Cutaneous Constitutive Nitric Oxide Synthase Activation in Postural Tachycardia Syndrome with Splanchnic Hyperemia

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Stewart Short Title: Constitutive NOS in POTS

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Abstract

Models of microgravity are linked to excessive constitutive nitric oxide synthase (NOS), splanchnic vasodilation and orthostatic intolerance (OI). Normal flow postural tachycardia syndrome (POTS) is a form of chronic OI associated with splanchnic hyperemia. To test the hypothesis that there is excessive constitutive NOS in POTS, we determined whether cutaneous microvascular neuronal NO (nNO) and endothelial NO (eNO) are increased. We performed two sets of experiments in POTS and control subjects aged 21.4±2 years. We used laser Doppler flowmetry to measure the cutaneous response to local heating as an indicator of bioavailable nNO. To test for bioavailable eNO, we infused intradermal acetylcholine through intradermal microdialysis catheters and used the selective neuronal NOS inhibitor L-\(\omega\)-Nitroarginine-2,4-L-diamino-butyric amide (N\(\omega\), 10 mM), the selective inducible NOS inhibitor aminoguanidine (AG, 10 mM), the non-specific NOS inhibitor nitro-L-arginine (NLA 10mM), or Ringer’s solution. The acetylcholine dose-response and the NO-dependent plateau of the local heating response were increased in POTS compared to control subjects. The local heating plateau was significantly higher, 98±1 %CVCmax in POTS compared to 88±2 %CVCmax in control, but decreased to the same level with N\(\omega\) (46±5 %CVCmax in POTS compared to 49±4 %CVCmax in control) or with NLA (45±3 %CVCmax in POTS compared to 47±4 %CVCmax in control). Only NLA blunted the acetylcholine dose-response indicating that NO produced by endothelial NOS was released by acetylcholine. AG was without effect. This is consistent with increased endothelial and neuronal NOS activity in normal flow POTS.
Key Words: nitric oxide synthase isoforms, laser Doppler flowmetry, acetylcholine, cutaneous blood flow,
Introduction

Orthostatic intolerance (OI) is defined by symptoms and signs relieved by recumbency. Symptoms include dizziness, fatigue, exercise intolerance, headache, memory problems, palpitations, nausea, blurred vision, pallor, and abnormal sweating while upright which improve with recumbence and which have no other medical explanation. Postural tachycardia syndrome (POTS) is the predominant form of chronic orthostatic intolerance (17; 28; 38; 52; 53) and is defined by symptoms of OI associated with the physical sign of excessive upright tachycardia (38). We previously described three subsets of POTS patients:

Low Flow POTS: characterized by pallor, absolute hypovolemia, reduced stroke volume, blunted orthostatic vascular responses, increased plasma angiotensin-II, and decreased bioavailability of cutaneous nitric oxide (NO) of nNOS origin (47) related to increased plasma angiotensin-II (44) consequent to ACE2 deficiency (50).

High Flow POTS: characterized by normovolemia, peripheral vasodilation, and increased peripheral blood flow, cardiac output and microvascular filtration accounting for postural tachycardia (42; 43). Evidence indicated a mechanism of defective adrenergic-mediated vasoconstriction possibly associated with a post-viral peripheral neuropathy (16; 27).

Normal Flow POTS: Less well characterized are, perhaps, the majority of our recent POTS patients who have normal peripheral blood flow, heart rate, and vascular resistance when supine. Upright, splanchnic vasodilation causes splanchnic hyperemia resulting in a redistributive reduction in central blood volume and reflex tachycardia due to excessive splanchnic pooling (45; 49).
Interestingly, splanchnic hyperemia also occurs in real and simulated microgravity and is often associated with orthostatic intolerance, adrenergic hyporeactivity (2; 26; 32).

We hypothesized therefore that microvascular NO production would be upregulated in normal flow POTS subjects with splanchnic hyperemia. To test this hypothesis, we examined the plateau phase of the cutaneous response to local heat that is largely NO dependent (19,27), and acetylcholine-induced increase in cutaneous blood flow that is due, in part to endothelial NO (47). We used different doses of acetylcholine alone and then combined with NOS inhibitors (NLA, Nω and AG) to elicit changes in skin blood flow responses thought to represent local signaling by NO.
Methods

To test this hypothesis we used skin as a surrogate microvasculature (14) and stimulated the production of NO in two ways:

1. Via the local cutaneous heating response reported by Kellogg (21) and Minson (29) which reaches a plateau that is highly sensitive to nitric oxide synthesis (NOS) inhibition.

2. Using the acetylcholine dose-response which is partly dependent on receptor mediated endothelial NO production.

In both cases we tested our hypothesis by employing a highly selective, isoform specific nNOS inhibitor, L-Nω-Nitroarginine-2,4-L-diamino-butyric amide (Nω), a highly specific iNOS inhibitor aminoguanidine (AG), and a non-isoform specific NOS inhibitor nitro-L-arginine (NLA) to examine individual roles for nNOS, eNOS and iNOS in the regulation of microvascular function in normal flow POTS patients.

Subjects

Enrolled POTS patients were referred to the Hypotension Center for investigation of signs and symptoms of chronic orthostatic intolerance lasting at least three months. Orthostatic intolerance was defined by the presence of dizziness, fatigue, exercise intolerance, headache, memory problems, palpitations, nausea, blurred vision, pallor, and abnormal sweating while upright relieved by recumbence. The diagnosis of POTS was made in these patients during a screening upright tilt table test to 70° for a maximum of 10 minutes. POTS was diagnosed by symptoms of orthostatic intolerance
during tilt associated with an increase in sinus heart rate of greater than 30 beats per minute or to a rate of greater than 120 beats per minute during the first 10 minutes of tilt (28; 36). During the same visit, POTS patients were partitioned on the basis of supine calf blood flow measured by venous occlusion strain gauge plethymography (12) into either “normal flow”, “high flow” or “low flow” POTS as we have previously defined (48).

In the current study, we only enrolled subjects with Normal Flow POTS. “Normal flow POTS” subjects are defined as those who had a minimum calf blood flow of 1.2ml/100ml tissue/min and a maximum calf blood flow of 4.0ml/100ml tissue/min. This is based on the range of calf blood flow that we have measured in healthy volunteers from determinations in over 150 healthy subjects. Splanchnic hyperemia was confirmed in these patients (45; 49).

Using these criteria, we recruited 14 normal flow POTS patients (10 female, 4 male all Caucasian, aged 15.5-24.1 years, median age 21.3 years). Twelve healthy Caucasian volunteers subjects were also recruited (8 female, 4 male all Caucasian, aged 17.0-25.7 years, median age 21.5 years) and were studied after a screening upright tilt at 70° demonstrated normal orthostatic response. Volunteer subjects served as a control group and were recruited from among adolescents and young adults referred for innocent heart murmur. Subjects with a history of syncope or orthostatic intolerance were specifically excluded from enrollment in this study.

All subjects were free from systemic, cutaneous, and cardiovascular diseases. Subjects refrained from alcohol and caffeinated beverages for 24 hours prior to study and were
not taking any medications. There were no smokers or competitive athletes. Informed consent was obtained from all participants, and the Committee for the Protection of Human Subjects (IRB) of New York Medical College approved all protocols. Female subjects were enrolled without regard to the phase of their menstrual cycle except that none were menstruating during testing procedures.

**Protocols**

Two sets of experiments were performed in which changes in cutaneous blood flow were measured.

The first series of experiments investigated the cutaneous response to local heating in POTS patients compared to healthy volunteers, in the presence and absence of NOS inhibition. We employed the highly selective neuronal NOS (nNOS, NOS-1) inhibitor L-$\omega$-Nitroarginine-2,4-L-diamino-butyric amide ($\omega$, 10 mM), the highly selective inducible NOS (iNOS, NOS-2) inhibitor L-aminoguanidine (AG, 10mM), and the nonselective NOS inhibitor nitro-L-arginine (NLA, 10mM). There is no highly selective endothelial NOS (eNOS, NOS-2) inhibitor and eNOS inhibition was assessed by inference (e.g. suppression by NLA but not by $\omega$ or AG) and by exploiting eNOS-specific stimulation using acetylcholine.

Past experiments have demonstrated that $\omega$ decreased cutaneous vascular conductance during local heating by an amount equivalent to the largest reduction produced by NLA (47). We have therefore used the local heating response as a bioassay for nNOS and do so in the current experimental design.
The second series of experiments investigated the response to increasing doses of acetylcholine, a receptor mediated eNOS agonist. This was measured in the presence and absence of NOS inhibition.

Experiment 1 The Effect of NOS Inhibitors on Local Heat Mediated Vasodilation

We compared the effects of the isoform selective nNOS inhibitor Nω, to a maximally plateau-suppressing dose of NLA, a nonselective NOS inhibitor and to AG, a selective iNOS inhibitor. Nω is a highly specific nNOS inhibitor which does not bind the iNOS isoform and has approximately 1500 fold selectivity for nNOS over eNOS (15). Aminoguanidine is a highly specific iNOS inhibitor which has approximately 100 fold selectivity for iNOS over nNOS or eNOS (57). All NOS inhibitors were dissolved in Ringer’s solution, and each having a low molecular weight, were freely permeable through the microdialysis dialysis catheters (see below).

Testing was conducted in a temperature controlled room (approximately 25°C) at least four hours after a light breakfast. Experiments began after a 30 minute acclimatization period and all experiments were performed while the subject was supine and resting comfortably. We used laser-Doppler flowmeters (Perimed, Sweden) with integrating laser-Doppler flow-probes (Probe 413, Perimed, Stockholm) placed on the lateral aspect of the right calf to measure cutaneous blood flow (19). The laser Doppler flow (LDF) probes were surrounded by a heating collar which enabled localized heating of the area under the flow probe. Measurements were made in the leg because prior
experiments from our laboratory consistently indicate significant findings in the lower
limb in POTS (16; 48). Blood pressure was monitored by Finometer (TNO, Amsterdam)
calibrated by automated oscillometry. Heart rate was monitored by continuous EKG.
Continuous LDF data were collected at a sampling rate of 200 Hz. During experiments,
data were multiplexed and interfaced to a personal computer through an A/D converter
(DI-720, DATAQ industries, Milwaukee, Wi) using custom software which generated
binary files for all measurements while simultaneously displaying collected data on a
computer screen.

LDF measurements were made on the right calf while supine with the leg at the level of
the heart. Subjects were instrumented with 4 microdialysis catheters placed at least 6
cm apart inserted in the dermal space of the lateral aspect of the calf after gentle hair
removal. Each probe (MD-2000 Linear Microdialysis Probes, Bioanalytical Systems,
West Lafayette, IN) has a 10 mm microdialysis membrane section that is placed in the
intradermal space using a 25 gauge needle as an introducer. Catheters were randomly
designated 1 - 4.

Following placement, all catheters were initially perfused with Ringer’s solution at 2
µl/min. An integrating laser Doppler flow probe was placed directly over each
microdialysis catheter to measure cutaneous LDF. There is a hyperemia following
catheter insertion. LDF was recorded until values were similar to those measured over
the same area prior to catheter insertion. The return of LDF to pre-insertion values
usually occurred by 60-90 minutes. When necessary, longer times were allowed until
pre-insertion LDF was achieved.

Once baseline LDF values were reached all subjects received perfusate containing
Ringer’s solution through catheter 1 (control), 10 mM NLA through catheter 2, 10 mM
N$\omega$ through catheter 3, and 10mM AG through catheter 4 each perfused at 2 µl/min for
45 minutes during this drug run-in phase. When run-in was complete, the area under
each laser was heated at 1°C/10seconds to 42 °C for at least 40 minutes until a plateau
was reached. Perfusion with Ringer’s solution or NOS inhibitors continued throughout
heating. At the end of experiments we maintained heating and perfused all catheters
with 28mM sodium nitroprusside to obtain maximum vasodilation and to compute the
maximum cutaneous vascular conductance (CVC$_{max}$). CVC was calculated as the ratio
of LDF to mean arterial blood pressure. CVC$_{max}$ was defined as CVC during sodium
nitroprusside infusion. Experimental results were thereafter expressed as %CVC$_{max}$
(100*CVC/CVC$_{max}$).

Doses of 10 mM of NLA and 10mM of N$\omega$ were chosen because these were the
smallest concentrations of drugs that gave maximum suppression of the local heating
plateau (47). N$\omega$ has a selectivity (Ki ratios) for nNOS/eNOS of 1538 (15). At the dose
used, and assuming similar microdialysis membrane permeability and interstitial
diffusion, this is equivalent to 0.25% of the binding to eNOS and 4-fold greater binding
to nNOS compared to equimolar NLA. The dose of AG used (10 mM) was based on
pilot experiments (not shown) that determined the smallest dose required for a maximal effect. This was equimolar to the other NOS inhibitors used in the present study.

Experiment 2. The Effect of NOS Inhibitors on the Acetylcholine-Mediated Vasodilation

On another day experiments were performed in the same subjects of Experiment 1. Experiments were not conducted as classical “dose-response” and “inhibition” studies as derivation of pharmacological characteristics was not our intent. Rather, different doses of agonist (acetylcholine) alone and then combined with NOS inhibitors (NLA, $N\omega$ and AG) were used to elicit changes in skin blood flow responses thought to represent local signaling by NO. We anticipated that NO would not completely suppress the acetylcholine response because there are effects of other local vasoactive agents, such as prostaglandins and endothelium-derived hyperpolarizing factor, and the influence of local axon reflexes.

Subjects were instrumented with 4 microdialysis catheters and integrative laser Doppler probes as in Experiment 1. Following recovery from catheter insertion, baseline LDF values were obtained. Subjects then received perfusate containing Ringer’s solution through catheter 1 (control), 10 mM NLA through catheter 2, 10 mM $N\omega$ through catheter 3, and 10mM of AG through catheter 4, each perfused at 2 µl/min for 45 minutes during a drug run-in phase. When run-in was complete subjects received perfusate containing acetylcholine dissolved in Ringer solution containing identical NOS inhibitors (or Ringer alone) as received during run-in. Acetylcholine was perfused in ascending doses (0.01, 0.10, 1.0, 10, and 100mM) in combination with NOS inhibitors.
through each of catheter at 2 μl/min. The range of concentration of acetylcholine used (0.01-100 mM) was based upon previous determinations in human skin utilizing delivery of this agonist through microdialysis catheters (24; 41). LDF monitoring continued and each dose was administered for 20 minutes during which steady state values of LDF were achieved. For purposes of analysis, only the last 5 minutes of data were averaged at each acetylcholine dose.

At the end of experiments all catheters were perfused with 28mM sodium nitroprusside to obtain CVC\textsubscript{max}.

**Statistics**

We used two way analysis of variance (2x4) to compare the plateau phases of the local heating response treatment of POTS with control subjects receiving N\textsubscript{ω}, NLA, AG or Ringer’s solution in Experiment 1. We used two way analysis of variance with repeated measures to compare dose-response curves of acetylcholine alone (acetylcholine + Ringer), acetylcholine + NLA, acetylcholine + N\textsubscript{ω}, and acetylcholine + AG for POTS and control subjects. Results were calculated using SPSS (Statistical Package for the Social Sciences) software version 14.0. Apart from representative figures, text, graphic and Table 1 results are reported as mean ± standard error. Significance require P<0.05.
Results

As shown in Table 1, POTS and control subjects had similar height, weight, supine heart rate, systolic blood pressure, diastolic blood pressure, pulse pressure, calf blood flow, calf arterial resistance. The baseline LDF, the maximum LDF elicited with 28 mM sodium nitroprusside and the calculated baseline $\%CVC_{\text{max}}$ were the same in POTS and control subjects. Microdialysis of drugs had no effect on systemic hemodynamics (heart rate, arm and leg blood pressure) in any patient.

Experiment 1: The Effect of NOS Inhibitors on Local Heat Mediated Vasodilation

The Local Heat Response is increased in Normal Flow POTS

Figure 1 shows representative heating responses for a POTS patient and a control subject during local heating while perfused with Ringer’s solution (no NOS inhibition).

The values for $\%CVC_{\text{max}}$ of the heat-induced plateau measured in the POTS subjects were nearly equal to 100%, while that of control subjects was significantly less.

$NLA$, $N^\omega$ and AG have no effect on baseline Cutaneous Blood Flow

As shown in Figure 2, and as previously reported for NLA (51) and $N^\omega$ (46), inhibition of NOS had no effect on the baseline cutaneous bloodflow compared to Ringer’s alone when measured prior to the application of local heat. This was also found for AG, which had no effect on baseline flow, compared to that measured with Ringer’s alone. When testing each of these NOS inhibitors, there was also no difference between baseline flows comparing control to POTS subjects.
NLA and $N^\omega$ are Equipotent Inhibitors of Local Heating in Normal Flow POTS and Control. AG has no Effect on the Local heating Response

Figure 2 shows heat responses averaged over all subjects for each NOS inhibitor in POTS and control subjects. On average the plateau phase conductance while receiving Ringer’s solution was $88\pm2 \%CVC_{\text{max}}$ for control compared to $98\pm1 \%CVC_{\text{max}}$ for normal flow POTS ($P<.01$); the plateau phase conductance while receiving NLA was $47\pm4 \%CVC_{\text{max}}$ for control compared to $45\pm3 \%CVC_{\text{max}}$ for normal flow POTS; the plateau phase conductance while receiving $N^\omega$ was $49\pm4 \%CVC_{\text{max}}$ for control compared to $46\pm5 \%CVC_{\text{max}}$ for normal flow POTS; and the plateau phase conductance while receiving AG was $86\pm2 \%CVC_{\text{max}}$ for control compared to $97\pm2 \%CVC_{\text{max}}$ for normal flow POTS ($P<0.025$).

Conductance was significantly increased during local heating in normal flow POTS and this increase was unaffected by AG. NLA and $N^\omega$ reduced the plateau conductance during local heating to a similar degree. During the perfusion of Ringer alone, the plateau conductance in normal flow POTS was larger than the plateau conductance in control subjects. Consequently, perfusion with either NLA or $N^\omega$ reduced the NO sensitive plateau by a larger amount in normal flow POTS compared to control subjects. $N^\omega$ is as effective as NLA in blunting the hyperemia of local heating in both normal flow POTS and control. AG has no effect on any phase of the heat response.
Experiment 2. The Effect of NOS Inhibitors on the Acetylcholine-Mediated Vasodilation

The Dose-Response to Acetylcholine is Increased in Normal Flow POTS

Figure 3 shows data averaged over all normal flow POTS subjects, and over all control subjects. Data showing the effect of acetylcholine dissolved in Ringer’s solution and free of NOS inhibitors is shown in the left upper panel. The ability of acetylcholine to increase cutaneous blood flow is significantly enhanced in POTS compared to control subjects (P<0.001).

The Dose-Response to Acetylcholine is Decreased by NLA but not N\textsubscript{\textomega} or AG in both Normal Flow POTS and Control Subjects

Figure 3 also demonstrates that NLA significantly (P<.0001) reduces the response to acetylcholine in both POTS and control subjects on the order of 50%. However, there was no significant difference in %CVCmax between control and POTS subjects when acetylcholine was administered in the presence of NLA. Consequently, perfusion with NLA reduced the response by a larger amount in POTS compared to control subjects.

There were no effects of selective nNOS and iNOS inhibitors on the acetylcholine dose-response. There were large reductions of non-isoform selective NOS inhibition with NLA on the acetylcholine dose-response.
Discussion

Summary and Discussion of Findings

Our main findings are that cutaneous nNOS and eNOS mediated production of NO are both increased in normal flow POTS patients compared to control subjects.

Experiment 1

nNOS Activity is Increased in Normal Flow POTS

Administration of a non-selective NOS inhibitor blunts the NO-dependent plateau of the local heating response. A selective nNOS inhibitor is equally effective in blunting this response at a dose that should exert a minimal effect on eNOS. AG has no effect on local heating indicating a lack of influence of iNOS under these experimental conditions. These findings indicate that the local heating plateau can be used as a bioassay for nNOS activity. The local heating response is enhanced in normal flow POTS compared to control subjects, reaching conductances close to CVC$_{\text{max}}$. This suggests that there is increased NO derived from nNOS in normal flow POTS.

The dependence of local heating response on nNOS is controversial. Kellogg has maintained that the local heating response is dependent on eNOS rather than nNOS. Those conclusions were based on observations made using 7-NI as a selective nNOS inhibitor and NNA as a selective eNOS inhibitor (22). However, 7-NI has no selectivity for nNOS in vitro (The IC$_{50}$ values for inhibition of nNOS, and eNOS are 0.71, 0.78 µM), and exhibits modest selectivity in vivo. L-NNA inhibits nNOS with K$_i$ values of 15 nM, and eNOS with K$_i$ value of 39 nM and cannot be regarded as eNOS selective.
Experiment 2

eNOS Activity is Increased in Normal Flow POTS

Our past work showed that acetylcholine increases cutaneous blood flow and that this increase is due, in part to nitric oxide (47). Our current results are similar and thus support the association between increased blood flow and NO. While NLA does not completely eliminate the response to increasing doses of acetylcholine, it attenuates this increase by some 50% (Figure 3).

Administration of a sufficient amount of the non-selective NOS inhibitor NLA blunts the response to acetylcholine. Neither the selective nNOS inhibitor Nω nor the selective iNOS inhibitor AG affects this acetylcholine-mediated response. These findings indicate that the blunting of the heat response by NLA is an indicator of local eNOS activity. The response to acetylcholine is enhanced in normal flow POTS compared to control subjects. This suggests that there is increased NO of endothelial origin in normal flow POTS. Other investigators have reported an increase in endothelial NO production during intradermal acetylcholine administration (5). In the current work we found no effect of Nω at any dose of acetylcholine used.

Significance of Increased Bioavailable Constitutive NO
Systemic illnesses typically have cutaneous manifestations. In particular, prior cutaneous findings in other variants of POTS (50) have been substantiated by systemic measurements (30). The skin is therefore a useful surrogate of the microvasculature that is likely reflective of systemic disease (14) in POTS.

Both neuronal and endothelial NOS are expressed constitutively (8), and require calcium-calmodulin for activation. nNOS was first discovered in the brain and therefore was initially associated with neurons, while eNOS was first discovered in endothelial cells. However, nNOS and eNOS have been found in diverse tissues. Thus, for example, nNOS has been found in keratinocytes (3), cardiac, skeletal muscle, splanchnic, renal and neurovascular tissue (6; 7; 18; 20) while eNOS has been found in every organ containing endothelium. nNOS has been shown to be important in the regulation of human basal forearm microvascular and coronary blood flow (39; 40).

Single nucleotide polymorphisms of eNOS have been explored in POTS and seem to be loosely related to disease (10), although studies did not subgroup patients on the basis of vascular physiology. Similar studies for nNOS single nucleotide polymorphisms and POTS have not yet been performed.

However, genetic differences are not necessary to alter expression of constitutive NOS as evidenced in hepatic cirrhosis where both nNOS and eNOS isoforms are increased and sustain NO-mediated vasodilation and the hyperdynamic circulation (35). Changes in the expression of nNOS and eNOS have been reported following conditions of
simulated microgravity and occur in both bed-rested humans and the rat hindlimb suspension models and are associated with splanchnic dilation (2; 56) and orthostatic intolerance (2; 54).

Increased NO can contribute to peripheral vasodilation through cGMP-mediated vasodilation (37), through its blunting of central sympathetic nerve activity (25), and through peripheral modulation of sympathetic neurotransmission. Modulated sympathetic neurotransmission may result from inhibitory effects of NO on the release of norepinephrine (11) chemical deactivation of norepinephrine by NO reducing its activity (23) or both. Of particular interest are the effects of nNOS on the central sympathetic nervous system and on nitrergic nerves innervating mesenteric vasculature (13; 34). Nitrergic nerves contain nNOS and are distributed with cholinergic parasympathetic nerves. nNOS may even colocalize with acetylcholine and VIP in the perivascular parasympathetic fibers (33), and modulate vascular tone and blood flow (31; 55).

**An Interpretation for the Mechanistic Pathophysiology of Normal Flow POTS Patients**

Data from these and other experiments can tentatively provide an explanation of the pathophysiology of normal flow POTS. Our previous data has shown an increase in splanchnic blood flow and splanchnic blood volume (45; 49) in normal flow POTS and the current data supports an increase in neuronal and endothelial nitric oxide. Globally Increased neuronal nitric oxide might act to reduce sympathoexcitation both supine and
upright, but there is no clinical evidence for a reduction of sympathetic nerve activity in POTS (4). Therefore the results are most consistent with interference with peripheral adrenergic vasoconstriction. This again is based on the assumption that results obtained in skin are reflective of microvascular function elsewhere. As we have reported previously, reduced vasoconstriction in normal flow POTS seems limited to the splanchnic circulation while patients are in an upright position. How adrenergic hyporeactivity may be limited by posture to regional vasculature remains to be verified in future systemic experiments.

**Summary**

We used laser Doppler flowmetry to measure the cutaneous response to local heating as an indicator of bioavailable nNO. The acetylcholine dose-response and the NO-dependent plateau of the local heating response were increased in POTS compared to control subjects. The local heating plateau was significantly higher in POTS compared to control, but decreased to the same level with \( N^\omega \) or with NLA. Only NLA blunted the acetylcholine dose-response indicating that NO produced by endothelial NOS was released by acetylcholine. This suggests increased endothelial and neuronal NOS activity in normal flow POTS subjects.

**Limitations**
We studied the cutaneous circulation which has unique autonomic control. Our recent work indicates that flow regulation abnormalities in POTS occur throughout the circulatory system (49) and that the flow abnormalities that occur in skin may be used as representation of systemic diseases (14).

We studied the calf cutaneous circulation. While flow abnormalities are widespread in POTS, whenever peripheral blood flows are studied, the most significant results occur in the lower extremities. It may be that dependence and gravitational exposure is important to observed findings. There may be inherent differences in forearm versus calf cutaneous microvascular control that may explain the role of various NOS isoforms thought to be responsible the mediation of the local heat response (22; 46).

While L-Nω-Nitroarginine-2,4-L-diamino-butyric amide has well documented in vitro selectivity for nNOS over eNOS (15), in vivo selectivity has not been well established. Thus there could be additional actions of this agent on choline transport, acetylcholinesterase, butyrocholinesterase, muscarinic, prostaglandin or NO mechanisms other than its effects of nitric oxide synthases.

We studied females without regard to menstrual cycle. The phase of the menstrual cycle can exert important effects on nitric oxide dependent mechanisms (1). Recent evidence suggests a relationship between phases of the menstrual cycle and changes in POTS symptoms and signs during a 2 hour stand due to altered response of the renal-angiotensin-aldosterone system that affects hemodynamics during orthostasis (9).
Since our determinations are made while the subject is supine it is unlikely that hormone-related differences play a major role in the response of skin to local heat, however this issue remains a concern.
Grants

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Disclosures

The authors have nothing to disclose to the APS Publications Office concerning any potential conflict of interest (e.g., consultancies, stock ownership, equity interests, patent-licensing arrangements, lack of access to data, or lack of control of the decision to publish).


48. **Stewart JM, Medow MS and Montgomery LD**. Local vascular responses affecting blood flow in postural tachycardia syndrome

49. Stewart JM and Montgomery LD. Regional blood volume and peripheral blood flow in postural tachycardia syndrome


Table 1 Dimensions and Supine Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Control (N=12)</th>
<th>POTS (N=14)</th>
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<td><strong>Age</strong> (years)</td>
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<td><strong>Weight</strong> (kg)</td>
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<td><strong>Supine HR (beats/min)</strong></td>
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<td><strong>Diastolic Systolic BP (mmHg)</strong></td>
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<td><strong>Pulse Pressure</strong> (mmHg)</td>
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<td><strong>Venous Occlusion Calf Blood Flow</strong> (ml/100ml/min)</td>
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<td>2.3±0.2</td>
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<tr>
<td><strong>Calf Arterial Resistance</strong> (ml/100ml/min/mmHg)</td>
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<td><strong>Maximum Laser Doppler Flow with Sodium Nitroprusside</strong> (pfu)</td>
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<td><strong>Baseline Laser Doppler Flow</strong> (pfu)</td>
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<td><strong>Baseline %CVCmax</strong></td>
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<td>11±1.3</td>
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%CVC = percent cutaneous vascular conductance.
1) The figure shows the local heating response in a representative normal flow POTS patient shown in gray, and in a healthy volunteer control subject shown in black. Key points along the curve are marked. The heat response plateau, dependent on bioavailable NO, is near maximum vasodilation in POTS patients, while the response in control subjects is significantly smaller.

2) The figure shows the local heating responses averaged over all POTS patients and all control subjects. Results are shown when the NOS inhibitors NLA, Nω, AG dissolved in Ringer’s solution are given and when only Ringer’s solution is given. POTS is shown in gray while control subjects are shown in black. The NO dependent plateau is larger than control in POTS (left upper panel). The plateau response is equally inhibited by the non-isoform specific NOS inhibitor NLA (right upper panel) and by the n-NOS specific NOS inhibitor Nω. (left lower panel). AG (right lower panel) has no effect on the heating response and results are similar to those using Ringer’s solution alone.

3) The figure shows the dose-response to logarithmic increases in perfused acetylcholine averaged over all POTS patients and all control subjects. Acetylcholine is perfused in combination with Ringer solution only or in combination with Ringer solution containing dissolved NOS inhibitors NLA, Nω, AG. POTS is shown in gray while control subjects are shown in black. Results for acetylcholine plus Ringer solution are shown as solid lines and are present in each panel for comparison with the NOS inhibitor results shown as dashed lines. POTS increases the response to acetylcholine compared to
control (left upper panel). Both POTS and control responses are similarly blunted by the addition of NLA but by neither N\(^\omega\) nor AG.