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Cutaneous neuronal nitric oxide is specifically decreased in postural tachycardia syndrome

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Stewart JM, Medow MS, Minson CT, Taneja I. Cutaneous neuronal nitric oxide is specifically decreased in postural tachycardia syndrome. *Am J Physiol Heart Circ Physiol* 293: H2161–H2167, 2007. First published July 27, 2007; doi:10.1152/ajpheart.00600.2007.—Low flow postural tachycardia syndrome (POTS), is associated with reduced nitric oxide (NO) activity assumed to be of endothelial origin. We tested the hypothesis that cutaneous microvascular neuronal NO (nNO) is impaired, rather than endothelial NO (eNO), in POTS. We performed three sets of experiments on subjects aged 22.5 ± 2 yr. We used laser-Doppler flowmetry response to sequentially increase acetylcholine (ACh) doses and the local cutaneous heating response of the calf as bioassays for NO. During local heating we showed that when the selective neuronal nNO synthase (nNOS) inhibitor N^{ω}-nitro-L-arginine-2,4-L-diaminobutyric amide (N^{ω}, 10 mM) was delivered by intradermal microdialysis, cutaneous vascular conductance (CVC) decreased by an amount equivalent to the largest reduction produced by the nonselective NO synthase (NOS) inhibitor nitro-L-arginine (NLA, 10 mM). We demonstrated that the response to ACh was minimally attenuated by nNOS blockade using N^{ω} but markedly attenuated by NLA, indicating that eNO largely comprises the receptor-mediated NO release by ACh. We further demonstrated that the ACh dose response was minimally reduced, whereas local heat-mediated NO-dependent responses were markedly reduced in POTS compared with control subjects. This is consistent with intact endothelial function and reduced NO of neuronal origin in POTS. The local heating response was highly attenuated in POTS [60 ± 6 percent maximum CVC(%CVC_{max})] compared with control (90 ± 4 %CVC_{max}), but the plateau response decreased to the same level with nNOS inhibition (50 ± 3 %CVC_{max} in POTS compared with 47 ± 2 %CVC_{max}), indicating reduced nNO bioavailability in POTS patients. The data suggest that nNO activity but not NO of endothelial NOS origin is reduced in low-flow POTS.

lasers; autonomic nervous system

POSTURAL TACHYCARDIA SYNDROME (POTS) is identified with chronic orthostatic intolerance (19, 27, 37, 44, 45). POTS is defined by symptoms of orthostatic intolerance associated with an excessive increase in heart rate during orthostatic challenge (37). Symptoms include dizziness, fatigue, exercise intolerance, headache, memory problems, palpitations, nausea, blurred vision, pallor, and abnormal sweating while upright, which improve with recumbence and have no other medical explanation. POTS thus combines symptoms of orthostatic intolerance with findings of excessive upright tachycardia. Stewart and Montgomery (42) previously described a subset of POTS patients designated "low-flow POTS" characterized by generalized pallor and acrocyanosis, often marked by resting as well as upright tachycardia, decreased cardiac output, mild hypovolemia, and widely decreased regional blood flows with severe vasoconstriction. These patients may correspond to subjects studied by other investigators, patients who have reduced blood volume (11, 18), abnormalities of the renin-angiotensin-aldosterone system (35), increased sympathetic outflow measured by microneurography (12), increased plasma angiotensin II (ANG II) (40), and widespread reductions of blood flow in regional circulations (42), including muscle and cutaneous circulation (28, 41).

Microvascular endothelial cell dysfunction has been proposed as a pathophysiological mechanism in POTS (7, 46). The results of prior work from our laboratory, where we used iontophoresis of the nonselective nitric oxide (NO) synthase (NOS) inhibitor N^{G}-nitro-L-arginine methyl ester (L-NAME) and local heating, support the hypothesis of reduced constitutive NO activity, which we assumed to be of endothelial origin (eNO) (28). This conjecture was based on the findings of Kellogg et al. (22, 23) and Minson et al. (29, 30), who demonstrated that local cutaneous heating produced an increase in cutaneous blood flow, which reaches a plateau that is highly sensitive to NOS inhibition. However, these investigations used nonisoform-specific NOS inhibition. Whether endothelial NOS (eNOS) or neuronal NOS (nNOS) is primarily involved has not yet been determined in healthy subjects or in POTS patients.

Thus, the goals of the present study were *1*) to test the hypothesis that nNOS, rather than eNOS, is specifically involved in the local heat response by using a highly selective isoform-specific nNOS inhibitor N^{ω}-nitro-L-arginine-2,4-L-diaminobutyric amide (N^{ω}) compared with the nonisoform specific NOS inhibitor nitro-L-arginine (NLA), *2*) to investigate the acetylcholine (ACh) dose response to N^{ω} and NLA to demonstrate that eNO is primarily responsible for the receptor-mediated NO release by ACh, and *3*) to test the hypothesis that the cutaneous NO deficiency in low-flow POTS is due to decreased production of NO from nNOS [neuronal NO (nNO)] rather than from eNOS (eNO) using local heating and ACh responses.

METHODS

Subjects

POTS patients recruited for the study were referred to the Hypotension Center for investigation of signs and symptoms of chronic orthostatic intolerance lasting at least 3 mon. 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, which were relieved by recumbence and had no other medical explanation. The diagnosis of

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POTS was made in these patients during a screening upright-tilt table test to 70° for a maximum of 10 min. POTS was diagnosed by symptoms of orthostatic intolerance during tilt associated with an increase in sinus heart rate of >30 beats/min or to a rate of >120 beats/min during the first 10 min of tilt (27, 36). During the same visit, POTS patients were partitioned on the basis of supine lower limb (calf) blood flow into patients who had decreased blood flow designated as low-flow POTS (<1.2 ml·100 ml tissue^{-1}·min^{-1}) and those that did not. It has been previously shown that POTS patients can be classified into normal-, low-, and high-flow groups based on venous occlusion plethysmography measurements of calf blood flow (41, 42). Our ongoing work indicates that 1.2 ml·100 ml tissue^{-1}·min^{-1} was the minimum calf blood flow found in healthy volunteers during experiments in more than 75 subjects. We measured calf blood flow by venous occlusion strain-gauge plethysmography (14) while supine. For the current study, patients were retained if they belonged to the low-flow POTS group.

Using these methods, we recruited 13 low-flow POTS patients (all women, Caucasian, and aged 16.6–26.3 yr, with median age of 22.5 yr). Thirteen healthy Caucasian volunteer subjects were also recruited (all women, Caucasian, and aged 17.2–26.9 yr, with median age of 23.6 yr) and were studied after a screening upright tilt at 70° demonstrated normal orthostatic response. Volunteer subjects served as the control group and were recruited from among adolescents and young adults referred for innocent heart murmur. This precluded the participation of subjects with mitral valve prolapse. Subjects with a history of syncope or orthostatic intolerance were specifically excluded.

For all experiments, subjects fulfilled certain criteria. Only subjects free from cutaneous, systemic, and cardiovascular diseases were eligible. Subjects were not taking any medications and refrained from drinking alcohol and caffeinated beverages for 24 h before the study. There were no smokers or trained competitive athletes involved. Informed consent was obtained, and the Committee for the Protection of Human Subjects (Institutional Review Board) of New York Medical College approved all protocols. Women were enrolled without regard to the phase of their menstrual cycle except that none were menstruating during testing procedures.

Protocols

Three sets of experiments were performed in which changes in cutaneous blood flow were measured. The first two sets of experiments investigated the response to local heating in healthy volunteers. The third set of experiments compared the responses to local heating and to ACh in POTS patients with responses in healthy control subjects.

Experiment 1: is nNOS rather than eNOS specifically involved in the local cutaneous heat response? Comparative effects of a highly selective nNOS inhibitor and a nonselective NOS inhibitor on the hyperemia of local heating. Healthy female volunteers ($N = 8$, all women, Caucasian, and aged 17.0–26.2 yr, with median age of 23.0 yr) were recruited. The ethnicity and age match were constrained by the composition of the POTS population.

We compared the effects of the highly isoform-selective nNOS inhibitor N^{ω} (546.38 Da mol wt) to a maximally plateau-suppressing dose of NLA (219.20 Da mol wt), a nonselective NOS inhibitor. N^{ω} is a highly specific nNOS inhibitor that does not bind the inducible NOS isoform and has ~1,538-fold selectivity for nNOS over eNOS (10, 16).

Testing was conducted in a temperature-controlled room (~25°C) at least 4 h after subjects ate a light breakfast. Experiments began after a 30-min acclimatization period, and all experiments were performed while each subject was supine and breathing spontaneously. We used laser-Doppler flowmeters (Perimed, Stockholm, Sweden) and integrating laser-Doppler flow (LDF) probes (Probe 413, Perimed) placed on the lateral aspect of the left calf to measure cutaneous blood flow (20). The 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 indicated significant findings in the lower limb in POTS (17, 41, 43).

Blood pressure was monitored by Finometer (TNO, Amsterdam, The Netherlands), and heart rate was monitored by continuous electrocardiogram. Continuous LDF data were collected at a sampling rate of 200 Hz during experiments multiplexed and interfaced to a personal computer through an analog-to-digital converter (DI-720, DATAQ, Milwaukee, WI) using custom data acquisition software and generating binary files and computer displays of simultaneously collected data from all lasers and blood pressure data.

LDF measurements were made on the left calf while supine with the leg at the level of the heart. Subjects were instrumented with two microdialysis catheters placed at least 6 cm apart and inserted in the dermal space of the lateral aspect of the left 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 as 1 or 2.

Following placement, catheters were initially perfused with Ringer solution at 2 μl/min. An integrating laser-Doppler flow probe was placed directly over each microdialysis catheter to measure cutaneous LDF. There was a hyperemia following catheter insertion. LDF was recorded until values were similar to those measured over the same area before catheter insertion. The return of LDF to preinsertion values usually occurred by 60–90 min (1). When necessary, longer times were allowed until preinsertion LDF was reached.

Once baseline LDF values were obtained, the areas under each laser were gradually heated at 1°C for 10 s to 42°C for at least 30 min until a plateau was reached. Heat was turned off to allow for recovery to baseline LDF. After heat recovery was complete, subjects received perfusate containing 10 mM NLA through *catheter 1* and 10 mM N^{ω} through *catheter 2* at a rate of 2 μl/min for 30 min. Local heating was repeated until a plateau was established while perfusion with NOS inhibitors continued. At that time, perfusates were switched so that *catheter 1* now received N^{ω} while *catheter 2* received NLA. Perfusion of catheters continued for 30 additional min. In preliminary studies, we demonstrated that the heat response at a given site is repeatable and that additional perfusion time with NOS inhibitors did not affect the plateau that was reached. Doses of 10 mM NLA and 10 mM N^{ω} were chosen because these were the least concentrations of drugs that gave maximum suppression of the local heating plateau. N^{ω} has a selectivity (K_i ratios) for nNOS/eNOS of 1,538 (10, 16). At the dose used and assuming similar transmicrodialysis transport and interstitial diffusion, this is equivalent to 0.25% of the binding to eNOS and fourfold greater binding to nNOS compared with equimolar NLA. This is discussed more fully in DISCUSSION. However, NLA is a smaller molecule and, therefore, probably reaches higher interstitial vasculature concentrations in higher concentrations than N^{ω}.

At the end of the experiments, we perfused both catheters with 28 mM sodium nitroprusside to obtain maximum endothelial-independent vasodilation and to compute the maximum cutaneous vascular conductance (CVC_{max}). Cutaneous vascular conductance (CVC) was calculated as the ratio of LDF to mean arterial blood pressure. CVC_{max} was defined as CVC during steady sodium nitroprusside infusion. Experimental results were thereafter expressed as $\%CVC_{max}$ ($100 \cdot CVC/CVC_{max}$).

Experiment 2: what are the relative effects of selective nNOS inhibition compared with nonisoform-specific NOS inhibition during cutaneous ACh dose response? For this purpose we recruited eight healthy female volunteers (all women, Caucasian, and aged 18.5–24.3 yr, with median age of 22.9 yr). The ethnicity and age match were constrained by the composition of the POTS population.

Experiments were performed on a day other than the day of *experiment 1*. Experiments were not conducted as classical dose-

response and inhibition studies since the derivation of pharmacological characteristics was not our intent. Rather, different doses of agonist (ACh) alone and then combined with NOS inhibitors (NLA and N^{ω}) 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 ACh response because there are effects of prostaglandins, endothelium-derived hyperpolarizing factors, and potentially local axon reflexes.

Subjects were instrumented with two microdialysis catheters and integrative laser-Doppler probes as in *experiment 1*. After recovery, patients had baseline LDF data collected for at least 10 min. Subjects then received perfusate containing 0.01, 0.10, 1.0, 10, and 100 mM ACh dissolved in Ringer solution in ascending doses through each catheter at a rate of 2 μl/min. The range of concentration of ACh used (0.01–100 mM) is based on previous determinations in human skin utilizing microdialysis delivery of this agonist (25, 39). LDF monitoring continued, and each dose was administered for 20 min during which steady-state values of LDF were achieved. For purposes of analysis, only the last 5 min of data were averaged at each ACh dose.

Subjects recovered from the initial ACh challenges for at least 1 h during which $\%CVC_{max}$ over each microdialysis catheter site fell to baseline values.

Subjects then received perfusate containing 10 mM NLA in *catheter 1* and 10 mM N^{ω} in *catheter 2* at a rate of 2 μl/min while LDF monitoring continued throughout a run-in period for 30 min. Subjects then underwent repeat ACh challenges while maintaining NLA and N^{ω} doses: (0.01, 0.10, 1.0, 10, and 100 mM ACh) + 10 mM NLA in *catheter 1* and (0.01, 0.10, 1.0, 10, and 100 mM ACh) + 10 mM N^{ω} in *catheter 2* at a rate of 2 μl/min while LDF monitoring continued. Each dose of ACh + blocker was maintained for 20 min. For purposes of analysis, only the last 5 min of data were averaged during the steady state.

At the end of the experiments, both catheters were perfused with 28 mM sodium nitroprusside to obtain CVC_{max}.

Experiment 3: is nNO rather than eNO bioavailability reduced in low-flow POTS? Microdialysis data from low-flow POTS patients compared with control subjects: local heating response and the response to ACh. We compared the response of POTS patients and control subjects to two stimuli: ACh, a receptor-mediated endothelium-dependent vasodilator, and local heating. After estimated baseline LDF were measured, two microdialysis catheters were inserted as in *experiment* 2, and subjects were allowed to recover. Subjects then received perfusate containing 0.01, 0.10, 1.0, 10, and 100 mM ACh dissolved in Ringer solution in ascending doses through *catheter 1* and underwent local heating of *catheter 2*. LDF monitoring continued, and each dose was administered for 20 min during which steady-state values of LDF were achieved. For purposes of analysis, only the last 5 min of data were averaged at each ACh dose.

When these tests were completed, subjects were allowed to fully recover from heat and ACh. Subsequently, each catheter received 10 mM NLA for 30 min after which the response to ACh was measured using the same ascending concentration of ACh (0.1–100 mM) with added 10 mM NLA. Local heating was repeated in *catheter 2* while NLA perfusion continued. At the end of the experiments, both catheters were perfused with 28 mM sodium nitroprusside to obtain CVC_{max}.

Statistics

We used two-way analysis of variance (2 × 2) to compare the plateau phases of the local heating response before and after treatment with N^{ω} or NLA in *experiment 1*. We used analysis of variance with repeated measures to compare dose-response curves of ACh alone, ACh + NLA, and ACh + N^{ω}. We also used two-way ANOVA to compare responses of POTS patients and control subjects with response from varying doses of ACh and to compare means of the plateau phases of local heating in *experiment 3*. Results were calculated using the statistical package for the social sciences software version 11.0. Apart from representative figures, text, graphic, and Table 1, results are reported as means ± SE. Significance required $P < 0.05$.

RESULTS

Experiment 1: a selective nNOS inhibitor is equally effective as a nonisoform-specific NOS inhibitor in blunting the hyperemia of local heating. Results are presented in Figs. 1 and 2. Figure 1 shows a representative heating response before and after nNOS inhibition with N^{ω}. Changes in plateau were comparable with those observed before and after NLA. On average, the plateau phase conductance before NLA was 92 ± 3 $\%CVC_{max}$ and after NLA was 47 ± 5 $\%CVC_{max}$ ($P < 0.001$). Similarly, the plateau phase conductance for N^{ω} was 89 ± 4 $\%CVC_{max}$ and after N^{ω} decreased to 44 ± 3 $\%CVC_{max}$ ($P < 0.001$).

There was no significant difference in the effects of NLA or N^{ω} on conductance during heating. We performed a crossover experiment in which the catheter initially receiving NLA was switched to N^{ω} while the catheter receiving N^{ω} was switched to NLA. No change was observed. Figure 2 shows a representative result. There is no difference in the blunted plateau phase or during crossover with either NLA or N^{ω}.

Experiment 2: a selective nNOS inhibitor minimally reduces the response to ACh. A nonselective NOS inhibitor greatly reduces the response to ACh. Results are presented in Fig. 3. While nNOS inhibition with N^{ω} reduces the dose response to ACh by a small but statistically significant amount ($P < 0.05$), NLA causes a much larger reduction of 50% or greater ($P < 0.0001$). There are thus clear differences in the responses to selective nNOS and nonisoform-selective NOS inhibition. These distinguish NO-dependent, endothelial receptor-mediated ACh response from nNOS-mediated ACh responses.

Experiment 3: POTS patients have dose responses to ACh similar to control, but the local heat response is blunted. Resting supine data for POTS patients and control subjects are shown in Table 1. Subjects were similar in size and in arm and leg blood pressure as with control subjects. In POTS patients, supine heart rate was increased ($P < 0.0025$) while pulse pressure was reduced ($P < 0.05$) . Resting LDF was signifi-

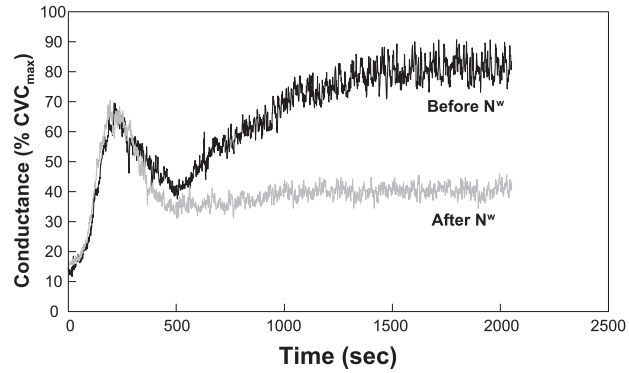

Fig. 1. Local heating response in a healthy volunteer control subject. The response before the neuronal nitric oxide synthase (nNOS) inhibitor N^{ω}-nitro-L-arginine-2,4-L-diaminobutyric amide (N^{ω}) is shown in black, and the response to local heating after N^{ω} is shown in gray. Similar responses to nonselective NOS inhibition also occur. $\%CVC_{max}$, percent maximum cutaneous vascular conductance.

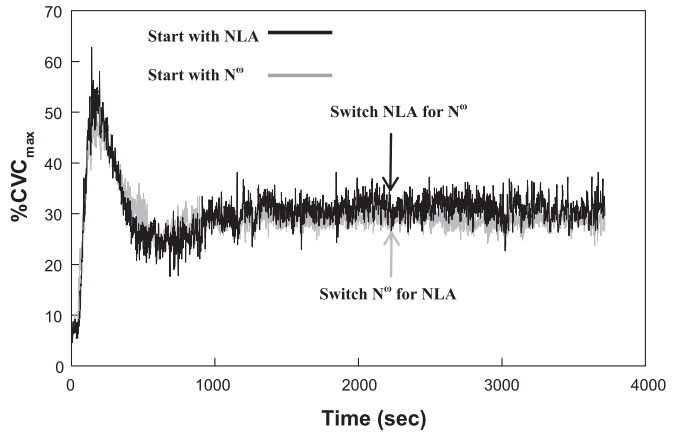

Fig. 2. Local heating response at 2 separate microdialysis sites. The site shown in black is perfused for 30 min with nitro-L-arginine (NLA) and then heated to 42°C. After the plateau is reached, NLA is switched for N^{ω} and heating is continued. The site shown in gray is perfused for 30 min with N^{ω} and then heated to 42°C. After the plateau is reached, N^{ω} is switched for NLA and heating is continued. There is similar blunting of the plateau phase at each site and with each NOS inhibitor.

Table 1. *Dimensions and supine hemodynamics*

	Control	POTS
	(N = 13)	(N = 13)
Age, yr	22.2±1.1	23.9±.08
Weight, kg	61±2	57±2
Height, cm	170±2	168±3
Body surface area, m^2	1.75±.04	1.62±.03
Supine HR, beats/min	68±2	88±3*
Supine systolic BP, mmHg	119±3	120±4
Diastolic systolic BP, mmHg	66±2	71±3
Pulse pressure, mmHg	58±2	46±3*
Venous occlusion calf blood flow, ml·100 ml min	2.5±0.2	0.84±0.11*
Calf arterial resistance, ml·100 ml min^{-1}·mmHg^{-1}	34±4	88±7*
Maximum laser-Doppler flow with sodium nitroprusside, pfu	177±12	164±18
Resting laser-Doppler flow, pfu	19.8±1.0	13.4±2.2*
Resting %CVC$_{max}$	13.0±1.0	7.4±1.4*

*$P < 0.05$ smaller than control. %CVC, percent cutaneous vascular conductance.

cantly decreased ($P < .0001$) in POTS as was %CVC$_{max}$ at rest. The maximum flow response to microdialyzed nitroprusside was similar for POTS and control patients. Since the overall peripheral blood flow is reduced (by definition) in low-flow POTS, it is reasonable to assume that low LDF actually reflects reduced cutaneous blood flow. Microdialysis of drugs had no effect on systemic hemodynamics (heart rate and arm and leg blood pressure) in any patient.

Figures 4 and 5 show results for *experiment 3*. On the one hand, as shown in Fig. 4, there is no significant difference in the response of POTS patients and control subjects to intradermal ACh, although there is a trend toward reduced hyperemia in POTS. This is consistent with similar receptor-mediated endothelial responses. On the other hand, as shown in Fig. 5, there is a marked decrease in the NO-sensitive plateau of the heat response in POTS patients ($P < 0.0001$) compared with control. On average, the plateau was 90 ± 4 %CVC$_{max}$ for control subjects but only 60 ± 6 %CVC$_{max}$ for POTS patients. When perfused with NLA, the plateau phase decreased to 47 ± 2 %CVC$_{max}$ for control subjects and to 50 ± 3 %CVC$_{max}$ in POTS patients. There was no significant difference in %CVC$_{max}$ of control subjects and POTS patients once NLA was given.

DISCUSSION

Summary and Discussion of Findings

Our main findings are as follows.

Administration of a sufficient amount of a nonselective NOS inhibitor blunts the NO-dependent plateau of the local heating response. A selective nNOS inhibitor does an equally good job of blunting at a dose that should exert minimal effect on eNOS. This observation suggests that nNOS is primarily responsible for the increase in bioavailable NO with local heating. Tissue concentrations of drugs delivered by microdialysis are mark-

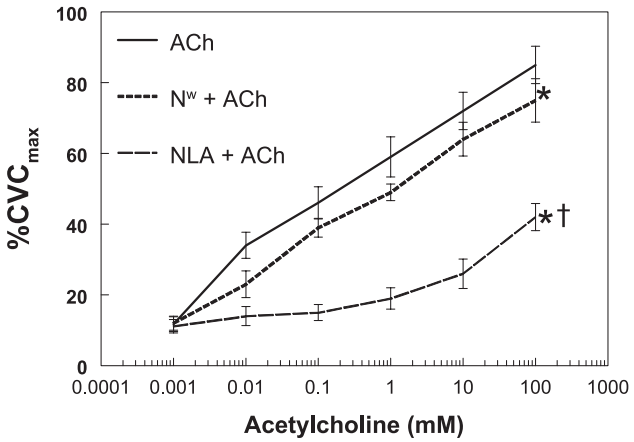

Fig. 3. Dose response of volunteer control subjects to a stepwise increase in acetylcholine (ACh) at 2 separate microdialysis sites. Solid line, response to ACh alone; short-dash line, response to ACh + N^{ω}; long-dash line, response to ACh + NLA. There is a small but significant reduction in overall dose response when N^{ω} is added. There is a much larger attenuation of the dose response when NLA is added. *$P < 0.05$ compared with ACh alone. †$P < 0.05$ compared with ACh + N^{ω}.

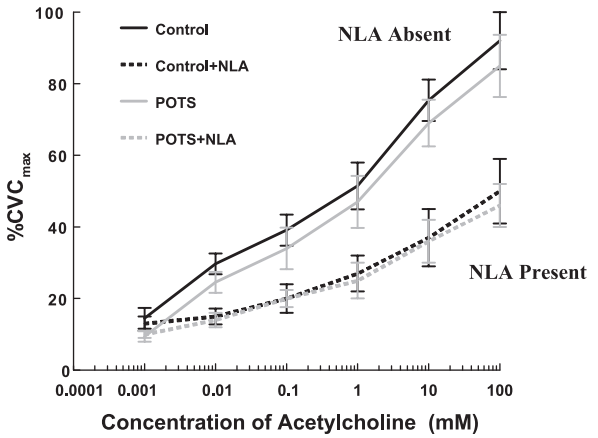

Fig. 4. Dose response of control subjects (black line) and postural tachycardia syndrome (POTS) patients (gray line) to a stepwise increase in ACh at 2 separate microdialysis sites. Both control subjects and POTS patients received ACh alone and combined with NLA. There is no difference between POTS and control results.

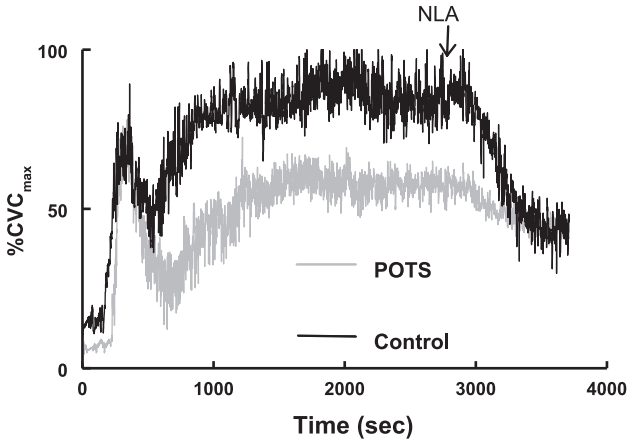

Fig. 5. Local heat response of a representative control subject (black line) and a representative POTS patient (gray line). The plateau is decreased in the POTS patient. Administration of NLA once a stable plateau was achieved resulted in a decrease to a similar %CVC_{max} for both subjects.

edly reduced compared with concentrations within the perfusate. Microdialysis relies on diffusion across the dialysis membrane and through the interstitium to deliver substances to the interstitial space. Quantitative measurements of the concentration of a substance in the interstitium by microdialysis cannot be made accurately without knowing factors such as membrane permeability to the specific molecule, interstitial properties, and local blood-flow rate. In general, tissue delivery decreases with increasing molecular weight. For NLA, the minimum concentration that yields maximum attenuation of the local heating plateau is 10 mM. This is much greater than the concentration needed to produce an in vitro 50% reduction (the K_i or IC_{50}) of nNOS or eNOS. For NLA, this is ~0.5 μM for both eNOS and nNOS. The ratio of 10 mM to 0.5 μM is equal to 20,000 and gives an approximation of the reduction of NLA concentration from perfusate to active binding site. For N^{ω}, the K_i for nNOS is ~0.13 μM whereas the K_i for eNOS is ~200 μM. With the assumption that roughly similar amounts of N^{ω} and NLA reach target NOS molecules, this implies that N^{ω} would have ~0.5 μM/200 μM (=0.25%) of the effect on eNOS compared with NLA. This would not reduce the local thermal plateau. This also implies that N^{ω} would have ~0.5 μM/0.13 μM (=4 times) the effect on nNOS compared with NLA.

A nonselective NOS inhibitor, NLA, greatly reduces ACh dose-response. Although Holowatz et al. (15a) suggest little role for NO in the response to ACh, the bulk of evidence indicates that a large reduction of ACh-augmented cutaneous blood flow occurs with the administration of nonselective NOS inhibitors independent of the route of delivery (5, 24). Evidence also supports an increase in eNO production with intradermal ACh administration (4). Recent data in other tissues support a role of nNO in the ACh response (13, 26), as shown in Fig. 3. This may be the first demonstration of nNOS dependence of the cutaneous ACh response and may indicate that the relationship of NO to ACh-mediated cutaneous hyperemia is not completely due to receptor-mediated NO release from the endothelium. The response to nNOS inhibition is quite small compared with the response to NLA, suggesting that the large majority of the NO-related ACh dose response relates to eNOS as described in other tissues.

Combined local heating and ACh dose response suggest a defect in nNO production in low-flow POTS. Microdialysis of ACh produces a similar response in control and low-flow POTS patients, although there is a trend toward a slightly smaller response in POTS. This suggests that NO from eNOS is intact in POTS patients (48). There is a trend toward a reduced ACh response in POTS compared with control, which is consistent with reduced nNO. The local heating response is blunted in POTS compared with control subjects. This suggests that there is a reduction of NO of nNOS origin in low-flow POTS. Taken together, these observations suggest that a defect in NO of nNOS origin accounts for the reduction of NO activity in low-flow POTS.

Significance of Reduced Bioavailable nNO

Initially, nNOS was believed to have primarily neurological as opposed to circulatory effects (31). Subsequently, nNOS has been found in various tissues, including keratinocytes (2), and has great importance for cardiac, skeletal muscle, renal, and neurovascular function (3, 8, 9, 21, 38). Of particular interest are its effects on the sympathetic nervous system, both in the central nervous system and in peripheral nerves (15, 34). It is now believed that nNO helps to regulate central sympathetic outflow and exerts important effects in sympathoexcited disease states such as heart failure and hypertension (49, 51). There is a reciprocal relationship between nNO and ANG II (51). It is interesting that ANG II is increased in the plasma of some low-flow POTS patients (40).

nNOS is present within parasympathetic nerves in the peripheral nervous system. It is sometimes colocalized with ACh and vasoactive intestinal peptide in the perivascular parasympathetic fibers (33) and modulates vascular tone and blood flow (32, 47). nNOS is present in skin keratinocytes and could hypothetically be coreleased from cutaneous sympathetic cholinergic nerves. Although we cannot define the specific cell of origin, overall vasoconstriction in low-flow POTS patients (42) would suggest a reduction of nNOS of neural origin.

An Interpretation for the Mechanistic Pathophysiology of Low-Flow POTS Patients

Data from these and other experiments can be tentatively assembled into a unified picture of the pathophysiology of low-flow POTS. Our previous data has shown an increase in ANG II (40) and now a reduction in nNO coupled with peripheral vasoconstriction, reduced cardiac output, and increased total peripheral resistance (42). Increased angiotensin and decreased nitrergic NO act reciprocally to increase central and peripheral sympathoexcitation (50). This is particularly important in states of excessive sympathetic activation such as heart failure (52) in which it appears to be related to oxidative stress (6). Whether a similar sympathoexcitation occurs in low-flow POTS remains to be determined.

Limitations

We studied the cutaneous circulation, which has unique autonomic control. Our recent work indicates that flow regulation abnormalities in low-flow POTS occur throughout the circulatory system and that the local flow abnormalities that occur in skin may be generalized. There is a paucity of quantitative comparisons of local regulatory mechanisms in

humans. However, it is clear that the myogenic response, venoarteriolar reflex, and reactive hyperemia occur in the skin and are abnormal in low-flow POTS patients (41).

We studied the calf cutaneous circulation. Our previous data indicate that flow abnormalities are widespread in low-flow POTS (42). However, whenever peripheral blood flows are studied, the most significant results occur in the lower extremities. It may be that dependence and gravitational exposure are important to observed changes.

Whereas N^{ω} has well-documented in vitro selectivity for nNOS over eNOS (10, 16), in vivo selectivity has not been well established. Indeed, there could be unexpected actions of this agent on choline transport, acetylcholinesterase, butyrocholinesterase, muscarinic, prostaglandin, or NO mechanisms other than effects of nitric oxide synthases.

We studied women without regard to menstrual cycle. The phase of the menstrual cycle can exert important effects on NO-dependent mechanisms. However, there is no evidence suggesting a relationship between menstrual cycling and changes in POTS symptoms or signs.

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