Blunted cerebral blood flow velocity in response to a nitric oxide donor in postural tachycardia syndrome

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Del Pozi AT, Pandey A, Medow MS, Messer ZR, Stewart JM. Blunted cerebral blood flow velocity in response to a nitric oxide donor in postural tachycardia syndrome. Am J Physiol Heart Circ Physiol 307: H397–H404, 2014. First published March 30, 2014; doi:10.1152/ajpheart.00194.2014.—Cognitive deficits are characteristic of postural tachycardia syndrome (POTS). Intact nitricergic nitric oxide (NO) is important to cerebral blood flow (CBF) regulation, neurovascular coupling, and cognitive efficacy. POTS patients often experience defective NO-mediated vasodilation caused by oxidative stress. We have previously shown dilation of the middle cerebral artery in response to a bolus administration of the NO donor sodium nitroprusside (SNP) in healthy volunteers. In the present study, we hypothesized a blunted middle cerebral artery response to SNP in POTS. We used combined transcranial Doppler-ultrasound to measure CBF velocity and near-infrared spectroscopy to measure cerebral hemoglobin oxygenation while subjects were in the supine position. The responses of 17 POTS patients were compared with 12 healthy control subjects (age: 14–28 yr), CBF velocity in POTS patients and control subjects were not different at baseline (75 ± 3 vs. 71 ± 2 cm/s, P = 0.31) and decreased to a lesser degree with SNP in POTS patients (to 71 ± 3 vs. 62 ± 2 cm/s, P = 0.02). Changes in total and oxygenated hemoglobin (8.83 ± 0.45 and 8.13 ± 0.48 μmol/kg tissue) were markedly reduced in POTS patients compared with control subjects (14.2 ± 1.4 and 13.6 ± 1.6 μmol/kg tissue), primarily due to increased venous efflux. The data indicate reduced cerebral oxygenation, blunting of cerebral arterial vasodilation, and heightened cerebral venules. We conclude, based on the present study outcomes, that decreased bioavailability of NO is apparent in the vascular beds, resulting in a downregulation of NO receptor sites, ultimately leading to blunted responses to exogenous NO.

METHODS

Patients and control subjects. Seventeen POTS patients and 12 control subjects were compared. Characteristics of the study participants are shown in Table 1. Potential POTS patients for the study were selected based on referral to our clinic for possible POTS, all having symptoms lasting longer than 6 mo; diagnosis was confirmed in our laboratory by means of completing a screening head-up tilt (HUT) test. The criteria for POTS in adults is a heart rate (HR) increase of ≥30 beats/min within 10 min of orthostasis; in children, a higher threshold is used of a HR increase of ≥40 beats/min or an absolute HR of ≥120 beats/min (26, 48). Only patients who fit the aforementioned POTS criteria were enrolled in the study. POTS enrollees were either therapeutically naïve (~85%) or were weaned off medication over 2 wk before the experiments. After withdrawal from medication, POTS patients were medication free for a minimum of 2 wk.

Healthy control subjects had no history of OI. Exclusionary criteria for participation in the study included any infectious or systemic disease, including diseases of the central nervous system, autonomic, endocrine, respiratory, metabolic, or cardiovascular diseases, competitive athletic training, and use of nicotine or any other chronic medication, excluding oral contraceptives. In addition, any subject with a history of fainting or experiencing syncope during a screening HUT test was excluded from participation.

This study was approved by the Committee for the Protection of Human Subjects (Institutional Review Board) of New York Medical College and conformed with The Declaration of Helsinki. All subjects 18 yr and older signed informed consent before participation, and those <18 yr old gave assent and their legal guardians signed informed consent forms.

Instrumentation. All participants arrived for testing at 9:30 AM after a 2-h fast. Participants were then instructed about the day’s experiment and outfitted with the instrumentation. An intravenous catheter was placed in the left antecubital vein. Beat-to-beat arterial blood pressure was measured using an oscillometric calibrated Finometer (FMS, Amsterdam, The Netherlands), TCD (Multigun, Yonkers, NY) insonated the right MCA, and the signal was optimized for depth and signal strength. Respiratory plethysmography (Respiratrace 200, NIMS, North Bay Village, FL) measured changes in respiration. A combined capnograph and integrated pulse oximeter (Smith Medical PM, Waukesha, WI) was used to measure end-tidal CO2 and

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arterial $O_2$ saturation. An ECG measured HR from the beat-to-beat cardiac electrical intervals.

Continuous-wave spatially resolved NIRS (Oxymon MKII, Artinis) was used to monitor changes in HbO$_2$, deoxyhemoglobin (Hb), and THb over the same volume of cerebral tissue throughout the protocol (40). Infrared signals produced by the cutaneous bed were resolved through spatial resolution, ensuring that the infrared signals from the brain were the only signals analyzed. NIRS was used. Because these are noninvasive measurements, we assumed that the notable changes in TCD representing CBF velocity (CBFv) measured by TCD. The modified Oxford method was performed and to the following solution for $\Delta Q_a$:

\[
\frac{dHbO_2}{dt} - \frac{dTHb}{dt} = \frac{(S_a - S_i)}{S_a} \Delta Q_a
\]

and to the following solution for $\Delta Q_a$:

\[
\frac{dHbO_2}{dt} - \frac{dTHb}{dt} = \frac{(S_a - S_i)}{S_a} \Delta Q_a
\]

A complete explanation of the mathematic equations has been previously published and can be found in Stewart et al. (51).

Statistics. Because there were no differences between the sexes, both male and female data were combined for group analysis. During the modified Oxford maneuver, measurements of arterial pressures, HR, CBFv, changes in Hb, HbO$_2$, and THb via NIRS, and NIRS-estimated changes in $Q_a$ and $Q_c$ ($\Delta Q_a$ and $\Delta Q_c$) were tabulated before SNP, at the peak response to SNP, and during the peak response to PE and were subsequently analyzed by a one-way ANOVA. When appropriate, post hoc evaluations were made using the Bonferroni procedure. Significance was set a priori, and differences were considered significant when $P$ values of <0.05 was achieved. All values are reported as means ± SE. Results were calculated using SPSS 16 (SPSS, Chicago, IL).

RESULTS

Hemodynamic responses to bolus SNP followed by bolus PE. Figure 1 is provided for illustrative purposes and shows a representative subject from both the control group and POTS group for TCD and NIRS tracings during the modified Oxford method. Comparative (control vs. POTS) hemodynamic responses to SNP and PE are shown in Table 1. Both control and POTS groups responded with a significant increase in HR after SNP ($P < 0.001$) and showed a significant decrease in HR after the PE bolus ($P < 0.001$).
In addition, MAP, SBP, and DBP decreased significantly ($P < 0.001$) after SNP and increased significantly ($P < 0.001$) after PE in both groups. The respiratory rate of the control group was unaffected; however, the POTS group experienced a significant increase in respiratory rate ($P < 0.001$) after SNP infusion, which returned to baseline values after the PE bolus ($P < 0.001$). For the POTS group, end-tidal CO$_2$ was relatively unchanged. Conversely, end-tidal CO$_2$ in the control group was increased after PE compared with SNP ($P < 0.05$). Neither group experienced a change in $S_a$ during any phase of the protocol. After the SNP and PE bolus injections, $Q_a$ in the POTS group was significantly lower than that of the control group ($P < 0.01$). Additionally, $Q_s$ in the POTS group was greater than that of the control group ($P < 0.01$), as measured by NIRS (Table 2).

$\Delta$THb in the POTS group was decreased significantly compared with the control group after SNP administration ($8.83 \pm 0.45$ vs. $15.2 \pm 1.4$ µmol/kg tissue, respectively, $P < 0.01$). $\Delta$HbO$_2$ was decreased significantly in the POTS group compared with the control group after SNP administration ($8.13 \pm 0.46$ vs. $13.6 \pm 1.6$ µmol/kg tissue, respectively, $P < 0.01$; Fig. 2). However, after the PE bolus, $\Delta$THb and $\Delta$HbO$_2$ in the POTS and control groups were not statistically different (Fig. 2). Both groups experienced significant ($P < 0.001$) decreases in CBFv in response to SNP administration. However, the POTS group did not experience a drop in CBFv as large as the control group (change of $-4 \pm 2$ vs. $-9 \pm 2$ cm/s, respectively, $P < 0.001$); these results are shown in Fig. 2.

**DISCUSSION**

One of the most debilitating symptoms of POTS is cognitive impairment (32, 44), sometimes referred to as “brain fog” (43). In past work, using an executive memory task in younger POTS patients, we demonstrated that POTS patients exhibit a progressive cognitive impairment during step-wise incremental orthostatic stress (36). Moreover, cognitive impairment was associated with neurovascular uncoupling (35, 52) such that the normal increase in CBF after neuronal activity (functional hyperemia) was absent in POTS patients (32, 52). Neurovascular uncoupling in the POTS group was indicated by a blunted response to NO donor (SNP), as shown in Fig. 2. This blunted response to NO donor in patients with POTS suggests impaired vasodilatory capacity in the cerebral circulation.
Table 2. Supine hemodynamic measurements before the Oxford maneuver and after bolus injections of SNP and PE

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Before the Oxford Maneuver</th>
<th>SNP</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POTS group</td>
<td>Control group</td>
<td>POTS group</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>73 ± 2*</td>
<td>58 ± 2</td>
<td>109 ± 3*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>118 ± 5</td>
<td>116 ± 2</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>51 ± 3</td>
<td>58 ± 3</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>71 ± 3</td>
<td>78 ± 2</td>
<td>52 ± 3*</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>15 ± 1</td>
<td>14 ± 2</td>
<td>18 ± 1*</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>43 ± 1</td>
<td>42 ± 1</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Cerebral blood flow velocity, cm/s</td>
<td>75 ± 3</td>
<td>71 ± 2</td>
<td>71 ± 3*</td>
</tr>
<tr>
<td>Estimated cerebral vascular resistance, mmHg·cm⁻¹·s⁻¹</td>
<td>0.96 ± 0.07</td>
<td>1.10 ± 0.08</td>
<td>0.75 ± 0.06*</td>
</tr>
<tr>
<td>Venous O₂ saturation</td>
<td>0.63 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>Arterial O₂ saturation</td>
<td>0.98 ± 0.02</td>
<td>0.97 ± 0.01</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Change in NIRS arterial inflow, µmol·kg tissue⁻¹·s⁻¹</td>
<td>0.025</td>
<td>0.025</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Change in NIRS venous outflow, µmol·kg tissue⁻¹·s⁻¹</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Change in oxyhemoglobin, µmol/kg tissue</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Change in deoxyhemoglobin, µmol/kg tissue</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Change in total hemoglobin, µmol/kg tissue</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values are means ± SE. SNP, sodium nitroprusside; PE, phenylephrine. Near-infrared spectroscopy (NIRS) flow and oxyhemoglobin and deoxyhemoglobin were set to zero before the Oxford recordings. Venous O₂ saturation was assumed constant during the modified Oxford maneuver, and therefore values during SNP and PE were estimated. *P < 0.05 compared with the control group.

cular coupling depends on the integrity of the neurotransmitters that link neuronal activity to increased CBF. NO is one of the most important of these neurotransmitters. Therefore, impaired NO responses may have dire consequences for neurovascular coupling.

We have previously shown that POTS patients experience a deficit in NO-dependent vasodilation within the peripheral microcirculation, linked to oxidative stress (28, 56). The deficit in NO has been associated with increased plasma levels of ANG II (50). The increase in ANG II was attributed to a defect in angiotension-converting enzyme-2 (54) and is known to produce ROS via binding to ANG II type 1 receptors (AT1R) on NADPH oxidase (6, 62) and scavenging NO by superoxide. Additional oxidative stress produced locally can cause augmented adrenergic-mediated vasoconstriction (20, 24).

Fig. 2. Top: ∆THb (solid lines) and ∆HbO₂ (dashed lines) averaged over all subjects for control (black) and POTS (gray) groups during the modified Oxford maneuver. Results are shown for illustrative purposes; statistical comparisons are shown in Table 1. NIRS, near-infrared spectroscopy. Middle: corresponding changes in arterial inflow (∆Qa; solid lines) and venous outflow (∆Qv; dashed lines) for both control (black) and POTS (gray) groups during the modified Oxford maneuver. Bottom: averaged ∆CBFv during the modified Oxford maneuver for control (black) and POTS (gray) groups. The first arrow indicates the administration of SNP, and the second arrow indicates the administration of PE.
When present, systemic circulatory abnormalities are also found in the brain (34, 55) and may account for impaired CBF autoregulation in the upright POTS patient (34). Impairment of cerebral autoregulation is associated with a reduction in neurovascular coupling (52) as well as cognition (33).

In the present study, we show that individuals diagnosed with POTS exhibit a blunted response to exogenous NO. This is shown in Fig. 2, where POTS subjects have reduced responses in THb and HbO2, concurrently showing an increase in $\Delta Q_v$ and a decrease in $\Delta Q_a$ while also exhibiting a blunted decrease in CBFv in response to SNP (see Table 2). Others, using NO synthase inhibitors, have stated that NO does not have an effect on the dimensions of the MCA measured using TCD (63). However, the present study shows that during exogenous NO administration, $Q_a$ and CBFv are directionally opposite, consistent with vascular dilation from NO donors. This is similar to our previous study using TCD (51) and those by others using PET scans (37, 49, 60), which showed a decrease in CBF under similar experimental conditions, indicating a dilation of the MCA in response to a NO donor.

We (28) have previously demonstrated that POTS patients experience a reduction in the amount of bioavailable NO in response to local warming of the cutaneous microvasculature, which was related to reduced neuronal NO (of neuronal NO synthase origin) activity in POTS (53). This is of interest as NO from neuronal NO synthase is believed to interact with central ANG II in the regulation of central sympathetic outflow (27), in the myogenic response of cerebral autoregulation (15), and with nitergic interneurons, which participate in the functional hyperemia of the neurovascular coupling (11). Impaired NO responses may also potentially affect the regulation of proximal CBF through the parasympathetic nitergic innervation of conduit cerebral vessels and pial arteries. Agassandian et al. (1) and Boysen et al. (4) have shown that direct projections from the cardiovascular nucleus tractus solitarii to the pontine preganglionic parasympathetic neurons, acting through the pterygopalatine ganglia, provide tonic dilatory influences on the cerebral vessels. This may provide a link between changes in central blood volume and arterial baroreflexes to influence cerebral vascular regulation via the extrinsic vasomotor innervation system (15). Thus, baroreflex unloading causes parasympathetic withdrawal; this is enhanced in POTS and may reduce NO-dependent dilation of the cerebral vasculature, contributing to cerebral vasoconstriction. In POTS, the sympathetic baroreflex is intact (29), whereas vagal withdrawal is enhanced (57). This conservation of the sympathetic baroreflex coupled with an enhanced vagal withdrawal implies attenuation of nitergic parasympathetic activity, leading to tonic cerebrovascular constriction and impaired myogenic dilation of the cerebral vessels (16). The present study shows significant reductions in MAP in POTS patients compared with control subjects, yet CBFv, while decreased, was higher in POTS patients than in control subjects. Previous investigations have shown a reduction of bioavailable NO in many POTS patients, and the present study may also imply a deficit in NO signaling. For example, the NO-cGMP receptor pathway (e.g., guanylyl cyclase) may be defective as exogenous NO did not produce the same response to CBFv as in the control group (9).

The present study found results similar to others who noted that cerebral perfusion persisted after HUT (18, 41, 47). Hermosillo et al. (18) compared CBFv of patients experiencing neurocardiogenic syncope with those diagnosed with POTS and showed that in POTS and syncope patients, CBFv did not differ significantly during HUT. They concluded that defects within the central nervous system play a crucial role in the pathophysiology of POTS (18). Razumovsky et al. (41) investigated if chronic fatigue syndrome patients, who have an exaggerated risk for POTS, experienced an abnormal CBFv response compared with healthy control subjects during HUT. They found no difference in CBFv through all phases of the tilt; however, hypotension was apparent in $\sim$61% of control subjects. Additionally, they noted that POTS was present in $\sim$30% of healthy control subjects (41). They concluded that there was not a characteristic CBFv pattern in chronic fatigue syndrome (41). Schondorf et al. (47) also compared POTS patients with healthy control subjects during HUT and found no differences in CBFv responses to orthostatic stress in systolic CBFv. Additionally, they noted that diastolic CBFv did not differ between the two groups until late in the tilt. They concluded that cerebral perfusion in POTS patients did not differ from healthy control subjects (47).

Other studies have shown significantly impaired CBF in POTS (19, 31). Novak et al. (31) compared POTS patients with healthy control subjects during HUT. They observed significant reductions in CBFv during all stages of the tilt and attributed these findings to concurrent hypocapnia and hyperventilation (31). Jacob et al. (19), who also used HUT, evaluated patients with OI compared with control subjects and found significant decrements in CBFv with increases in tilt angle in the OI group but not in the control group. They concluded that the regulation of cerebrovascular tone is impaired in OI (19).

Lavi et al. (21) studied the role of NO in the regulation of CBF and noted a drop in end-tidal CO2 after a steady-state infusion of SNP to achieve a stable drop in blood pressure (5–10 mmHg). They concluded that NO affects basal cerebrovascular tone and is not involved in the blood-pressure-dependent portion of cerebral autoregulation but does affect the chemoregulatory mechanism of cerebral autoregulation (21). In contrast, subjects in the present study had no significant drop in end-tidal CO2 and the modified Oxford maneuver was used, which consisted of a bolus injection of SNP followed by a bolus injection of PE. The differences in the administration of SNP (bolus vs. continual infusion) could potentially explain the observed differences in end-tidal CO2 values.

Additionally, Zhang et al. (63) investigated cerebral autoregulation after NO inhibition using $N^\omega$-monomethyl-l-arginine (l-NMMA). Their results indicated that l-NMMA poorly crossed the blood-brain barrier, whereas NO crossed freely. They concluded that tonic production of NO did not alter cerebral autoregulation. Zhang et al. (63) noted no significant differences between end-tidal CO2 under conditions of NO inhibition. This is in agreement with the present study; however, Zhang et al. did not use a NO donor. This could potentially explain the differences in our conclusions.

In the present study, during the administration of SNP, the POTS group experienced a decrease in MAP from 71 to 52 mmHg. A MAP of 52 mmHg falls below the described range of blood pressure where autoregulation is thought to be most effective (60–160 mmHg) (21). Based on this, we would expect an interruption in cerebral autoregulation; however, this was not observed, as CBFv after SNP was not different than the control group (10).
CBFv before administration of this NO donor. Despite the fall in MAP, autoregulation was maintained. It is possible that what we observed was due to a decrease in the critical lower limit of the autoregulation curve in POTS. Alternatively, this may have been due to diminished NO-dependent vasodilation, for it has been suggested that for pressures that fall moderately below the lower limit of the autoregulation regulation curve, pharmacologically induced vasodilation could normalize CBF (58). The present study, however, was not designed to distinguish between these possibilities.

The differences in CBF found in the present study could be explained by the differences in patient populations as well as differing methodologies. The present study required that all patients must fit the POTS criteria, having symptoms for a minimum of 6 mo. While POTS is the most common form of chronic OI (2), a key feature in POTS is that patients do not experience orthostatic hypotension. By requiring that all of our patients had POTS and by excluding subjects with OI, including vasovagal syncope in which hypotension is the hallmark, we reduced variability in our study population.

The present study describes decreased cerebral circulation responsiveness to exogenous NO. Previous work (28, 53, 56) indicated reductions in the peripheral bioavailability of NO. Based on this, we conclude that deficits in NO in POTS may be manifold and that research into the mechanisms responsible for these decrements in NO is warranted.

**Limitations.** The present study is limited by the use of TCD and NIRS, which are indirect methods for the estimation of changes in CBF, and they have low regional specificity. However, these methods have superior time resolution compared with functional MRI or PET scans. Thus, because of the experimental arrangement and the rapidity of changes during bolus administration of SNP and PE, the use of large-scale imaging is precluded.

It is possible that interference from the cutaneous vascular bed could influence the results of NIRS; however, the infrared signals produced by the cutaneous bed were resolved through spatial resolution, assuring that the infrared signals from the brain were the only signals analyzed (3). Additionally, if cutaneous blood flow was interfering during the bolus injection of SNP, then an increase in blood flow, as measured by NIRS, would have been observed; no such increase was observed. We (54) have previously shown that a subset of POTS patients experience augmented vasoconstriction in the dependent periphery. This heightened vasoconstriction was due to increased ANG II and reduced angiotension converting enzyme-2. The present study, however, did not distinguish between the subsets of POTS patients. Any differences in peripheral vasoconstrictors would have little effect in the present study because peripheral ANG II has limited access to the central circulation (59). We also assumed that the MCA is uniform and cylindrical. Most studies using TCD to measure CBFv within conduit vessels use this assumption, and it has been a useful approximation (23, 30, 51).

We assumed that the concentrations of THb, Hb, and HBO2 in the vessels illuminated by NIRS are the same as that in the MCA. However, this assumption applies only if other conduit arteries that might contribute to the illuminated sample would be equally affected by SNP and PE.

Due to the rapidity of the modified Oxford maneuver, we assumed that Sv was unaffected by the bolus injection of SNP (17) and PE (5). In addition, Sa and Sv are indirectly measured, and this too could possibly affect the results. However, Ross et al. (45), who studied noninvasive measurements of arterial O2 saturation, compared different pulse oximeters with direct arterial blood gas measurement and found no statistical differences among them. Finally, we assumed that the cerebral rate of O2 consumption and Sv of Hb during the modified Oxford maneuver remained constant, and, under the conditions of the present study, we feel that these assumptions are reasonable (5, 14).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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