 Seven patients with LBPS, 6 with NBPS, and 7 healthy control subjects underwent the procedure. A skin incision was performed on the dorsum of the forearm, and a 10-mm length of vein was removed (2–3 mm diameter) and placed in liquid nitrogen, then stored at −80°C until assay.
Tyrosine Hydroxylase, Vesicular Monoamine Transporter 2, Dynamin I, and NE Transporter Protein Immunoblotting Procedure

The SNPs analyzed were tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), Dynamin I, and NE transporter (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-TH (NET17–1, Abcam), anti-TH (AB152, Chemicon), anti-Dynamin I (3G4B6) (sc-53877, Santa Cruz biotechnology, Inc), anti-VMAT2 (AB1767, Chemicon), and anti-GAPDH (sc-32233, Santa Cruz biotechnology, Inc). Scanned signals were analyzed with BIO-RAD Quantity One software.

Statistics

Data were analyzed using SigmaStat Version 3.5 and STATA 10. One-way ANOVA was used to analyze baseline data and 2-way repeated-measures ANOVA was used to investigate changes in hemodynamics, NE spillover, and MSNA. Multiple comparison procedures (Holm-Sidak) were applied when the overall significance between groups was $P<0.05$. A mixed-effects model (XTmixed-STATA) was also used to test for significant differences in NE spillover and MSNA between groups during HUT and to determine the influence of variables such as sex and age. Testing was 2-sided, and results are reported as mean ± SEM for repeated measures and mean ± SD for nonrepeated measures (age, body mass index [BMI], office BP, and heart rate). Statistically significant differences are reported for $P<0.05$.

Results

Baseline Measurements

Baseline values for patients with LBPS, NBPS, and control subjects 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 ± 2 mm Hg versus 132 ± 2 mm Hg in NBPS subjects and 129 ± 2 mm Hg in control subjects; $P=0.005$). SBP was substantially higher in patients with LBPS on the day of the invasive HUT compared with clinic cuff pressures (70–100 mm Hg), representing a response to the invasive procedure.

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

Tilt Test Outcomes

Of the control subjects, 1 had VVS at tilt 30° and 2 at tilt 40°. In the LBPS group, syncope occurred at 20° in 1 subject, 30° in 2 subjects, 40° in 1 subject, and 60° 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. Syncpe developed in 1 subject at 20°, 2 subjects at 30°, 10 subjects at 40°, and 5 subjects at 60°.

| Table 1. Baseline Values in Healthy Control Subjects and Syncope Groups: Supine Rest |
|--------------------------|--------------------------|--------------------------|
| Control Subjects | LBPS | NBPS |
| Age, y | 22 ± 4 | 29 ± 9 | 27 ± 9 |
| Body mass index | 24 ± 4 | 24 ± 3 | 25 ± 4 |
| Office systolic blood pressure, mm Hg, *$P=0.008$ | 118 ± 3 | 98 ± 3 | 122 ± 3 |
| Office diastolic blood pressure, mm Hg, *$P=0.007$ | 70 ± 1 | 62 ± 2 | 70 ± 1 |
| Office heart rate, bpm | 64 ± 1 | 67 ± 2 | 66 ± 2 |
| Intraarterial systolic blood pressure, mm Hg, *$P=0.006$ | 129 ± 4 | 120 ± 2 | 132 ± 2 |
| Intraarterial diastolic blood pressure, mm Hg, *$P=0.01$ | 72 ± 2 | 67 ± 2 | 71 ± 1 |
| Heart rate, bpm at the time of invasive tilt table testing | 67 ± 1 | 69 ± 3 | 68 ± 2 |
| Respiration frequency, breaths per min | 19 ± 1 | 18 ± 1 | 18 ± 1 |
| MSNA, bursts per min | 19 ± 1 | 15 ± 2 | 15 ± 2 |
| Plasma NE spillover, ng/min, *$P=0.003$ | 441 ± 26 | 201 ± 23 | 370 ± 48 |

LBPS indicates low blood pressure syncope; NBPS, normal blood pressure syncope; MSNA, muscle sympathetic nerve activity; and NE, norepinephrine. *Overall significant difference between groups ($P<0.05$).

Hemodynamics

Hemodynamic responses, NE spillover, and MSNA (bursts/100 heart beats) at stable time points before the development of syncope are summarized in Table 2. Progressive HUT increased heart rate significantly in all 3 groups. At tilt 40° LBPS and NBPS subjects had a mean heart rate of 90 ± 4 bpm and 94 ± 2 bpm, respectively; significantly higher than control subjects (81 ± 2 bpm, $P<0.001$). SBP was significantly lower in LBPS patients compared with both control subjects 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 sex had no independent effect on the HUT data.

Sympathetic Nerve Firing

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

NE 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 at tilt 40°; $P<0.001$) (Figure 4). In contrast, NBPS patients had a severely blunted NE spillover response to HUT, with no
increase in NE spillover during HUT \((P=0.32, \text{Figure 4})\), indicating a failure of SNS response to postural change.

NE spillover was significantly lower at all tilt angles in LBPS subjects compared with control subjects and NBPS subjects (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.

**Sympathetic Nerve TH, VMAT2, Dynamin I, and NET Proteins**

Figure 5 illustrates representative Western blots of 7 control subjects, 7 LBPS patients, and 6 NBPS patients. Analysis of TH protein revealed reduced intensity of the 50-kDa band in LBPS compared with control subjects 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 with control subjects \((P=0.001)\). Because 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 control subjects revealed the 80-kDa 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 with control subjects (Figure 5). In contrast, NBPS subjects demonstrated normal TH and increased abundance of NET (mean, 135%) compared with control subjects \((P=0.019)\). VMAT 2 and dynamin I 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 2 distinct clinical groups, based on their supine SBP: normal \((>100 \text{ mm Hg})\) or low \((70–100 \text{ mm Hg})\). Recognition of low BP as a specific clinical phenotype of VVS has not received emphasis before, only Mathias having identified this variant.\(^4\)

Our expectation is that better understanding of the neural

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**Table 2. Responses to Head-Up Tilting**

<table>
<thead>
<tr>
<th>Diagnosis and No. of Subjects*</th>
<th>Tilt Angle (Degrees)</th>
<th>Heart Rate</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>MSNA, bursts/100 heart beats</th>
<th>NE Spillover (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy, n=18</td>
<td>0</td>
<td>67±2</td>
<td>129±4</td>
<td>72±2</td>
<td>28±3 (n=18)</td>
<td>441±48</td>
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<tr>
<td>n=18</td>
<td>20</td>
<td>68±2</td>
<td>133±4</td>
<td>74±3</td>
<td>33±3 (n=18)</td>
<td>438±38</td>
</tr>
<tr>
<td>n=18</td>
<td>30</td>
<td>75±1</td>
<td>127±5</td>
<td>73±3</td>
<td>38±3 (n=16)</td>
<td>558±48</td>
</tr>
<tr>
<td>n=18</td>
<td>40</td>
<td>81±2</td>
<td>127±4</td>
<td>74±3</td>
<td>39±3 (n=15)</td>
<td>592±61</td>
</tr>
<tr>
<td>LBPS, n=15</td>
<td>0</td>
<td>71±3</td>
<td>120±2</td>
<td>67±1</td>
<td>21±2 (n=15)</td>
<td>201±23</td>
</tr>
<tr>
<td>n=15</td>
<td>20</td>
<td>75±3</td>
<td>119±2</td>
<td>65±2</td>
<td>40±4 (n=11)</td>
<td>265±27</td>
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<tr>
<td>n=14</td>
<td>30</td>
<td>82±3</td>
<td>116±2</td>
<td>66±2</td>
<td>43±3 (n=10)</td>
<td>268±31</td>
</tr>
<tr>
<td>n=12</td>
<td>40</td>
<td>90±4</td>
<td>113±1</td>
<td>66±1</td>
<td>44±3 (n=9)</td>
<td>298±37</td>
</tr>
<tr>
<td>NBPS, n=21</td>
<td>0</td>
<td>70±3</td>
<td>132±2</td>
<td>68±2</td>
<td>24±3 (n=11)</td>
<td>370±48</td>
</tr>
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<td>n=21</td>
<td>20</td>
<td>74±3</td>
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<td>70±2</td>
<td>25±2 (n=11)</td>
<td>363±42</td>
</tr>
<tr>
<td>n=20</td>
<td>30</td>
<td>84±3</td>
<td>127±2</td>
<td>68±1</td>
<td>35±3 (n=9)</td>
<td>386±39</td>
</tr>
<tr>
<td>n=13</td>
<td>40</td>
<td>94±2</td>
<td>122±2</td>
<td>69±2</td>
<td>35±4 (n=5)</td>
<td>450±53</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MSNA, muscle sympathetic nerve activity; NE, norepinephrine; LBPS, low blood pressure syncope; and NBPS, normal blood pressure syncope.

*No. of subjects refers to the number of patients who tolerated the given tilt angle sufficiently to provide stable hemodynamic data before syncope. n refers to the number of subjects with available microneurography data.
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 stabilizing BP during standing. 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. 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 3 key levels.

Electric: 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.

Neurochemical: Neurotransmitter Release
We measured the overflow of NE from sympathetic nerves to arterial plasma (NE spillover) using radiotracer techniques that we developed. This is a well-validated measure of sympathetic activity 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.

Cellular: SNPs
A previous limitation to investigation of the human SNS has been the lack of access to sympathetic nervous tissue. In the present study, we used biopsied forearm subcutaneous veins as a source of SNPs to overcome this deficiency. 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 used 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 and NE release until total disappearance.

*Error bars represent SEM.

**Figure 3.** Sample traces of muscle sympathetic nerve activity (MSNA) and associated hemodynamics in a patient with normal blood pressure phenotype (NBPS) (**A**) and low blood pressure phenotype (LBPS) (**B**). **A**, Sample trace of heart rate, BP, and MSNA of a patient with NBPS. **B**, 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.** Norepinephrine (NE) spillover during head-up tilt (HUT) in control subjects, low blood pressure phenotype (LBPS), and normal blood pressure phenotype (NBPS) subjects. NE spillover (ng/min) increased progressively during HUT in control subjects. LBPS patients demonstrated significantly lower NE spillover throughout HUT (P=0.001), on average 39% to 52% lower than control subjects. NBPS patients had a higher mean NE spillover compared with LBPS but lower than control subjects (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.
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 5 syncopal events per month, which is more severe than in our cohort. Interestingly, BP 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 BP 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.20 The hemodynamic 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 had presyncope.18 Tilting differs from LBNP in that in addition to reduction in filling pressures, it activates the vestibular system and found attenuated MSNA responses in the group that had presyncope.18 Tilting differs from LBNP in that in addition to reduction in filling pressures, it activates the vestibular system 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 support 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 control subjects. This implies a mismatch between the electric and neurotransmitter components of the sympathetic neural response. The NBPS patients exhibited a different pathophysiology. In these patients, MSNA increased normally during HUT, but NE spillover failed to increase at all. Thus, despite differences between the 2 VVS variants, in both, there is a “mismatch” between nerve firing and neurotransmitter release.

We sought to explain this “mismatch” with analysis of SNPs 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 vesicles21,22; Dynamin I, vesicle formation and recycling23; and NET, clears 60% to 95% of NE released into the synaptic cleft.24 Analysis of SNPs in LBPS patients revealed low levels of TH. In these patients, 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 I were normal, whereas 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-β-hydroxylase (DβH) deficiency, a rare genetic disorder that also impairs NE synthesis. Patients with DβH deficiency typically have low-normal BP, postural syncope, and near-zero NE spillover. Nerve firing is high 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 patients, 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% to 95%) into the sympathetic varicosity through NET. 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 concentration, may predispose patients to VVS. The importance of excessive action of NET as a driver for postural VVS has been intimated in a prior study, in which antagonism of NET by Reboxetine was shown to protect against syncope during prolonged HUT.

The origins of low TH levels in LBPS patients and increased NET in NBPS patients remain 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 1 meter. 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 counterpressure maneuvers should still be universally applied, with administration where indicated of medication, such as fludrocortisone, to expand plasma volume and pressor agents such as midodrine. However, 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 must be augmented. This might be achieved with L-DOPS (l-threo-3,4-dihydroxyphenylserine), a NE prodrug, which is converted to NE by DOPA-decarboxylase, bypassing TH. L-DOPS has been used to advantage in patients with dopamine-β-hydroxylase deficiency, 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, which we believe reflects the natural distribution of this condition and is consistent with the observations by Mathias. Our data do not allow us to comment on LBPS in male subjects, other than the fact that it is probably a particularly rare phenotype. It is uncertain whether the abundance of SNPs 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 2 sites to be approximately in parallel. Hemodynamic patterns seen during VVS vary widely. None of our patients exhibited the asystolic variant, which might have a differing underlying pathophysiology.

Conclusions
Patients with recurrent VVS can be phenotyped into 2 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.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Recurrent postural syncope is a common and potentially disabling problem that is frequently seen in clinical practice. Vasovagal syncope, although well recognized, is often misdiagnosed and poorly treated. In our report, we identify a new phenotype of vasovagal syncope: the patient with low supine systolic blood pressure. This variant has not received emphasis in the medical literature to date, and clinicians often regard low blood pressure to be “healthy” and of little clinical consequence. It is our experience that a significant subset of patients with recurrent vasovagal syncope has low blood pressure, and our study demonstrates distinct physiological differences between these patients and their counterparts with normal blood pressure syncope. Low blood pressure in an apparently healthy young patient presenting with syncope is a valuable clue to the etiology of the fainting episodes. In our experience, patients with low blood pressure more commonly describe frequent dizzy spells and fatigue between episodes of significant presyncope or syncope, whereas those with normal blood pressure syncope seem to have far fewer intercurrent symptoms. Patients with low blood pressure syncope may respond more favorably to a high fluid and salt intake; they may also benefit from pharmacological volume expansion with fludrocortisone and/or vasoconstrictor therapy with α-agonists such as midodrine.
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