

# Altered Sympathetic Nervous Reactivity and Norepinephrine Transporter Expression in Patients With Postural Tachycardia Syndrome

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**Background**—Clinical observations in patients with postural tachycardia syndrome (POTS) suggest abnormal sympathetic nervous system activity and a dysfunction of the norepinephrine (NE) transporter (NET).

**Methods and Results**—We examined sympathetic nervous system responses to head-up tilt by combining NE plasma kinetics measurements and muscle sympathetic nerve activity recordings and by quantifying NET protein content in peripheral sympathetic nerves in patients with POTS compared with that in controls. POTS patients had an elevated heart rate during supine rest ( $81 \pm 2$  bpm versus  $66 \pm 2$  bpm in healthy subjects [HS],  $P < 0.01$ ). Head-up tilt to  $40^\circ$  induced a greater rise in heart rate in patients with POTS ( $+24 \pm 4$  bpm versus  $+13 \pm 2$  bpm in HS,  $P < 0.001$ ). During rest in the supine position, muscle sympathetic nerve activity, arterial NE concentration, and whole-body NE spillover to plasma were similar in both groups. Muscle sympathetic nerve activity response to head-up tilt was greater in the POTS group ( $+29 \pm 3$  bursts/min in patients with POTS and  $+13 \pm 2$  bursts/min in HS,  $P < 0.001$ ), but the NE spillover rise was similar in both groups (51% in the POTS subjects and 50% in the HS). Western blot analysis of NET protein extracted from forearm vein biopsies in patients with POTS and HS demonstrated a decrease in the expression of NET protein in patients with POTS.

**Conclusion**—Patients with POTS exhibit a decrease in NET protein in their peripheral sympathetic nerves. Paradoxically, whole-body NE spillover to plasma during rest in the supine position and in response to head-up tilt is not altered despite excessive nerve firing rate in response to the head-up tilt. (*Circ Arrhythmia Electrophysiol.* 2008;1:103-109.)

**Key Words:** tachycardia ■ norepinephrine ■ nervous system, sympathetic

The postural tachycardia syndrome (POTS) is a form of orthostatic intolerance characterized by a dramatic rise in heart rate (HR) on standing that is typically not accompanied by a decrease in blood pressure (BP). Common symptoms include fatigue, palpitations, exercise intolerance, and light-headedness. Overall, patients with POTS experience clear limitations across multiple domains of quality of life, including physical and social functioning.<sup>1</sup>

## Clinical Perspective see p 109

Numerous mechanisms have been invoked in the pathogenesis of POTS, such as hypovolemia,<sup>2</sup> inadequate vasoconstriction, peripheral denervation,<sup>3</sup> and excessive venous pooling.<sup>4,5</sup> Moreover, it has been suggested that the functional distribution of central sympathetic tone to the heart and vasculature is abnormal in POTS.<sup>6</sup> The examination of the sympathetic nervous system, estimated by measuring direct muscle sympathetic nervous activity (MSNA), has yielded

equivocal results, with various reports showing high,<sup>6</sup> low,<sup>7</sup> or normal<sup>8</sup> sympathetic activity in subjects with POTS. High levels of circulating norepinephrine (NE) have been reported in a number of studies,<sup>6,9,10</sup> but this is perhaps due to decreased NE clearance<sup>11</sup> rather than increased NE release.

An alternative explanation for increased NE levels may be an impairment of the clearance of the NE from the synaptic cleft by the NE transporter (NET), the presynaptic transmembrane pump responsible for neuronal reuptake of NE. This hypothesis is supported by a previous finding of a functionally significant genetic mutation in the NET in a family kindred with POTS.<sup>12</sup> Furthermore, selective NET blockade in healthy subjects induces a HR response to head-up tilt similar to that of POTS patients.<sup>13</sup> The present study explores the sympathetic nervous system response to progressive head-up tilt in subjects with POTS compared with that of healthy subjects, examines the NET protein content in peripheral sympathetic nerves assessed by subcutaneous vein

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biopsy, and explores the possibility that a defect in NET underpins the POTS phenotype.

## Methods

### Subjects

The research protocol conformed to the relevant guidelines of the National Health and Medical Research Council of Australia and was approved by the Alfred Hospital Ethics Review Committee. A total of 18 healthy subjects (10 men, 8 women,  $27 \pm 3$  years old) and 14 POTS patients (4 men, 10 women,  $31 \pm 3$  years old) participated in the study after giving their written informed consent. The subjects with POTS underwent an exhaustive medical evaluation to exclude any other relevant medical condition. All patients with POTS shared the common clinical characteristics central to the diagnosis, such as recurrent episodes of presyncope while standing, freedom from postural hypotension (decrease in systolic BP while standing in the clinic  $< 20$  mm Hg), and the presence of posture-related tachycardia (mean HR increase on standing recorded in the clinic  $= 41 \pm 3$  bpm). The most common symptoms of the POTS patients included fatigue ( $n=10$ ), palpitations ( $n=9$ ), cognitive impairment ( $n=8$ ), syncope ( $n=7$ ), and chest pain ( $n=4$ ). All patients with POTS were unmedicated. They were either newly diagnosed and never treated or had stopped any medication for at least 7 days (7 days for  $\beta$ -adrenergic-blocking drugs, 21 days for fludrocortisone). The reference population had none of the clinical characteristics listed earlier, and their mean HR increase on standing recorded in the clinic was  $11 \pm 2$  bpm.

### Experimental Protocol

Participants were placed in the supine position on a tilt table and were instrumented for plasma NE kinetics measurements and microneurography recordings. Tilt testing was performed in 12 subjects with POTS (3 men and 9 women,  $27 \pm 1$  years old) and 15 healthy controls (7 men and 8 women,  $24 \pm 2$  years old). All subjects were tested in the morning, after a light breakfast. Caffeine and alcohol intake was excluded from 7 PM on the evening before the study. The radial artery was cannulated percutaneously (3F, 5 cm, Cook) to enable arterial BP monitoring and blood sampling for catecholamine measurement. Subjects were instrumented with an intravenous cannula in an antecubital vein. A lead III ECG was recorded, and respiration measurements were determined by using a transducer based on a piezoelectric device (ADI Instruments, Castle Hill, NSW, Australia). After instrumentation, subjects were allowed to rest for at least 30 minutes. BP, ECG, respiration rate, and MSNA were measured for 10 minutes, and blood samples were collected from the subjects at rest. Subjects were tilted to angles of  $20^\circ$ ,  $30^\circ$ , and  $40^\circ$  for 10 minutes at each angle. Blood samples were taken at the end of each tilt angle, and all other parameters were collected continuously.

### Microneurographic Measurements

Multinunit sympathetic nerve firing rates in postganglionic fibers distributed to the skeletal muscle vasculature were recorded by using clinical microneurography as previously described.<sup>14</sup> The common peroneal nerve was located by palpation and electrical stimulation by using a surface probe. A tungsten microelectrode (FHC, Bowdoinham, Maine) was inserted percutaneously and adjusted until satisfactory spontaneous MSNA was observed in accordance with previously described criteria.<sup>14</sup> MSNA was expressed as burst frequency (bursts per minute) and burst incidence (bursts per 100 heart beats). BP, ECG, and MSNA were digitized with a sampling frequency of 1000 Hz (PowerLab recording system, model ML785/8SP, ADI Instruments).

### Cardiac Baroreflex Sensitivity

Baroreflex sensitivity was assessed by the sequence method,<sup>15</sup> using BaroCor software (AtCor Medical, West Ryde, NSW, Australia). This procedure identifies "spontaneous" sequences of 3 or more consecutive beats in which systolic BP progressively rises (by at least 1 mm Hg) and cardiac interval lengthens, or systolic BP progressively decreases (by at least 1 mm Hg) and cardiac interval progressively shortens, with a lag of

1 beat. For each sequence, the linear correlation coefficient between cardiac interval and systolic BP was computed, and the sequence was validated when  $r > 0.80$ . The slope between cardiac interval and systolic BP was calculated for each validated sequence, and an average slope was calculated for each recording.

### Assessment of Spontaneous Arterial Baroreflex Control of MSNA

Over a 3- to 5-minute period, diastolic pressures of individual heart beats were grouped in intervals of 2 mm Hg, and for each interval, the percentage of diastoles associated with a sympathetic burst was plotted against the mean of the pressure interval. Muscle sympathetic bursts were advanced by 1.3 second to compensate for baroreflex delay.<sup>16</sup> The reflex gain was defined as the slope of the regression line<sup>17</sup> and was assessed for each subject in the supine position.

### Catecholamine Determinations

The NE appearance, or spillover, rate to plasma was determined by using the principle of isotope dilution during an intravenous infusion of a tracer dose of tritiated NE. Participants were infused with a tracer infusion of  $^3\text{H}$ -labeled NE via the peripheral venous cannula at 0.6 to 0.8  $\mu\text{Ci}/\text{min}$ , after a priming bolus of 12  $\mu\text{Ci}$ . Arterial blood sampling for measurement of endogenous and radiolabeled NE was done after a minimum of 30 minutes' infusion time to ensure that steady-state plasma conditions had been reached and that plasma NE-specific activity could be determined.<sup>18</sup> Blood was collected into chilled tubes containing reduced glutathione and EGTA. Plasma was separated by refrigerated centrifugation ( $4^\circ\text{C}$  at 3000g) and stored at  $-80^\circ\text{C}$  until assayed. The plasma concentrations of NE and its intraneuronal metabolite, 3,4-dihydroxyphenylglycol (DHPG), were measured in all patients with POTS and 11 of the healthy subjects.

The total-body NE spillover to plasma and the total-body clearance rate of NE were determined according to the following formulas:

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

and

$$\text{Total Body Clearance} = \frac{[^3\text{H}]\text{NE Infusion Rate (dpm/min)}}{\text{Arterial } [^3\text{H}]\text{NE Concentration (dpm/mL)}}$$

### Plasma Renin Activity Determination

Blood samples were drawn in prechilled tubes and centrifuged at 2000g at  $4^\circ\text{C}$ . Plasma was stored at  $-80^\circ\text{C}$  until analysis. Plasma renin activity (PRA, expressed as nanograms per milliliter per hour) was determined by incubating the plasma samples at  $37^\circ\text{C}$  for 90 minutes and then by measuring the amount of angiotensin I generated with a commercial radioimmunoassay kit (Ren-CT2; CIS Bio International, France).

### Noradrenaline Transporter Expression From Vein Biopsies of Healthy Subjects and POTS Patients

A small vein biopsy was performed in 6 patients with POTS (3 men, 3 women) and 3 healthy controls (2 men, 1 woman). Four of the subjects with POTS but none of the 3 healthy subjects had completed the tilt test. A skin incision was performed on the dorsum of the forearm under local anesthesia to identify a vein with a diameter of approximately 1 mm. One centimeter of the vein was removed after ligation at both ends with absorbable suture material. One to 3 skin sutures were used to provide adequate closure of the skin. After removal, the vein was frozen in liquid nitrogen.

The tissue samples were homogenized in RIPA buffer, containing 50 mmol/L Tris-HCl (pH 7.5), 100 mmol/L NaCl, 2 mmol/L EDTA (pH 8.0), 0.1% SDS, 0.5% NA deoxycholate, 1% Triton X-100, and protease inhibitors (leupeptin, PMSF, aprotinin, and pepstatin). Western blots were prepared by using lysates from vein biopsies. Proteins were separated on 7.5% SDS-acrylamide gel and transferred onto polyvinylidene fluoride (PVDF) membranes. Immunodetection was performed by using anti-NET antibody (NET17-1; MAb Technologies, Inc). Immunoreactive bands were visualized by using a

chemiluminescence system (NEN Life Science). Two specific bands at 80 and 50 kDa were detected.  $\alpha$ -Tubulin (Sigma) was used as a marker protein to assess protein loading of the gel. Quantification of band density was performed on scanned images, with NIH Image (version 1.62) software used to perform densitometry analyses.

### Noradrenaline Transporter Expression From Vein and Heart Biopsies of Deceased Donors

Left ventricles and forearm veins used in this study were obtained during autopsy at the Victorian Institute of Forensic Medicine. Informed consent of donor's next of kin, arranged by the Donor Tissue Bank of the Victorian Institute of Forensic Medicine, was acquired before the autopsy. The Alfred Hospital Ethics Review Committee approved tissue retrieval performed at the Victorian Institute of Forensic Medicine.

The tissue samples were obtained from donors with no known history of heart disease. Donors were also screened with regard to their known medical history, particularly to exclude diabetes and other endocrine disorders. For all tissues acquired at autopsy, time elapsed since death did not exceed 72 hours. All tissues were snap-frozen in liquid nitrogen on site and kept at  $-80^{\circ}\text{C}$  until processed. The source of obtained tissue included 2 men (ages 40 and 67 years; body mass index 49 and 20  $\text{kg}/\text{m}^2$ ; causes of death, motor accident and ruptured ventricle, respectively) and 1 woman (age 34 years; body mass index 36  $\text{kg}/\text{m}^2$ ; cause of death, multiple systems failure).

The tissues were homogenized in a similar fashion to vein biopsies. GAPDH (sc-32233; Santa Cruz Biotechnology, Inc) was used as a housekeeping protein.

### Statistics

Data were analyzed with 2-way ANOVA for repeated measures. Pairwise multiple-comparison procedures were used when appropriate. Results are reported as mean  $\pm$  SEM. Values of  $P < 0.05$  were considered statistically significant differences.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

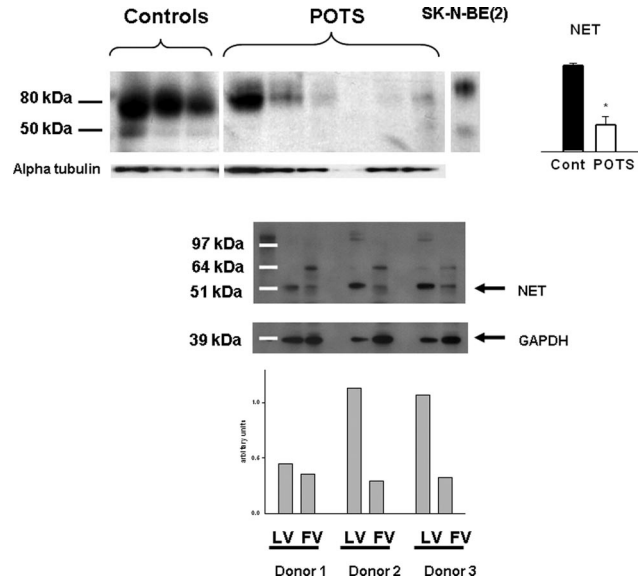
## Results

### NET Expression

Western blot analysis revealed the presence of an 80-kDa band in the positive control, a human neuroblastoma cell line, and in the 3 controls. This band was less visible in 5 of the 6 patients with POTS. Quantification by using  $\alpha$ -tubulin indicated a significant reduction in NET protein content in the POTS group ( $P = 0.0017$ ; Figure 1). The NET expression in the heart and veins extracted from the deceased donors indicated the presence of NET in both regions. There was a 2.8-fold increase in NET expression in the heart compared to that in the forearm vein (1.28, 3.86, and 3.30, respectively; Figure 1).

### Baseline Sympathetic Parameters

Baseline averages for the 2 groups of subjects who underwent the tilt test are summarized in the Table. There was no difference in either systolic or diastolic BP during rest in the supine position. Average HR at rest was significantly higher in the POTS groups ( $P < 0.001$ ). There was no difference between the 2 groups in baseline MSNA, regardless of whether expressed as burst incidence or burst frequency. Likewise, plasma NE, plasma DHPG concentration, whole-body NE spillover, and PRA did not differ between the 2 groups. Plasma NE clearance tended to be lower in POTS patients, but this did not reach significance ( $P = 0.09$ ). The ratio of DHPG to NE plasma concentration was lower in the



**Figure 1.** Top, Western blot indicating abundance of sympathetic nerve NET protein in forearm vein biopsies from healthy volunteers and patients with POTS. NET protein was in 80- and 50-kDa forms, which is typical. Loading conditions were identical, with  $\alpha$ -tubulin as the loading marker. Protein extracted from a human neuroblastoma cell line, SK-N-BE,<sup>2</sup> was used as a positive control. \* $P < 0.05$ , POTS vs controls. Bottom, Western blot indicating NET expression in hearts and forearm veins from 3 human tissue donors. NET was expressed at higher rates in the heart samples of all 3 donors. GAPDH was used as a housekeeping protein. For the heart samples, 2  $\mu\text{g}$  of total protein was loaded in wells, whereas 20  $\mu\text{g}$  of total protein was loaded in wells of the corresponding forearm vein samples.

POTS group ( $P = 0.04$ ). Patients with POTS, when compared with the healthy subjects, displayed reduced cardiovascular baroreflex function ( $P < 0.001$ ). The sympathetic baroreflex gain was not significantly different between the 2 groups.

### Tilt Tests

The tilt test had to be aborted in 1 healthy subject who developed some symptoms of orthostatic intolerance at the  $40^{\circ}$  angle and in 3 patients with POTS (2 during the  $40^{\circ}$  and 1 during the  $30^{\circ}$  angle).

Progressive head-up tilt increased HR in healthy subjects (+7 bpm at  $30^{\circ}$ ,  $P < 0.005$ ; +13 bpm at  $40^{\circ}$ ,  $P < 0.001$ ) and in POTS patients (+8 bpm at  $20^{\circ}$ ,  $P < 0.05$ ; +15 bpm at  $30^{\circ}$ ,  $P < 0.001$ ; +24 bpm at  $40^{\circ}$ ,  $P < 0.001$ ). As expected, the HR response was more marked in the POTS patients at each angle ( $P < 0.001$ ; Figures 2 and 3). Systolic BP, diastolic BP, and respiration rate remained unaltered during the tilt test in both groups. Muscle sympathetic nerve recording was done during rest in the supine position in all patients and healthy subjects. The recording site was maintained up to the highest angle reached in all but 1 healthy subject and 1 POTS patient (site lost during the early part of tilt  $40^{\circ}$  and at  $20^{\circ}$ ). MSNA, as expressed in burst incidence, increased during tilting in healthy subjects (+12 bursts per 100 heartbeats at a tilt of  $40^{\circ}$ ;  $P < 0.001$ ) and in POTS patients (+12 bursts per 100 heartbeats at a tilt of  $20^{\circ}$ ,  $P < 0.001$ ; +19 bursts per 100 heartbeats at a tilt of  $30^{\circ}$ ,  $P < 0.001$ ; +24 bursts per 100 heartbeats at a tilt of  $40^{\circ}$ ,  $P < 0.001$ ). The MSNA response to head-up tilt was greater in the POTS patients than in healthy



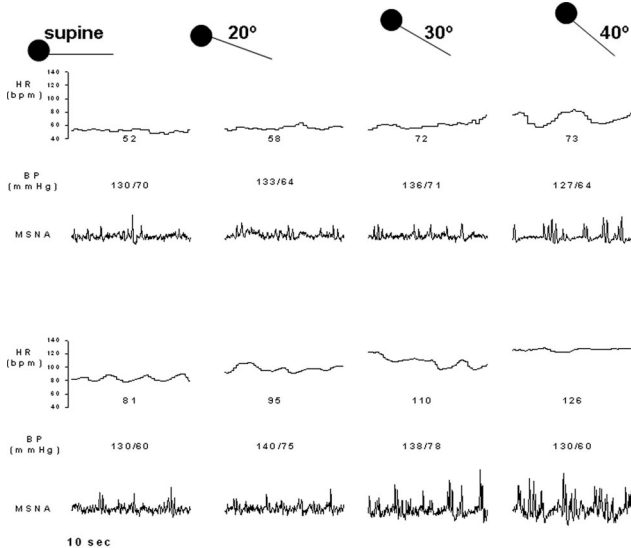
**Table. Baseline Average Values of the Participants Who Underwent the Tilt Test**

	Controls	POTS
Sex, female/male	8/7	9/3
Body mass index, kg/m <sup>2</sup>	24.9±1.1	25.4±1.1
Systolic BP, mm Hg	127±4	134±5
Diastolic BP, mm Hg	71±2	72±2
HR, bpm	66±2	81±2†
Respiration frequency, breaths/min	17±1	18±1
MSNA, bursts/min	17±2	20±1
MSNA, bursts per 100 heartbeats	26±2	27±2
Plasma NE, pg/mL	177±19	223±15
Plasma NE spillover, ng/min	431±42	424±39
Plasma NE clearance, L/min	2.68±0.24	1.99±0.29
Plasma DHPG, pg/mL	1318±80	1132±72
Plasma DHPG/NE	7.85±1.38	5.36±0.35*
Epinephrine, pg/mL	30±8	7±22
Plasma renin activity, ng/(mL · h)	0.88±0.36	0.80±0.17
Cardiac baroreflex gain, ms/mm Hg	17.5±1.7	8.8±0.8†
Sympathetic baroreflex gain, burst incidence, mm Hg	-7.74±0.99	-5.31±0.95

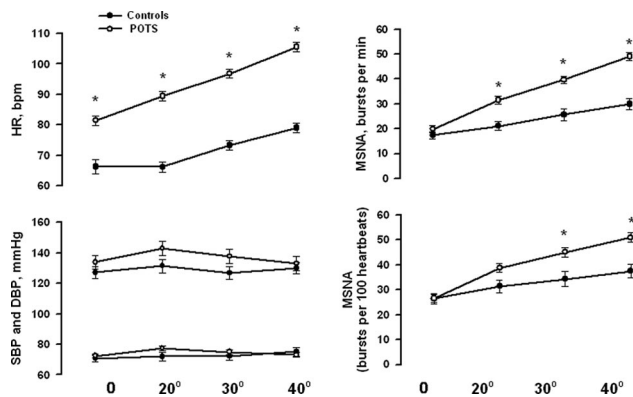
\**P*<0.05; †*P*<0.001.

subjects (30°, *P*<0.05; and 40°, *P*<0.01). Similarly, when MSNA was expressed as burst frequency, differences between patients and controls were also evident after tilt (Figures 2 and 3).

Plasma NE concentrations, whole-body NE spillover to plasma, and NE clearance from plasma were measured in 9 patients with POTS and in 11 healthy subjects at all tilt angles and in 1 POTS patient up to 30° only. Plasma NE concen-



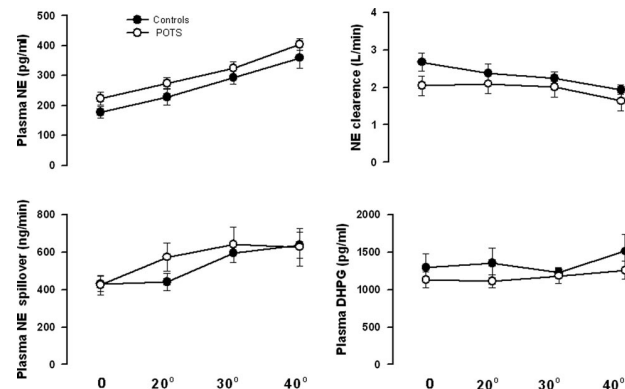
**Figure 2.** HR, BP, and MSNA responses at supine rest and head-up tilt in 1 healthy subject (top) and in a subject with POTS (bottom).



**Figure 3.** HR, systolic BP, diastolic BP, and MSNA responses at supine rest and head-up tilt in the control subjects (●) and in subjects with POTS (○). \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, POTS vs controls.

tration increased during head-up tilt similarly in healthy subjects and in POTS patients (Figure 4, healthy subjects +115 pg/mL at 30°, *P*<0.001, and +182 pg/mL at 40°, *P*<0.001; POTS patients +101 pg/mL at 30°, *P*<0.001, and +184 pg/mL at 40°, *P*<0.001). NE clearance decreased to the same extent in both groups (to 1.94±0.13 L/min in healthy subjects at 40°, *P*<0.001, and to 1.53±0.12 L/min in POTS subjects, *P*=0.022). NE spillover to plasma increased similarly in both groups (healthy subjects +206 ng/min at 40°, *P*=0.001; POTS patients +216 ng/min at 30°, *P*=0.002, and +182 ng/min at 40°, *P*=0.016). Plasma DHPG concentrations remained unaffected by the tilt test in both groups. The ratio of plasma DHPG to plasma NE decreased equally in both groups (healthy subjects to 4.34±0.38 at 30°, *P*<0.001, and to 4.48±0.38 at 40°, *P*<0.001; POTS patients to 3.45±0.37 at 30°, *P*=0.003, and to 3.01±0.37 at 40°, *P*<0.001).

PRA increased similarly during tilting in the 2 groups of subjects (from 0.88±0.36 to 1.75±0.48 ng/(mL · h) at tilt 40°, *P*<0.05 in healthy subjects and from 0.80±0.17 to 1.73±0.45 ng/(mL · h), *P*<0.01 in the POTS patients). Cardiac baroreflex function progressively decreased during the tilt test in both groups, but the values remained higher in the healthy subjects until 30° (12.9±0.7 for the healthy group and 5.4±0.8 ms/mm Hg for the POTS patients, *P*=0.002).



**Figure 4.** Plasma NE concentrations, NE spillover to plasma, NE clearance, and plasma DHPG concentrations at supine rest and in response to head-up tilt in control subjects (●) and in subjects with POTS (○).

## Discussion

Two striking new observations about the pathophysiology of POTS emerge from this study. First, NET protein content is decreased in peripheral sympathetic nerves of POTS patients. The abnormal ratio between plasma concentration of dihydroxyphenylglycol and NE, found in the present study and previously described,<sup>9,12</sup> provides some evidence of impaired NE uptake. Second, the sympathetic nervous system response to head-up tilt is disturbed in patients with POTS, who displayed a preferential and enhanced sympathetic activation in the outflow to the skeletal muscle vasculature during head-up tilt, not evident in the total-body NE spillover response, indicative of overall sympathetic activation that was not different from that of normal subjects.

We investigated NET protein content in POTS patients, given previous strong evidence that NET dysfunction may be implicated in this syndrome. Shannon et al<sup>12</sup> showed the existence of a specific genetic mutation in exon 9 of the NET, which resulted in a 98% loss of function in 1 patient with POTS. Second, reboxetine, a highly selective NE uptake inhibitor, induces a phenotype that resembles POTS, increasing HR in the supine position and inducing a dramatic increase in HR in the upright position.<sup>13</sup> The genetic mutation identified by Shannon et al<sup>12</sup> was not found in any of our patients (data not presented) and has indeed not been observed in any other patients with POTS.<sup>19</sup> The origin of decreased NET protein in peripheral sympathetic nerves in our POTS patients remains unknown. Decreased NET abundance is unlikely to result from regulation of NE itself, not only because POTS patients did not display higher plasma NE concentrations, but also because experimental data support the idea that NE does not play a role in the regulation of NET.<sup>20,21</sup> Whether decreased NET is a cause or a consequence of POTS remains to be unequivocally demonstrated.

Given NET impairment in peripheral sympathetic nerves in POTS patients, one would expect that this would be associated with higher NE spillover to plasma in the supine position and in response to head-up tilt. At a given level of sympathetic nerve firing, impairment of NE neuronal reuptake would be expected to lead to higher levels of transmitter overflow to plasma. Paradoxically, NE spillover to plasma was normal in the supine position in patients with POTS, and the increase with head-up tilt was of similar magnitude to that observed in control subjects.

A number of reports have drawn attention to the presence of high sympathetic nervous activity in the supine position in subjects with POTS because plasma NE concentrations have commonly been found to be elevated.<sup>6,9,12</sup> However, more accurate estimation of sympathetic nervous activity by using total-body NE spillover, as used in the present study, has demonstrated normal sympathetic activity in POTS patients.<sup>10,11,22</sup> Elevated plasma NE concentrations may arise from a reduction in plasma NE clearance<sup>11,22</sup> rather than the activation of the sympathetic nervous system, given that NE clearance from plasma is dependent on NE neuronal uptake. We found plasma NE clearance to be marginally but not significantly decreased in POTS patients. The decrease in NET abundance we describe in POTS, together with a decrease in the ratio of plasma concentration of DHPG and

NE, would be expected to be paralleled by an increase in plasma NE concentration and NE spillover to plasma. Without such an increase in plasma NE, our cohort of patients with POTS clearly are not "hyperadrenergic." Whether there occurs a specific activation of the cardiac sympathetic outflow remains to be determined. The reason for a lack of a direct effect of NET dysfunction on plasma NE concentration and NE spillover is unclear. Vincent et al<sup>23</sup> demonstrated that NET inhibition by duloxetine caused a dose-dependent increase in the levels of plasma venous NE both in the supine and the upright positions. However, selective NET inhibition by reboxetine did not alter NE plasma concentration in the supine and upright positions in another study.<sup>24</sup> The mismatch between NET abundance and NE spillover to plasma in patients with POTS may reflect profound changes in NE content within adrenergic nerves or abnormalities in NE release, although this remains to be demonstrated. Indeed, Jacob et al<sup>22</sup> found that sympathetic challenge induced by the cold pressor test, sodium nitroprusside infusion, and tyramine infusion increased NE spillover in the arms to a similar extent in the POTS and control groups, although the increases in the legs were smaller in the patients with POTS than in the normal subjects. Another study reported that NE spillover to plasma failed to increase in patients with POTS in response to standing or to a tyramine injection.<sup>11</sup>

In agreement with normal NE spillover to plasma, direct sympathetic nerve recording to the skeletal muscle confirmed normal sympathetic firing activity in patients with POTS during rest in the supine position. Muentzer Swift et al<sup>8</sup> also found normal MSNA; however, discordant MSNA results have previously been observed in patients with POTS.<sup>6,7</sup>

Our finding of an excessive increase in MSNA during head-up tilt in patients with POTS is in agreement with a previous report.<sup>8</sup> Increased sympathetic nerve activity to the skeletal muscle was also observed in patients with POTS in response to a hypotensive challenge induced by sodium nitroprusside.<sup>7</sup> The fact that MSNA increased disproportionately to the rise in plasma NE concentration and NE spillover during head-up tilt suggests the existence of a mismatch between the nerve traffic and NE release. There have been some suggestions that patients with POTS may have distal sympathetic denervation,<sup>22,25</sup> whereby reduction in the number of nerves could perhaps increase the neural activity in the remaining ones. Alternatively, there could be a functional defect of postganglionic sympathetic neurons. For example, patients with POTS tend to have a greater chronotropic response to isoproterenol than do normal volunteers, suggesting adrenoceptor hypersensitivity,<sup>9</sup> and similarly, they are hypersensitive to the pressor effect of phenylephrine.<sup>11</sup>

Our finding that patients with POTS do not display overall increased sympathetic nervous activity as a whole but greater response to postural stimulation, together with a mismatch between nerve traffic and NE release, suggests the presence of abnormal neuronal function that goes beyond NET dysfunction.

It is important to note that it is not known whether the reduced NET protein that we document in the peripheral sympathetic nerves is paralleled by such a reduction in the sympathetic nerves in the legs and in the heart. Tissue samples obtained from 3 deceased subjects enable us to

demonstrate that both peripheral veins and heart tissue were rich in NET, therefore supporting to a degree the use of peripheral veins in the assessment of NET abundance. The heart is more dependent on the activity of the NET to clear the NE from synaptic clefts than is the case in the vascular beds,<sup>26</sup> and therefore, a reduction in NET function in the heart could underlie a greater HR rise during sympathetic activation in patients with POTS. Goldstein et al<sup>10</sup> documented higher cardiac NE spillover to plasma in subjects with POTS than in controls and noted that increments in HR during yohimbine infusion correlated significantly positively with that in cardiac NE spillover. However, cardiac extraction of <sup>3</sup>H-labeled NE, taken as an index of the activity of the NET, was not reduced, which suggests that high cardiac NE spillover was more likely due to increased cardiac sympathetic firing and high NE release rather than decreased NE reuptake. Our results favor the idea that NET impairment is also present in cardiac sympathetic nerves in patients with POTS. If NET impairment is present in most sympathetic nerves in patients with POTS, it is probable that it would affect the regional sympathetic system differentially. It is important to recognize that POTS is a heterogeneous condition, with patients presenting physiological and clinical differences. Hence, our results of reduction in NET protein cannot necessarily be generalized to all patients with POTS. Because of the relatively small number of patients in whom vein biopsies were obtained, we are unable at this point in time to quantitatively link the defect in NET to their clinical characteristics.

Other mechanisms could contribute to the abnormal cardiovascular response to the upright posture in patients with POTS. The marked impairment of cardiac baroreflex sensitivity seen in patients with POTS when compared with healthy control subjects, described by others,<sup>8</sup> suggests that vagal impairment also contributes to the excessive tachycardia seen in patients with POTS. Impairment of the renin-angiotensin system may induce disturbed vasoconstrictor response to head-up tilt in patients with POTS.<sup>27</sup> Our results of normal PRA in patients with POTS agree with previous observations.<sup>28</sup> However, PRA in patients with POTS seems inappropriately low, given the degree of hypovolemia that they exhibit.<sup>28</sup> Another important mechanism that could induce excessive sympathetic response in patients with POTS is the increase in venous pooling in the lower extremities.<sup>29</sup> Such an increase in venous pooling would require augmented sympathetic activation to the lower extremities to maintain arterial pressure, which may perhaps translate into increased sympathetic nerve firing in the skeletal muscle, as we described. Stewart et al<sup>5</sup> demonstrated that venous pooling in patients with POTS occurs as a result of blunted arterial vasoconstriction rather than a defect in venous compliance.

In summary, we have documented that patients with POTS exhibit decreased NET protein in their peripheral sympathetic nerves. However, during rest and head-up tilt, whole-body NE spillover to plasma was not accentuated. Further investigations should aim at delineating the molecular mechanism underlying the defect of NET expression and should specifically target the heart to establish whether a cardiac defect in NET is responsible for the magnification of the cardiac sympathetic response and tachycardia elicited during upright posture.

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## Disclosures

None.

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### CLINICAL PERSPECTIVE

The simple act of standing can be a challenge for some people, being accompanied by weakness, dizziness, or fainting. The postural orthostatic tachycardia syndrome (POTS) is one specific disorder causing problems of this type. The defining characteristic of POTS is an excessive rise (increases by 30 bpm or more or heart rate in excess of 120 bpm) in heart rate upon standing. Often the rise in heart rate is accompanied by presyncope or fainting in the absence of postural hypotension. Although the underlying pathophysiology of POTS remains unclear, there is evidence to suggest that elevated norepinephrine spillover from the heart correlates with heart rate in POTS patients. Reuptake of norepinephrine into sympathetic nerves after its release terminates the neural signal. A fault in transmitter inactivation augments the effects of sympathetic nerve traffic. In the heart, approximately 80% to 90% of released norepinephrine is recaptured into sympathetic nerves, making the heart more sensitive than all other organs to impairments of transmitter reuptake. Indeed, an abnormality in neuronal norepinephrine reuptake could sensitize the heart to sympathetic activation and its consequences. In this study, we found that patients with POTS exhibit decreased norepinephrine transporter protein in their peripheral sympathetic nerves. Future studies should concentrate on the molecular mechanism underlying the defect in norepinephrine transporter and establish whether it is responsible for the excessive cardiac sympathetic response and tachycardia elicited during the upright posture.



## Altered Sympathetic Nervous Reactivity and Norepinephrine Transporter Expression in Patients With Postural Tachycardia Syndrome

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