

Defects in Cutaneous Angiotensin-Converting Enzyme 2 and Angiotensin-(1-7) Production in Postural Tachycardia Syndrome

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Abstract—Postural tachycardia syndrome (POTS) is associated with increased plasma angiotensin II (Ang II). Ang II administered in the presence of NO synthase inhibition with nitro-L-arginine (NLA) and Ang II type 1 receptor blockade with losartan produces vasodilation during local heating in controls. We tested whether this angiotensin-mediated vasodilation occurs in POTS and whether it is related to angiotensin-converting enzyme 2 (ACE2) and Ang-(1-7). We used local cutaneous heating to 42°C and laser Doppler Flowmetry to assess NO-dependent conductance at 4 calf sites in 12 low-flow POTS and in 12 control subjects 17.6 to 25.5 years of age. We perfused Ringer's solution through intradermal microdialysis catheters and performed local heating. We perfused one catheter with NLA (10 mmol/L)+losartan (2 µg/L) and repeated heating, and NLA+losartan+Ang II (10 µmol/L), repeating heating a third time. A second catheter received NLA+losartan+Ang II, heated, perfused NLA+losartan+Ang II+DX600 (1 mmol/L; a selective ACE2 inhibitor), and reheated. A third catheter received NLA+losartan+Ang II, heated, perfused NLA+losartan+Ang II+Ang-(1-7) (100 µmol/L), and reheated. The fourth catheter received Ang-(1-7) then reheated a second time only. Angiotensin-mediated vasodilation was present in control but not POTS. Ang-mediated dilation was eliminated by DX600, indicating an ACE2-related effect. Ang-mediated vasodilation was restored in POTS by Ang-(1-7). When administered alone during locally mediated heating, Ang-(1-7) improved the NO-dependent local heating response. ACE2 effects are blunted in low-flow POTS and restored by the ACE2 product Ang-(1-7). Data imply impaired catabolism of Ang II through the ACE2 pathway. Vasoconstriction in POTS may result from a reduction in Ang-(1-7) and an increase in Ang II. (*Hypertension*. 2009;53:767-774.)

Key Words: angiotensin ■ lasers ■ autonomic nervous system

Chronic orthostatic intolerance is related to postural tachycardia syndrome (POTS).¹⁻⁵ POTS is defined by excessively increased heart rate during orthostatic challenge associated with symptoms of orthostatic intolerance,³ including dizziness, exercise intolerance, headache, fatigue, memory problems, nausea, blurred vision, pallor, and sweating, all of which improve with recumbence. Findings have been ascribed to increased sympathetic activity.^{6,7} We described a subset of low-flow POTS, in which marked upright tachycardia is associated with supine pallor, acrocyanosis, and hypovolemia, tachycardia, decreased cardiac output, and increased peripheral resistance.⁸ We observed increased plasma angiotensin II (Ang II)^{9,10} not accounted for by changes in angiotensinogen, renin, or angiotensin-converting enzyme (ACE). Increased Ang II and decreased NO increase central^{11,12} and peripheral sympathetic activity.^{13,14}

Recently, we investigated a model of Ang II–NAD/NADPH oxidase superoxide production, which scavenges NO, reducing its bioavailability.^{15,16} Experiments made extensive use of the vasodilation response of nonglabrous skin

to local heating,^{17,18} which has 3 distinct phases: an initial peak, a nadir, and an increase to a plateau. All phases, especially the plateau phase, are NO dependent.^{17,18} The plateau has been used to test for bioavailability of NO during intradermal microdialysis.¹⁹⁻²¹ The local heating response is blunted in low-flow POTS,²² and blunting is reversed by Ang II type 1 receptor (AT1R) blockade.²⁰

We studied intradermal perfusion of exogenous Ang II in healthy volunteers to replicate blunted vasodilation to local heating.²² Perfusion of Ang II in the presence of NO synthase inhibition and AT1R blockade caused vasodilation during local heating (Figure 1). We denote this response as angiotensin-mediated vasodilation. We previously showed that angiotensin-mediated vasodilation was unrelated to AT2R stimulation.²³ We hypothesized that the carboxydipeptidase ACE2 could account for angiotensin-mediated vasodilation. ACE2 is the main catabolic enzyme for Ang II, synthesizing the heptapeptide Mas receptor agonist Ang-(1-7).²⁴ A deficit in ACE2 might account for increased Ang II and decreased local heating response in POTS.

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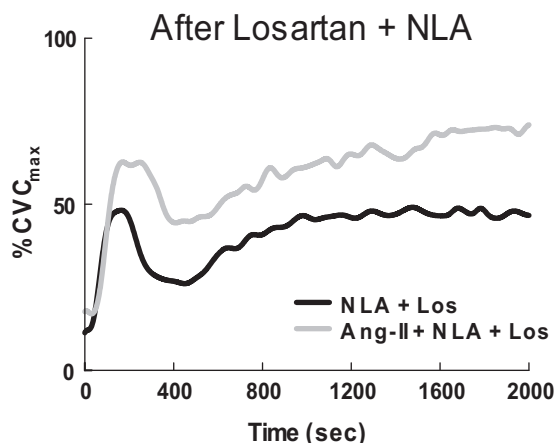


Figure 1. Heating response expressed as %CVC_{max} after administration of NLA+losartan (Los) and after addition of Ang II+NLA+Los averaged over all healthy control subjects. Addition of Ang II to NLA+Los produces a relative vasodilation during local heating.

Therefore, the current study was designed to explore the following aims: (1) to test whether angiotensin-mediated vasodilation is present in POTS; (2) to test whether ACE2 is involved in angiotensin-mediated vasodilation in control subjects and POTS patients; (3) to test whether perfusion with Ang-(1-7) helps to restore angiotensin-mediated vasodilation in low-flow POTS; and (4) to test whether perfusion with Ang-(1-7) helps to restore the local heating response in low-flow POTS.

Methods

Subjects

We studied the effects of Ang II, NO synthase inhibition, and AT1R blockade, ACE2 blockade and Ang-(1-7) administration on the local heating response in 12 healthy volunteers 18.1 to 25.5 years of age (median age 21.1 years; 3 males and 9 females) and in 12 low-flow POTS patients 17.6 to 24.5 years of age (2 males and 10 females). Subjects were excluded as controls for a history of orthostatic intolerance. All subjects were free from cutaneous, systemic, and cardiovascular diseases, were not taking medications, and had refrained from alcohol and caffeinated beverages for ≥ 24 hours before study. There were no smokers or trained competitive athletes. There were no bed-rested subjects. POTS patients were referred for chronic orthostatic intolerance lasting ≥ 3 months. Symptoms included day-to-day dizziness, exercise intolerance, headache, fatigue, memory problems, nausea, blurred vision, pallor, and abnormal sweating while upright, which was relieved by recumbence. The diagnosis of POTS was made during a 10-minute screening 70° upright. POTS was diagnosed when symptoms of orthostatic intolerance were associated with an increase in heart rate exceeding 30 bpm or to a rate exceeding 120 bpm during 10 minutes of tilt.^{4,25} POTS patients were subgrouped by supine calf blood flow measured with venous occlusion plethysmography²⁶ into low-flow POTS with decreased blood flow and non-low-flow POTS.^{8,27} Patients were retained if they belonged to the low-flow POTS group. Female subjects were enrolled without regard to the phase of their menstrual cycle.

Protocol

General

Experiments were performed during 1 day. Four microdialysis catheters were placed to infuse drugs locally into the intradermal space of the leg. Before microdialysis catheter insertion, laser

Doppler flow (LDF) was measured over each of the 4 insertion sites to estimate baseline flows for later use in determining recovery from catheter insertion. Laser probes were removed, and 4 microdialysis catheters were inserted. After recovery, LDF was measured for 10 minutes while perfusing lactated Ringer's solution and again during local heating at each site. Subjects completely recovered from heating for 30 to 60 minutes until the preheat baseline flow was achieved. Thereafter, catheters were sequentially perfused with vasoactive drugs for 40 minutes, local heating repeated, heat recovery repeated, and catheters 1 through 3 were perfused with additional drugs for 40 minutes, and local heating repeated a third time. At the end of experiments, all catheters were perfused with 28 mmol/L sodium nitroprusside for the determination of maximum cutaneous vascular conductance (CVC).²⁸ All chemicals were highly purified to United States Pharmacopeia standards and were produced under sterile conditions. The catheters were sterilized. Microdialysis membranes act as micropore filters, preventing any possibility of infection. Informed consent was obtained. The institutional review board of New York Medical College approved all protocols.

To determine whether angiotensin-mediated vasodilation is present in POTS, we perfused the first catheter with the AT1R antagonist losartan combined with the nonisoform-specific NO synthase inhibitor nitro-L-arginine (NLA) and repeated local heating. After recovery from the second heating response, we perfused the same catheter with losartan+NLA+Ang II and repeated local heating a third time.

To test whether ACE2 is involved in the angiotensin-mediated vasodilation in control subjects and POTS patients, we perfused the second catheter with losartan+NLA+Ang II and repeated local heating. After recovery, we perfused the catheter with losartan+NLA+Ang II to which the specific ACE2 inhibitor DX600 was added and repeated local heating repeated a third time.

To test whether perfusion with Ang-(1-7) restores angiotensin-mediated vasodilation in POTS, we perfused the third catheter with losartan+NLA+Ang II and repeated local heating. After recovery, we perfused the catheter with losartan+NLA+Ang II, to which Ang-(1-7) was added, and repeated local heating a third time.

To test whether perfusion with Ang-(1-7) restores the local heating response in POTS, we studied whether Ang-(1-7) alleviates blunting of the local heating response. Therefore, we perfused the fourth catheter with Ang-(1-7) and repeated local heating a second time only.

Use of Heat-Reheat Assessment

We used a heat-reheat scheme in which the heating response was assumed unaffected by time or repeat measurements. We verified this assumption in studies showing that intracatheter differences of plateau phase measurements using heat-reheat are far smaller than intercatheter differences of local heating plateaus in a given subject.¹⁰

Instrumentation

All testing was conducted in a temperature-controlled room ($\approx 25^\circ\text{C}$) at least 2 hours after a light breakfast. Supine subjects were instrumented in the dermal space of the lateral aspect of the left calf after hair was gently removed. The leg was always at heart level. Microdialysis insertion sites were cooled with ice packs before insertion to reduce discomfort. Each probe (MD-2000 Linear Microdialysis Probes; Bioanalytical Systems) has a 10-mm microdialysis membrane section that is placed in the intradermal space using a 25-gauge needle as an introducer. Catheters were designated randomly. The molecular weight cutoff is nominally 10 000 Da.

After placement, catheters were initially perfused with Ringer's solution at $2 \mu\text{L}/\text{min}$. A 7-element integrating LDF probe (Probe 413; Perimed) was placed directly over the center of each microdialysis catheter to measure LDF. LDF was thereafter recorded until values returned to preinsertion baseline, which indicated recovery from catheter placement trauma and usually occurred by 60 to 90 minutes.²⁹

Drug Infusions

Once returned to baseline, subjects received perfusate containing lactated Ringer's solution in all catheters, and local heating was performed. After recovery from local heating, $2 \mu\text{g}/\text{L}$ losartan + $10 \text{ mmol}/\text{L}$ NLA was perfused through the first catheter; $2 \mu\text{g}/\text{L}$

Table 1. Dimensions and Supine Hemodynamics

Quantity Measured	Control Subjects (n=12)	POTS Patients (n=12)
Age, y	21.1±1.5	20.7±1.1
Weight, kg	63±2	55±3*
Height, cm	168±3	168±3
Body mass index, kg/m ²	23.1±0.8	19.0±0.8
Supine heart rate, bp	63±3	84±4*
Supine systolic blood pressure, mm Hg	118±3	112±5
Diastolic systolic blood pressure, mm Hg	62±4	73±4
Pulse pressure, mm Hg	55±4	42±3*
Venous occlusion calf blood flow, mL/100 mL per min	2.4±0.3	0.80±0.11*
Calf arterial resistance, mL/100 mL/min per mm Hg	32±7	79±9*
Maximum LDF with sodium nitroprusside averaged, perfusion units	215±15	198±21
Resting LDF averaged, perfusion units	20.1±1.0	12.3±2.6*
Resting %CVC _{max} averaged	12.0±1.0	6.7±1.1*

LDFs and %CVC_{max} are averaged over all catheters and subjects in control and POTS groups.

**P*<0.05 different from control subjects.

losartan+10 mmol/L NLA+10 μmol/L Ang II was perfused through the second and third catheters; and 100 μmol/L Ang-(1-7) was perfused through the fourth catheter. All drugs were dissolved in lactated Ringer's solution and were perfused for ≥40 minutes. Heating was repeated. After recovery from local heating, 2 μg/L losartan+10 mmol/L NLA+10 μmol/L Ang II was perfused through the first catheter; 2 μg/L losartan+10 mmol/L NLA+10 μmol/L Ang II+1 mmol/L DX600 was perfused through the second catheter, and 2 μg/L losartan+10 mmol/L NLA+10 μmol/L Ang II+100 μmol/L Ang-(1-7) was perfused through the third catheter.

Doses of NLA and Ang II were chosen on the basis of pilot studies showing this to be the minimum concentration yielding maximum attenuation of the NO-dependent local heating plateau.^{17,18} Losartan (2 μg/L) was chosen based on previous human testing.³⁰ A 100-μmol/L dose of Ang-(1-7) was chosen based on pilot studies showing this to be the lowest concentration needed to restore the angiotensin-mediated vasodilation in POTS patients. DX600 (1 mmol/L) was chosen as the lowest concentration that suppressed the angiotensin-mediated vasodilation in control subjects.

Local Heating

Once baseline LDF values were obtained, the areas under each laser were gradually heated at 1°C/10 s to 42°C until a plateau was reached. Heat was turned off to allow for recovery to baseline LDF. Investigators^{17,18} demonstrated that the first heating peak is mediated by neurogenic reflexes, NO, and neuropeptides.^{31,32} The first peak is followed by a nadir and then an NO-dependent plateau, which is blunted by NO synthase inhibition.

Monitoring

Heart rate was monitored by electrocardiography, and right extremity blood pressure was measured by finger plethysmography (Finometer), intermittently recalibrated against oscillometry. Mean arterial pressure was obtained by averaging the signal over 5 minutes and compared with oscillometry (using the formula mean arterial pressure=(systemic arterial pressure+2×diastolic arterial pressure)/3). Finometer and oscillometric blood pressure were in agreement.

Data and Statistical Analysis

LDF was measured in arbitrary perfusion units. Continuous LDF data were sampled at 200 Hz. LDF data were multiplexed and interfaced to

Table 2. Heat Responses (%CVC_{max}) Catheter 1

Heat Event	Medication	Control Subjects (n=12)	POTS Patients (n=12)
Baseline	Before drugs	10±1	6±1*
	NLA+losartan	12±4	7±2
	NLA+losartan+Ang II	15±3	8±1*
First thermal peak	Before drugs	75±9	49±4*
	NLA+losartan	50±5†	49±6
	NLA+losartan+Ang II	63±4	48±4*
Nadir	Before drugs	48±5	34±4*
	NLA+losartan	27±4†	29±5
	NLA+losartan+Ang II	48±4†	28±3*
Plateau	Before drugs	92±6	51±5*
	NLA+losartan	48±4†	48±4
	NLA+losartan+Ang II	73±4†	49±5*

**P*<0.05 different from control subjects; †*P*<0.05 different from previous drug combination or before drugs.

a personal computer through an A/D converter (DI-720; DATAQ Industries) using custom acquisition software. LDF data were converted to units of CVC by dividing by the mean arterial pressure. CVC measurements were then converted to percent maximum conductance (%CVC_{max}) by dividing CVC by the CVC_{max} achieved after the administration of 28 mmol/L sodium nitroprusside at the end of experiments. This fraction was converted to a percentile by multiplying by 100.

Changes in baseline LDF before and after drugs were compared by 2-way ANOVA. Results are reported as mean±SE. Comparisons were made by repeated-measures ANOVA to look at differences in the local heating response between predrug and postdrug infusion using the particular microdialysis catheter as the within factor. We also compared postdrug responses to NLA+losartan with responses to Ang II+losartan+NLA and compared postdrug responses to Ang II+losartan+NLA with postdrug responses to Ang II+losartan+NLA+DX600 and to Ang II+losartan+NLA+Ang-(1-7) using catheters as the between factor and subjects as the within factor. Graphical representations comparing POTS and control subject data were obtained by averaging heat responses over all local heating curves for all subjects. All averaged numeric data±SE at baseline, first thermal peak, nadir, and plateau are tabulated in Tables 2 through 5. Results were calculated using SPSS software version 11.0. The minimum value for α was <0.05.

Results

Dimensions and Hemodynamic Data

Data are shown in Table 1. POTS patients had reduced body mass index (*P*<0.025) because they weighed less than control subjects (*P*<0.025). Supine heart rate was increased in POTS (*P*<0.01). Pulse pressure was reduced in POTS (*P*<0.025). Systolic and diastolic pressures were not different. Upright heart rate was increased, and calf blood flow was decreased in POTS by definition. Calf arterial resistance was increased in POTS patients (*P*<0.001). Resting LDF (perfusion units) and resting %CVC_{max} were decreased in POTS compared with control subjects (*P*<0.025). There was no difference in maximum LDF.

Tables 2 through 5 show results from individual catheter experiments. We compared the first peak, the nadir, and the plateau phase before and after drug treatments. Results are also depicted in Figures 2 through 5, which show averages over all POTS patients and control subjects.

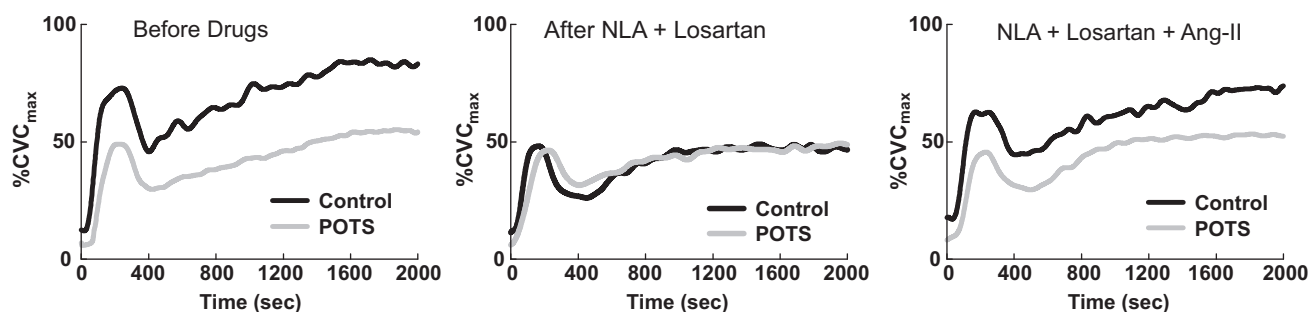


Figure 2. Heating response expressed as %CVC_{max} before (left panel) and after administration of NLA+losartan (middle panel) and NLA+losartan+Ang II (right panel) averaged over all control subjects and all POTS patients. The middle panel demonstrates that the heating response is significantly attenuated in control subjects by NLA+losartan with only a small effect on POTS patients. The right panel shows that addition of Ang II increases the heating response in control subjects but not in POTS patients.

Is Angiotensin-Mediated Vasodilation Present in POTS?

As shown in Table 2 and Figure 2, %CVC_{max} of the predrug baseline ($P<0.01$), the first peak ($P<0.025$), the nadir ($P<0.05$), and the plateau phase ($P<0.001$) were decreased in POTS compared with control subjects. After NLA+losartan was administered, control heating responses were decreased ($P<0.025$ first peak; $P<0.01$ nadir; $P<0.001$ plateau) compared with before drugs. POTS and control heating responses became similar. After Ang II was added, all heated phases increased in healthy control subjects ($P<0.05$ first peak; $P<0.01$ nadir; $P<0.01$ plateau) but not in POTS patients, for whom all heating phases remained less than for control subjects. Thus, an increase in NO- and AT1R-independent angiotensin-mediated vasodilation with local heating (angiotensin-mediated vasodilation) occurs in control subjects but not in POTS patients.

Is ACE2 Involved in the Angiotensin-Mediated Vasodilation in Control Subjects?

The Response to DX600

Table 3 and Figure 3 compare the response to DX600 after administration of NLA+losartan+Ang II. The addition of DX600 produced a significant decrease in the control heating response, with decreased first peak ($P<0.05$), nadir ($P<0.001$), and plateau ($P<0.001$). There was no significant

change in the heating response for POTS. After addition of DX600, POTS and control heating responses were similar, with no significant differences observed during any phase of heating. Thus, ACE2 inhibition causes a decrease in angiotensin-mediated vasodilation in control but not in POTS subjects.

Does Ang-(1-7) Restore Angiotensin-Mediated Vasodilation in Low-Flow POTS?

Table 4 and Figure 4 compare the response to Ang-(1-7) after administration of NLA+losartan+Ang II. The combination of NLA+losartan+Ang II produced a decrease in plateau flow for control subjects ($P<0.025$). The addition of Ang-(1-7) produced a significant increase in the POTS heating response, with increased first peak ($P<0.01$), nadir ($P<0.05$), and plateau ($P<0.025$). There was no significant change in the control subjects; POTS and control subject heating responses were not different after Ang-(1-7) was added. Ang-(1-7) significantly increases angiotensin-mediated vasodilation in POTS but not in control subjects.

Does Perfusion With Ang-(1-7) Help to Restore the Local Heating Response in Low-Flow POTS?

Table 5 and Figure 5 compare the response to Ang-(1-7) during a simple local heating experiment in control and POTS subjects. The addition of Ang-(1-7) produced a small nonsig-

Table 3. Heat Responses (%CVC_{max}) Catheter 2

Heat Event	Medication	Control Subjects (n=12)	POTS Patients (n=12)
Baseline	Before drugs	12±2	7±2
	NLA+losartan+Ang II	20±3†	14±5†
	NLA+losartan+Ang II+DX600	13±1†	12±1
First thermal peak	Before drugs	72±7	48±6*
	NLA+losartan+Ang II	66±5	44±5*
	NLA+losartan+Ang II+DX600	50±3†	49±2
Nadir	Before drugs	54±5	38±4*
	NLA+losartan+Ang II	49±2	33±4*
	NLA+losartan+Ang II+DX600	33±2†	33±3
Plateau	Before drugs	89±8	48±4*
	NLA+losartan+Ang II	71±2†	44±3*
	NLA+losartan+Ang II+DX600	52±3†	48±4

* $P<0.05$ different from control subjects; † $P<0.05$ different from previous drug combination or before drugs.

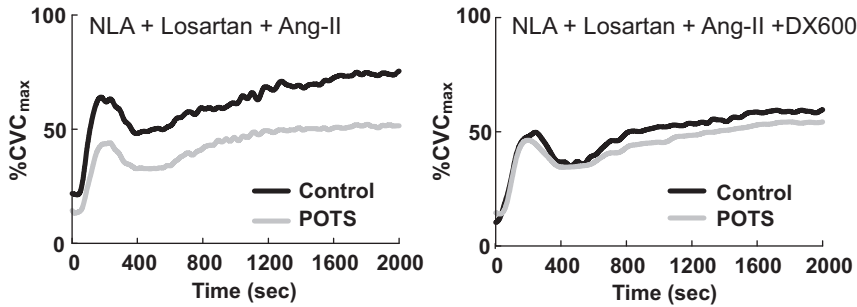


Figure 3. Comparison of heating responses expressed as %CVC_{max} after administration of NLA+losartan+Ang II (left panel) and after DX600 has been added to yield NLA+losartan+Ang II+DX600 (right panel) in the perfusate. Data are averaged over all control subjects and all POTS patients. Addition of DX600 causes a reduction of the local heating response in control subjects. No significant change occurs in POTS patients.

nificant increase in the control heating response. On the other hand, the baseline ($P<0.05$), nadir ($P<0.001$) and plateau phase ($P<0.001$) of POTS patients increased significantly. POTS and control subject heating responses were similar after Ang-(1-7) was added, although there remained a small difference in the plateau phase ($P<0.05$). Thus, Ang-(1-7) significantly increases the local heating response in POTS but not in control subjects.

Discussion

We studied the low-flow POTS subgroup using the cutaneous microcirculation to investigate potential angiotensin-related defects. Our results suggest a deficit in ACE2 resulting in impaired metabolism of Ang II, producing increased local concentrations of Ang II and decreased concentrations of Ang-(1-7) and contributing to excessive vasoconstriction characteristic of this disorder. These conclusions are based on the following findings.

The first aim of this study was to determine whether NO- and AT1R-independent angiotensin-mediated vasodilation with local heating (angiotensin-mediated vasodilation) is present in POTS. The data in Table 2 and in Figure 2 show no effect of addition of Ang II to NLA+losartan in POTS, whereas there is a significant effect in control subjects. We reasoned that the absent response may help explain angiotensin-related abnormalities in POTS.

Past work indicated that cutaneous angiotensin-mediated vasodilation was unrelated to AT2R stimulation. Our second aim was to test whether ACE2 could be involved. Results

shown in Table 3 and Figure 3 demonstrate that cutaneous ACE2 inhibition eliminates the angiotensin-mediated vasodilation in control subjects but not in POTS patients. The results of aims 1 and 2 suggest that angiotensin-mediated vasodilation is produced by cutaneous ACE2 in healthy control subjects and that the healthy response is absent in POTS. ACE2 metabolizes Ang II, producing Ang-(1-7).²⁴ ACE2 also produces Ang-(1-7) via Ang-(1-9) by the enzymatic degradation of Ang I.³³ The data imply a deficit of ACE2. If generalized, this could account for increased Ang II and reduced Ang-(1-7) in POTS patients.

Data from aims 1 and 2 predict aim 3: that restoring Ang-(1-7) with exogenous Ang-(1-7) could enhance angiotensin-mediated vasodilation with local heating in POTS. Data in Table 4 and Figure 4 demonstrate restoration of angiotensin-mediated vasodilation with local heating in POTS once Ang-(1-7) is perfused without significant effect in control subjects.

Aim 4 reinforces the importance of Ang-(1-7) and ACE2 because Ang-(1-7) is able to improve the blunted heating response in POTS. The data support previous observations of cutaneous NO deficiency in POTS¹⁰ caused by excessive Ang II.²⁰

ACE2 has assumed increasing importance as an enzyme that converts Ang I to Ang-(1-9), which is thereafter converted to Ang-(1-7). ACE2 also removes Ang II by catabolism to Ang-(1-7).^{33,34} ACE2 is expressed in most tissues, including skin.³⁵ Ang-(1-7) is regarded as the principal counter-regulatory mechanism for Ang II.³⁶ Binding of Ang-

Table 4. Heat Responses (%CVC_{max}) Catheter 3

Heat Event	Medication	Control Subjects (n=12)	POTS Patients (n=12)
Baseline	Before drugs	13±1	7±1*
	NLA+losartan+Ang II	23±4	19±2
	NLA+losartan+Ang II+Ang-(1-7)	22±2	22±3
First thermal peak	Before drugs	73±9	45±5*
	NLA+losartan+Ang II	69±7	48±3*
	NLA+losartan+Ang II+Ang-(1-7)	75±8	70±7†
Nadir	Before drugs	44±5	29±5*
	NLA+losartan+Ang II	49±4	34±7
	NLA+losartan+Ang II+Ang-(1-7)	52±2	54±5†
Plateau	Before drugs	93±5	53±5*
	NLA+losartan+Ang II	77±2†	49±7*
	NLA+losartan+Ang II+Ang-(1-7)	79±7	72±6†

* $P<0.05$ different from control subjects; † $P<0.05$ different from previous drug combination or before drugs.

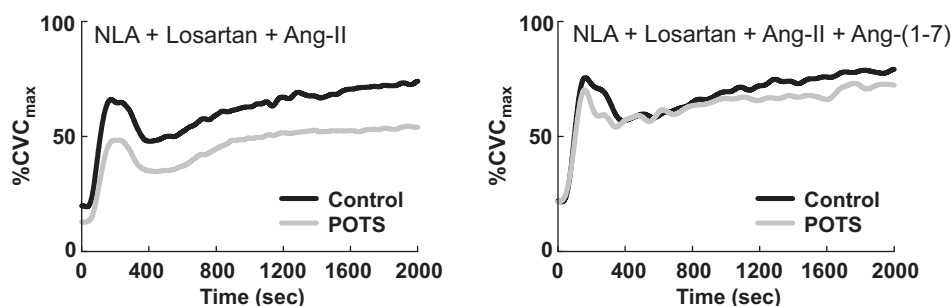


Figure 4. Comparison of heating responses expressed as %CVC_{max} after administration of NLA+losartan+Ang II (left panel) and after Ang-(1-7) has been added to yield NLA+losartan+Ang II+Ang-(1-7) (right panel). Data are averaged over all control subjects and all POTS patients. Addition of Ang-(1-7) produces an increase of the local heating response in POTS patients to equal control. No change occurs in control subjects.

(1-7) to Ang-(1-7) receptors produces vasodilation and anti-proliferative antihypertrophic effects. Ang (1-7) may interfere with Ang II directly at the AT1R^{37,38} or through other pathways, including antagonism of ACE.³⁹ Although Ang-(1-7) may exert dilator effects through bradykinin,⁴⁰ its principal ligand is the recently discovered Mas receptor.⁴¹ During our experiments, NO synthase was inhibited and AT1Rs were blocked; actions mediated through ACE2 and Ang-(1-7) should have been independent of NO or AT1R. Ang-(1-7)-Mas receptors have multiple vasodilating effects; these include responses mediated by AKT-NO upregulation, direct Ang II antagonism, and reduction of cyclooxygenase-2.^{42,43} It is probable that the vasodilating effects of Ang-(1-7) repletion are not evidenced completely during angiotensin-mediated vasodilation.

Limitations

Experiments using direct intradermal administration of ACE2 would restore endogenous Ang-(1-7) and the local heating response. Unfortunately, ACE2 was not available.

Skin can be a representative microvascular surrogate in illnesses such as low-flow POTS, hypertension, hypercholesterolemia, heart failure, and diabetes mellitus, in which there are widespread and generalized pathophysiological microvascular effects.⁴⁴ It has the advantages of ubiquity and easy access, and experiments lack systemic perturbation. Our work indicates that vascular abnormalities occur throughout the circulation in POTS; local flow abnormalities in skin can be generalized. However, the cutaneous circulation has unique

autonomic control and may not be the best representative organ for understanding Ang II relationships.

We studied females without regard to menstrual cycle. The phase of the menstrual cycle can affect-NO dependent mechanisms. However, NO was blocked throughout experiments. In addition, we found directionally consistent and similar results across all subjects. There was no evidence suggesting a relationship between menstrual cycling and changes in POTS symptoms or signs.

Endogenous angiotensin was not considered. Many tissues produce Ang II, but there are no skin data. However, data from skeletal muscle microvessels suggest local concentrations on the order of 100 pmol/L.⁴⁵ This was similar to our lowest dose of Ang II administered during previous dose-response measurements but was far less than exogenously administered Ang II in current experiments. Alternatively, we could have sought to eliminate local Ang II production with an ACE inhibitor. However, because ACE is also bradykininase, use of an ACE inhibitor enhances bradykinin release, altering microvascular conductance.

Microdialysis is invasive and alters the interstitial milieu. The work of Anderson et al suggests that flow responses return to baseline levels within ≈ 1 hour.²⁹ In pilot experiments, we measured baseline flows, removed the LDF probes, instrumented the same site with microdialysis catheters, replaced the probes, waited ≥ 1 hour, and repeated the LDF measurements with similar results (on average).

The local cutaneous heating model has been useful in dissecting biochemical pathways, but its relevance to POTS is uncertain. Specifically, all of the results entail vasodilating responses to heat, not at baseline. POTS patients are often intolerant to heat. In addition, it is unclear how vasoconstriction can lead to POTS. However, eutermic skin is highly vasoconstricted, with very small changes occurring with further vasoconstriction. Experiments concerning constrictor mechanisms often include a predilation step. Local heating is particularly useful because it produces a near-maximum increase in LDF in control subjects but a much smaller increase in POTS patients. Local skin heating is distinct from core heating, which diverts blood from the systemic circulation into the skin, worsening orthostatic tolerance. Local heating produces only local effects, primarily attributable to NO. Our findings suggest that an ACE2 deficit explains increased Ang II in POTS. An ACE2 deficit also

Table 5. Heat Responses (%CVC_{max}) Catheter 4

Heat Event	Medication	Control Subjects (n=12)	POTS Patients (n=12)
Baseline	Before drugs	12 \pm 1	5 \pm 1*
	Ang-(1-7)	10 \pm 3	15 \pm 5†
First thermal peak	Before drugs	63 \pm 6	45 \pm 4*
	Ang-(1-7)	70 \pm 4	58 \pm 7
Nadir	Before drugs	35 \pm 5	23 \pm 5
	Ang-(1-7)	43 \pm 6	56 \pm 7†
Plateau	Before drugs	84 \pm 5	29 \pm 5*
	Ang-(1-7)	91 \pm 5	74 \pm 4†

* $P < 0.05$ different from control subjects; † $P < 0.05$ different from before drugs.

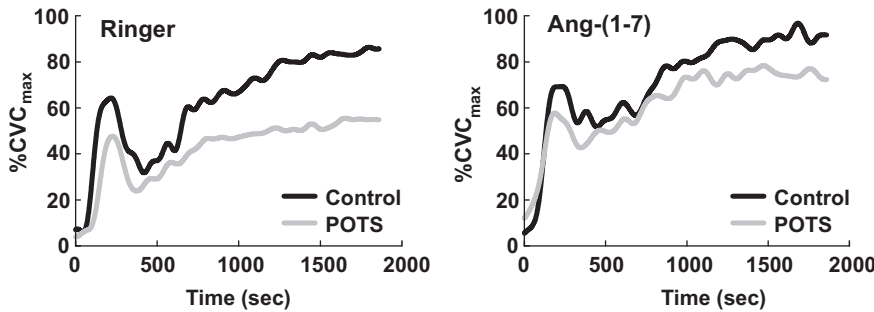


Figure 5. Comparison of the heating response expressed as %CVC_{max} before (left panel) and after (right panel) administration of Ang-(1-7) averaged over all control subjects and all POTS patients. The left panel shows the reduced NO-dependent heat response in POTS patients compared with control subjects. The right panel demonstrates that addition of Ang-(1-7) markedly improves the local heating response in POTS. There is also a small increase in the heat response of control subjects.

decreased Ang-(1-7). The increase in Ang II and decrease in Ang-(1-7) are associated with increased vascular resistance and decreased vascular capacitance, blood volume, and cardiac output, which respond poorly to orthostasis, causing orthostatic intolerance akin to the physiology of pheochromocytoma.

Perspectives

POTS is associated with a hyperadrenergic state of sympathoexcitation and widespread vasoconstriction. Our previous work showed that cutaneous and systemic Ang II is increased in low-flow POTS, in which NO bioavailability is reduced. We have now shown that there is blunted activity of ACE2, which is the central pathway for Ang II degradation. This may be the first human illness in which this deficit has been detected. The findings transcend the tachycardia syndrome and point to mechanisms for Ang II excess and sympathetic activation that are potentially present in diverse systemic illnesses of vascular regulation.

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Disclosures

None.

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