Acetylcholinesterase Inhibition Improves Tachycardia in Postural Tachycardia Syndrome

Satish R. Raj, MD; Bonnie K. Black, RN, NP; Italo Biaggioni, MD; Paul A. Harris, PhD; David Robertson, MD;

Background—Postural tachycardia syndrome (POTS) induces disabling chronic orthostatic intolerance notable for an excessive increase in heart rate that occurs on standing. Many current therapeutic strategies focus on sympatholysis, but the alternative strategy of enhancing cardiovagal tone has not been studied. The objective of this study was to test the hypothesis that acetylcholinesterase inhibitors will attenuate the tachycardia and improve symptom burden in patients with POTS.

Methods and Results—Seventeen patients with POTS underwent acute drug trials of pyridostigmine 30 mg orally and placebo, on separate mornings, in a randomized crossover design. Blood pressures, heart rate, and symptoms were assessed while patients were seated and after they had been standing for up to 10 minutes both before the study drug was given and at 2 and 4 hours after study drug. The heart rate was significantly lower at 2 hours after pyridostigmine than after placebo (100±16 versus 111±14 bpm, P=0.001). Pyridostigmine significantly decreased the standing heart rate from baseline (119±16 bpm) at 2 hours (104±16 bpm, P<0.001) and 4 hours (100±16 bpm, P<0.001) after administration. There was no significant change in blood pressure. The decrease in symptom burden within 4 hours after study drug was significantly greater with pyridostigmine than placebo (−10.4±14.0 AU versus 0.6±7.5 AU, P<0.025).

Conclusions—Acute acetylcholinesterase inhibition significantly attenuated tachycardia in POTS. There was also an improvement in symptom burden with this promising therapy. (Circulation. 2005;111:2734-2740.)

Key Words: tachycardia ■ acetylcholine ■ nervous system, autonomic ■ drugs ■ patients

Patients with POTS often have an exaggerated increase in plasma norepinephrine on standing.3 This finding has driven a focus on primarily sympatholytic therapeutic strategies with central4 or peripheral5 targeting, or secondarily by increasing peripheral resistance6 or by blood volume expansion.5 There are no data addressing the alternative strategy of enhancing cardiovagal tone.

Acetylcholinesterase inhibition is a commonly used treatment for myasthenia gravis.7 Pyridostigmine, a peripheral acetylcholinesterase inhibitor, should increase the availability of acetylcholine at both ganglionic nicotinic acetylcholine receptors (with a resultant increase in both sympathetic and parasympathetic tone) and postganglionic muscarinic acetylcholine receptors (which increases parasympathetic tone). Several studies have suggested a clinically beneficial role for acetylcholinesterase inhibitors in other autonomic disorders,8,9 with one study noting an incidental bradycardia. We hypothesized that this “double hit” of increasing parasympathetic tone at 2 points on the autonomic pathway might result in increased cardiovagal tone and a decreased heart rate. Acetylcholinesterase inhibition has been shown to decrease tachycardia in some other clinical circumstances,10 but to the best of our knowledge, there are no published reports of acetylcholinesterase inhibition in POTS. In the present study, we sought to test the hypothesis that acetylcholinesterase inhibition would decrease the tachycardia and symptoms in patients with POTS.

Subjects
Patients referred to the Vanderbilt University Autonomic Dysfunction Center with POTS between January 2004 and June 2004 were...
candidates for inclusion in this study. Patients met criteria for POTS\textsuperscript{11} in that they developed symptoms of orthostatic intolerance accompanied by a heart rate rise $\geq 30$ bpm (or a rate that exceeded 120 bpm) within the first 10 minutes of standing or head-up tilt in the absence of orthostatic hypotension (a fall in blood pressure of $>20/10$ mm Hg) and with an elevated standing norepinephrine value ($>2.81$ nmol/L [475 pg/mL]). All patients had at least a 6-month history of symptoms in the absence of other chronic debilitating disorder or prolonged bed rest, were at least 18 years of age, were free of medications that could impair autonomic tone,\textsuperscript{13} and had not been taking fludrocortisone for at least 5 days before testing. The Vanderbilt University Investigational Review Board approved this study, and written informed consent was obtained from each subject before the study was initiated.

### Study Diet and Baseline Characterization

Study investigations were performed at the Elliot V. Newman Clinical Research Center at Vanderbilt University. For at least 3 days before testing, subjects consumed a diet that contained 150 mEq of sodium per day and 70 mEq of potassium per day. Chronic medications were discontinued 5 half-lives before the study. The diet was free of caffeine-containing beverages. Heart rate, blood pressure, and fractionated plasma catecholamines were assessed after overnight rest in the supine position and again after standing for up to 30 minutes (as tolerated) as part of baseline characterization. For catecholamine measurements, blood was collected in plastic syringes, immediately transferred to chilled vacuum tubes with EGTA and reduced glutathione (Amersham International PLC), and immediately placed on ice. The plasma was separated by centrifugation at $-4^\circ$C and stored at $-70^\circ$C until the assay was performed. Concentrations of norepinephrine and epinephrine were measured by batch alumina extraction followed by high-performance liquid chromatography for separation with electrochemical detection and quantification.\textsuperscript{13}

### Drug Trials

All drug trials were started in the morning at least 2 hours after breakfast (to avoid any acute hemodynamic effects from eating) in a postvoid state. On separate days, patients with POTS were given a tablet of pyridostigmine 30 mg (Watson Pharmaceuticals) or placebo in a randomized, single-blind, crossover fashion. The dose of pyridostigmine was chosen because this is the lowest recommended starting dose for this medication.\textsuperscript{14} Placebo was prepared by the Vanderbilt Investigational Drug Services. The patients were seated comfortably in a chair for the duration of the data collection except during the prescribed periods of standing. Brachial cuff blood pressures and heart rates were measured with an automated vital signs monitor (Dinamap Vital Signs Monitor, Critikon Corp) and digitally acquired into a custom designed database (Microsoft Access, Microsoft Corporation). Seated heart rates and blood pressures were measured every 10 minutes for at least 30 minutes before and for 4 hours after administration of the study drug. Immediately before and at 2 and 4 hours after study drug administration, each patient was asked to stand for 10 minutes while their standing heart rates and blood pressures were recorded.

### Results

#### Patient Characteristics

Study inclusion criteria were met by 17 subjects with POTS (14 females) who were 37$\pm$11 years of age. One female subject dropped out of the study after completing the pyridostigmine study day but not the placebo study day, and another female subject dropped out after completing the placebo day but not the pyridostigmine day. Because both sets of data were not available for analysis, these subjects were excluded from all paired data analyses.

The data from the baseline supine and standing study are presented in Table 1. The supine heart rate was $75\pm14$ bpm with a blood pressure of $112\pm9/70\pm11$ mm Hg. The supine plasma norepinephrine and epinephrine values were within the normal range (norepinephrine $<2.81$ nmol/L [$<475$ pg/mL] and epinephrine $<0.41$ nmol/L [$<75$ pg/mL]). On standing, there was a marked increase in heart rate (124$\pm22$ bpm, $P<0.001$) and norepinephrine (6.27$\pm3.97$ nmol/L [1060$\pm671$ pg/mL], $P<0.001$) compared with respective supine values. Systolic blood pressure (112$\pm19$ versus 127$\pm18$ mm Hg, $P=0.005$) and diastolic blood pressure (70$\pm11$ versus 77$\pm9$ mm Hg, $P=0.022$) also increased on standing. Mean$\pm$SEM differences are shown in Table 1.
Two- and Four-Hour Seated and Standing Heart Rate and Blood Pressure Measurements

The standing heart rate before study drug administration was not different between placebo and pyridostigmine (120±14 versus 119±16 bpm, P=0.722; Figure 1B). Compared with placebo, pyridostigmine effected a lower standing heart rate at 2 hours (P=0.001) but not at 4 hours (P=0.160) after study drug administration. This was the primary outcome measure. Pyridostigmine effected a marked decrease in the standing heart rate (ANOVA P<0.001) that was maximal at 2 hours (100±16 bpm, P<0.001 versus preadministration) and still very significant at 4 hours after the dose (104±16 bpm; P<0.001 versus preadministration). The standing heart rate also decreased after administration of placebo (ANOVA P<0.001).

Immediately before administration of the study drug, there was no difference in seated heart rate between the placebo day (87±10 bpm) and the pyridostigmine day (87±11 bpm; P=0.815; Figure 1A). The seated heart rate did not change after placebo administration (ANOVA P=0.833), nor did it decrease significantly after pyridostigmine administration (P=0.293; Table 2). In contrast, there was a trend toward a lower seated heart rate 2 hours after pyridostigmine administration (80±18 bpm, P=0.125 versus preadministration), before it recovered slightly at 4 hours after pyridostigmine (81±14 bpm, P=0.171 versus preadministration). Compared with placebo, pyridostigmine effected a significantly lower seated heart rate at 4 hours (P=0.011) after study drug (Figure 1A).

Before study drug administration, the POTS patients had a large acute postural increase in heart rate with standing (delta heart rate), a cardinal hemodynamic feature of the syndrome (pyridostigmine day 33±13 bpm versus placebo day 33±12 bpm, P=0.903). There was a significant decrease in the delta heart rate with both pyridostigmine (ANOVA P=0.003) and placebo (ANOVA P=0.012). The reduction in delta heart rate was not significantly different between pyridostigmine and placebo at 2 hours (19±14 versus 25±10 bpm; P=106) or at 4 hours (23±12 versus 22±10 bpm; P=0.662) after study drug administration. Neither pyridostigmine nor placebo significantly altered the systolic blood pressure (Table 2; Figures 1C and 1D).

Serial Seated Heart Rate and Blood Pressure Measurements

As a secondary analysis, seated heart rates, systolic blood pressures, diastolic blood pressures, and mean arterial pressures were measured every 10 minutes starting immediately before study drug administration until 230 minutes after study drug administration. These data for both pyridostigmine and placebo are shown in Figures 2A through 2D. Heart rate trends over time show that pyridostigmine effected a greater reduction in heart rate than placebo. The heart rate data were superimposed for the
first 50 to 60 minutes after study drug ingestion before the curves separated to indicate a greater reduction with pyridostigmine. These data were analyzed with a general linear model repeated-measures ANOVA. Because the sphericity assumptions were not valid (Mauchly’s test probability value < 0.001), the Huynh-Feldt probability value was used. The interaction between heart rate over time and study drug was significant (P<0.007), with a lower heart rate over time in response to pyridostigmine. This interaction was primarily quadratic in nature (quadratic P<0.001). Paired t tests at each time point revealed significant differences (P<0.05) at 100, 110, 120, 140, 150, 170, and 230 minutes after study drug administration.

The systolic blood pressures, diastolic blood pressures, and mean arterial pressures were also analyzed with a similar model over the same time frame. There was no significant interaction between seated blood pressure over time and the study drug.

### Table 2. Standing and Sitting Heart Rate and Systolic Blood Pressure Before and After Pyridostigmine and Placebo

<table>
<thead>
<tr>
<th></th>
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<th>4 Hours After Study Drug</th>
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</table>

Pre indicates before study drug; SBP, systolic blood pressure. ANOVA was used to determine the overall P value for the change in a variable over time. Contrasts were used to make pairwise comparisons with baseline (2 hours vs Pre and 4 hours vs Pre).

*ANOVA P<0.05 was deemed to be significant. P<0.025 was deemed to be significant for these pairwise comparisons to account for the 2 comparisons.

**Figure 2.** Seated hemodynamics over 4 hours from study drug administration. Heart rates (A), systolic blood pressures (SBP; B), diastolic blood pressures (DBP; C), and mean arterial pressures (MAP; D) measured every 10 minutes for 4 hours after study drug administration are presented for pyridostigmine (●) and placebo (○). Probability values are reported for interaction between effect of study drug over time (Pint) by general linear model repeated-measures ANOVA. Error bars represent SEM. Probability value <0.05 was defined as significant.
Acetylcholinesterase Inhibitor

Figure 4. Role of acetylcholinesterase inhibition in heart rate and blood pressure control. See text for details. SNS indicates sympathetic nervous system; PNS, parasympathetic nervous system; ACh, acetylcholine; NE, norepinephrine; BP, blood pressure; and HR, heart rate.

POTS at 2 hours after administration compared with placebo, (2) improved the symptom burden both over 4 hours and compared with placebo, (3) decreased the seated heart rate compared with placebo over 230 minutes after drug administration, and (4) significantly decreased the standing heart rate of the patients with POTS at both 2 and 4 hours after administration compared with baseline

Pyridostigmine Hemodynamics

There was a highly significant decrease in the standing heart rate at 2 hours compared with placebo ($P=0.001$) and at both 2 and 4 hours after drug administration compared with baseline ($P<0.001$ for both), as seen in Figure 1B and Table 2. These data provide a proof of concept for the use of acetylcholinesterase inhibition for the restraint of standing tachycardia in POTS. Other drugs that are commonly used in POTS to decrease the heart rate (eg, $\beta$-adrenergic blockers, clonidine, and methyl-dopa) are also antihypertensive agents. The pyridostigmine was able to effect heart rate control without causing a significant change in blood pressure (Table 2).

In addition to decreasing the standing heart rate, pyridostigmine decreased seated heart rates in patients with POTS. Figure 2A shows the seated heart rate over 230 minutes from medication administration for both the pyridostigmine and placebo days. The curves separate at ≈60 minutes. Pyridostigmine significantly decreased the seated heart rate over time compared with placebo, as demonstrated by the significant interaction between study medication and heart rate over time ($P_{INT}=0.007$). Similar interactions were not significant for systolic blood pressure (Figure 2B), diastolic blood pressure (Figure 2C), or mean arterial pressure (Figure 2D).

Placebo Effects

Placebo also had a significant effect on controlling the heart rate in POTS patients. The reasons for these significant effects are not clear. One possibility is that they result from a circadian heart rate variation in these patients. We started each study in the morning 2 hours after breakfast (to remove the influence of digestion on heart rate and blood pressure). It is possible that
both the seated and standing heart rates are always lower at midday than earlier in the morning. If this is true, then it is of vital importance that when studies such as this are performed, they be conducted at the same times of day.

Another possibility to explain the heart rate reduction on the placebo day is that this represents a true “placebo effect.” It is well accepted that surgical procedures can have placebo effects independent of the actual care received. More recently, this effect has been seen in the treatment of neurally mediated syncope. Multiple unblinded, randomized trials of dual-chamber pacing showed a large reduction in the recurrence of syncope (>90% at 1 year). When a placebo controlled trial was finally performed, the benefits of pacing for neurally mediated syncope were not found to be statistically significant. These data emphasize the importance of including a placebo arm in therapeutic studies on patients with POTS.

Despite the remarkable placebo effects noted in the present study, it is noteworthy that pyridostigmine was able to decrease the seated and standing heart rates to a significantly greater degree than did placebo. These findings are strengths of the present study.

**Symptom Burden**

There was an improvement in symptom burden in POTS patients who received pyridostigmine. In contrast to the heart rate response, placebo did not improve the symptom burden of the POTS patients. The change in symptoms score from baseline to 4 hours after study drug (Figure 3) ranged from an improvement in symptoms with pyridostigmine to a slight worsening of symptoms with placebo.

**Acetylcholinesterase Inhibition: Proposed Model of Action**

The mechanism of the salutary action of pyridostigmine is not entirely clear, but we propose a model based on neurotransmission in the autonomic nervous system (Figure 4, top). Acetylcholine is the primary neurotransmitter in the autonomic ganglia in both the sympathetic and parasympathetic nervous systems. The parasympathetic nervous system uses acetylcholine again as the neurotransmitter at the postganglionic synapse. This results in an inhibitory effect on heart rate. In contrast, the sympathetic nervous system uses norepinephrine as the postganglionic neurotransmitter. This results in a direct increase in heart rate and blood pressure (Figure 4, solid lines); however, the increase in blood pressure is modulated by the baroreflex, which leads to a reduction in sympathetic tone, an increase in parasympathetic tone, and a subsequent decrease in heart rate (Figure 4, dashed lines).

In the presence of an acetylcholinesterase inhibitor (Figure 4, bottom), synaptic acetylcholine is increased in the autonomic ganglia of both the sympathetic and parasympathetic nervous systems, which results in increased cholinergic transmission in both limbs. At the postganglionic synapse of the parasympathetic nervous system, the augmented level of acetylcholine (due to both increased transmission and decreased acetylcholine degradation) has a strong inhibitory effect on heart rate. Because the sympathetic nervous system uses norepinephrine as the postganglionic neurotransmitter, the acetylcholinesterase inhibitor has no effect at this level. There is slightly more sympathetic nervous system traffic (thicker lines) as a result of the ganglionic acetylcholine augmentation. The resulting increase in blood pressure leads to augmented baroreceptor activity, which also contributes to a restraining effect on heart rate. In support of this model, there are recent data that pyridostigmine has been found to increase baroreflex sensitivity in both a murine model and in patients with POTS.

It is likely that the major effect of acetylcholinesterase inhibition in patients with POTS is the reduction in heart rate through augmentation of parasympathetic tone. It is possible, however, that other mechanisms also play a role in the apparent benefit of acetylcholinesterase inhibition. Jacob et al have reported that some patients with POTS have a “partial dysautonomia,” with focal impairment of sympathetic tone in the lower extremity, which leads to impaired vasoconstriction. Stewart et al have shown that intravenous phenylephrine, an α-1 adrenoreceptor agonist, acutely improved orthostatic tolerance through peripheral vasoconstriction in a cohort of patients with POTS. Augmentation of sympathetic tone due to ganglionic acetylcholinesterase inhibition might increase peripheral vascular resistance through α-1 receptor stimulation. It is tempting to speculate that this “non–heart rate” mechanism may play an important role in the symptomatic improvement seen with acetylcholinesterase inhibition in POTS; however, vascular resistance was not measured in the present study.

The augmentation in sympathetic tone with acetylcholinesterase inhibition has proven clinically useful in patients with orthostatic hypotension. We found that peripheral acetylcholinesterase inhibition increased blood pressure among patients with autonomic failure in a dose-dependent fashion. Singer et al reported that pyridostigmine decreased orthostatic hypotension in patients with neurogenic orthostatic hypotension by increasing the peripheral resistance in response to head-up tilt and consequently improved their orthostatic symptoms.

**Study Limitations**

The main limitation of the present study is the relatively small sample of patients. Although the sample was not large, our primary outcome of reduction in standing heart rate was highly significant (P<0.001). The small sample should have biased against our ability to show significance, but it did not. This emphasizes the large signal (with relatively little noise) of the pyridostigmine data.

The short duration of follow-up in the present study (4 hours) makes it difficult to intelligently project the long-term efficacy of this treatment. This study has provided a proof of concept for the use of acetylcholinesterase inhibition for the control of tachycardia in patients POTS. We do not know how well tolerated this treatment will be in this population. For example, pyridostigmine is known to increase gastrointestinal motility, and this could be a limiting side effect. Ultimately, there is no way to determine this with certainty without a long-term, well-powered, outpatient clinical trial.
We chose pyridostigmine because it was a peripheral acetylcholinesterase inhibitor with a relatively short half-life and a long history of use for other disorders. It is possible that other drugs in this class might be equally if not more effective in patients with POTS. Furthermore, they may be more beneficial clinically owing to a longer half-life.

Conclusions
We found that acetylcholinesterase inhibition with pyridostigmine was a highly effective method of acutely decreasing the tachycardia in patients with POTS. It is also exciting to note the acute improvement in symptom burden in these patients. Longer-term studies are needed to assess this promising therapy.

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References
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