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Nitric oxide synthase inhibition attenuates cutaneous vasodilation during postmenopausal hot flash episodes

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Abstract

Objective: The purpose of this study was to test the hypothesis that local inhibition of nitric oxide and prostaglandin synthesis attenuates cutaneous vasodilator responses during postmenopausal hot flash episodes.

Methods: Four microdialysis membranes were inserted into the forearm skin (dorsal surface) of eight postmenopausal women (mean ± SD age, 51 ± 7 y). Ringer solution (control), 10 mM ketorolac (Keto) to inhibit prostaglandin synthesis, 10 mM N^G-L-arginine methyl ester (L-NAME) to inhibit nitric oxide synthase, and a combination of 10 mM Keto + 10 mM L-NAME were each infused at the separate sites. Skin blood flow at each site was indexed using laser-Doppler flowmetry. Cutaneous vascular conductance (CVC) was calculated as laser-Doppler flux/mean arterial blood pressure and was expressed as a percentage of the maximal calculated CVC (CVC_{max}) obtained after infusion of 50 mM sodium nitroprusside at all sites at the end of the study. Data from 13 hot flash episodes were analyzed.

Results: At the control site, the mean ± SD peak increase in CVC was 15.5% ± 6% CVC_{max} units. This value was not different relative to the peak increase in CVC at the Keto site (13.0% ± 5% CVC_{max} units; $P = 0.09$). However, the peak increase in CVC during hot flash episodes were attenuated at the L-NAME and L-NAME + Keto sites (7.4% ± 4% and 8.7% ± 7% CVC_{max} units, respectively) relative to both the control and the Keto sites ($P < 0.05$ for both comparisons). There were no significant differences in the peak increases in sweat rate between any of the sites ($P = 0.24$).

Conclusions: These data demonstrate that the mechanism for cutaneous vasodilation during hot flash episodes has a nitric oxide component. Increases in CVC despite the inhibition of prostaglandin synthesis suggest that prostaglandins do not contribute to cutaneous vasodilation during hot flash episodes.

Key Words: Hot flash – Nitric oxide – Skin blood flow – Cutaneous vasodilation.

Hot flashes are one of the most prominent symptoms of female menopause. They affect approximately 70% of women during the first 5 years after the onset of the menopausal transition.[1-3] A hot flash is defined as a sudden feeling of heat usually accompanied by flushed skin and perspiration. These sensations frequently begin in the chest and often include the face, head, and arms. Hot flashes can range in severity and can cause additional symptoms such as anxiety, embarrassment, depression, and nausea.[4-7] In addition, hot flashes can decrease quality of life by negatively affecting concentration, quality of sleep, mood, and sexual function.[8,9]

Previous studies have reported transient increases in blood flow in the finger, hand, forearm, and calf during hot flash episodes.[10-13] Blood flow in these studies was measured using plethysmography, which is unable to distinguish between the vascular beds of the skin and the muscle; however, the authors of these studies speculated that the increases in blood flow were due to increases in skin blood flow (SkBF). A recent study in our laboratory confirmed that hot flashes are accompanied by increases in sternal and forearm SkBF.[9]

The mechanism responsible for cutaneous vasodilation during hot flash episodes is unknown. Previous research has shown that ~85% to 95% of the increase in SkBF during a whole-body heat stress occurs via a neurally mediated active vasodilator system.[14] Moreover, both nitric oxide (NO)– and prostaglandin-dependent mechanisms contribute to this neurally mediated vasodilatory response.[15,16] It may be that similar mechanisms are responsible for the increases in SkBF during hot flash episodes, but this remains unknown. Therefore, the first objective of this study was to test the hypothesis

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that NO and/or prostaglandins are responsible for, or contribute to, cutaneous vasodilator responses during postmenopausal hot flash episodes.

Increases in sweating also occur during hot flash episodes.[4-7,17] This response presumably occurs via similar mechanisms as sweating in hyperthermic humans in which acetylcholine is released from sympathetic cholinergic nerves to stimulate sweat production.[18] Because NO has been shown to sensitize sweat glands,[19-21] it is possible that sweating occurring during hot flash episodes may be modified by NO-related mechanisms. Therefore, a secondary purpose of this study was to test the hypothesis that sweating during hot flash episodes is modulated by NO mechanisms.

METHODS

Participants

Eight healthy postmenopausal women participated in this study. Their mean ± SD age, height, and weight were 51.2 ± 7.4 y, 164.8 ± 6.3 cm, and 68.1 ± 8.7 kg, respectively. All women were amenorrheic for at least 1 year and had a minimum of four episodes per day. Participants completed a 7-day journal before participation to verify the average number of hot flash episodes per day. Participants were healthy, with no history of cardiovascular or metabolic disease, and were not taking hormone therapy or any other treatments for hot flashes. Institutionally approved (University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas) written informed consent was obtained from all women before they enrolled in the study.

Instrumentation

Upon arrival at the laboratory, each participant donned water-perfused tube-lined pants (Med-Eng, Ottawa, ON, Canada). Participants rested quietly in a semirecumbent position while four microdialysis membranes (Bioanalytical Systems, West Lafayette, IN) were inserted into the dorsal forearm skin. An integrating laser-Doppler probe (model DP7a; Moor Instruments, Wilmington, DE), secured in an acrylic sweat rate capsule, was placed over each microdialysis membrane (Fig. 1). This setup allowed the simultaneous evaluation of sweating from a 0.78-cm^2 area, with SkBF being indexed from the same location. Sweat rate was measured using the capacitance hygrometry ventilated-capsule method (Vaisala, Woburn, MA), with compressed nitrogen delivered at a rate of 150 mL/minute. Two additional larger sweat rate capsules (2.83 cm^2) were placed on the participants, one on the chest and one on the forearm, neither of which was over a microdialysis membrane. Sweat rate in these capsules was measured using the same method, but with compressed nitrogen delivered at a rate of 300 mL/minute. Continuous beat-by-beat arterial blood pressure was recorded from a finger (Finapres Medical Systems, Amsterdam, The Netherlands). Arterial blood pressure was also measured intermittently using electrosphygmomanometry of the brachial artery (Tango, SunTech Medical Instruments, Raleigh, NC).

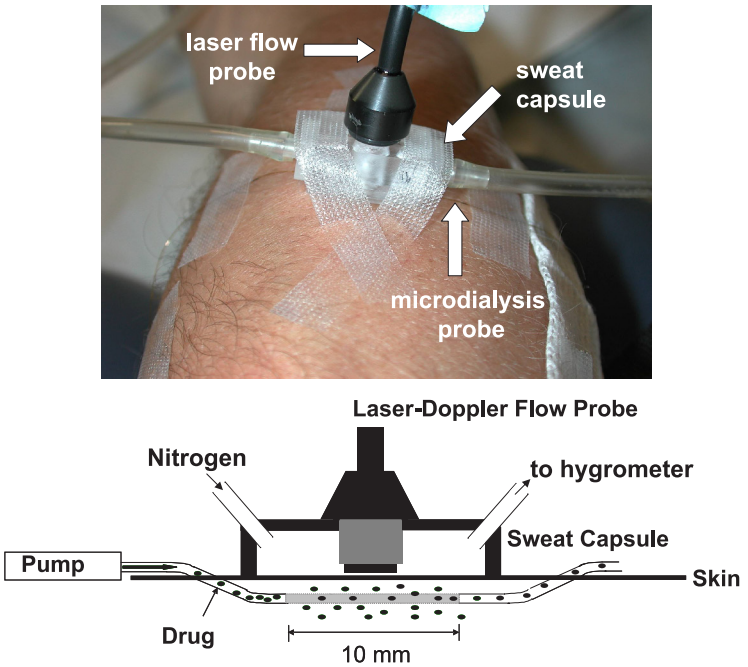

FIG. 1. Intradermal microdialysis, laser-Doppler probe, and sweat capsule setup (upper panel). A pump perfuses the drug solution through the microdialysis probe (lower panel). Some of the drug leaves the probe through the 10-mm semipermeable membrane in the middle of the probe. In the present experiment, the drugs L-NAME and ketorolac were infused at sufficient doses to inhibit nitric oxide synthase and prostaglandin synthesis, respectively, while the effects of those drugs on skin blood flow and sweat rate were simultaneously measured during hot flash episodes. Skin blood flow was indexed via laser-Doppler flowmetry, and sweat rate was measured via capacitance hygrometry using compressed nitrogen as the perfusion gas. L-NAME, N^G-L-arginine methyl ester.

Protocol

Immediately after microdialysis probe insertion, Ringer solution (control), 10 mM ketorolac (Keto), 10 mM N^G-L-arginine methyl ester (L-NAME), and a combination of 10 mM Keto + 10 mM L-NAME was each infused at the separate microdialysis sites at a rate of 2 μL/minute using perfusion pumps (Harvard Apparatus, Holliston, MA). Keto was perfused to inhibit prostaglandin synthesis, whereas L-NAME was perfused to inhibit NO synthase synthesis. These drugs and doses were selected given previous findings where successful blockade of NO and prostaglandin synthesis were achieved in human skin.[15,22,23] Participants rested quietly and were monitored during a 4- to 5-hour evaluation period. Hot flashes are reported to be more frequent in higher ambient temperatures and during peripheral warming.[1,8] Therefore, if a participant did not experience a hot flash after a few hours of monitoring, attempts were made to induce hot flashes with a mild heat stress by perfusing the suit pants with moderately warm (43°C) water. At the end of the protocol, 1 M methacholine was infused through the four microdialysis sites to obtain maximal sweat responses followed by infusion of 50 mM sodium nitroprusside at all sites to obtain maximal skin blood flux.

Data analysis

Data were sampled at 50 Hz with a data acquisition system (Biopac Systems, Santa Barbara, CA) and analyzed using a statistical software package (SigmaStat 3.11; Systat Software, Inc., San Jose, CA). Cutaneous vascular conductance (CVC) was calculated as laser-Doppler flux/mean arterial pressure. CVC was normalized relative to the percentage of the maximal CVC (CVC_{max}) obtained during sodium nitroprusside administration. Sweat rate was expressed as a percentage of maximal sweat rate (SR_{max}) normalized upon methacholine administration. The onset of a hot flash episode was defined as an increase in sternal sweating of at least 0.002 (mg sweat cm^{-2} min^{-1})/second, as has been previously reported.[9] Because of the variability in the length of hot flash episodes, each hot flash episode was divided into eight equal segments (each segment representing 12.5% of the hot flash duration). Five-second periods of data at the end of each segment and every 15 seconds over a period of 2 minutes before and after each hot flash episode were used in the statistical analysis. The highest CVC and sweat rate responses during each flash were identified. Differences in the peak change in CVC responses from pre–hot flash baseline between sites were evaluated using one-way repeated-measures analysis of variance followed by a Student-Neuman-Keuls test when a main effect was identified. Differences in the peak change in sweat rate responses between sites were analyzed via Friedman repeated-measures analysis of variance on ranks given that these data failed a normality test. All values are reported as means ± SD. P values less than 0.05 were considered statistically significant.

TABLE 1. *Participant and hot flash characteristics*

Participant	Age, y	Body mass, kg	Number of flashes	Average duration, sec	Occurrence[a]
A	56	75	2	135.5	N, H
B	51	52	2	306.5	N, H
C	48	72	1	91	N
D	53	66	3	84.3	N, N, N
E	66	64	2	204.5	H, H
F	42	77	1	207	N
G	49	64	1	152	H
H	45	80	1	719	H

[a]Indicates if the flash occurred naturally (N) or was induced by mild heat stress (H).

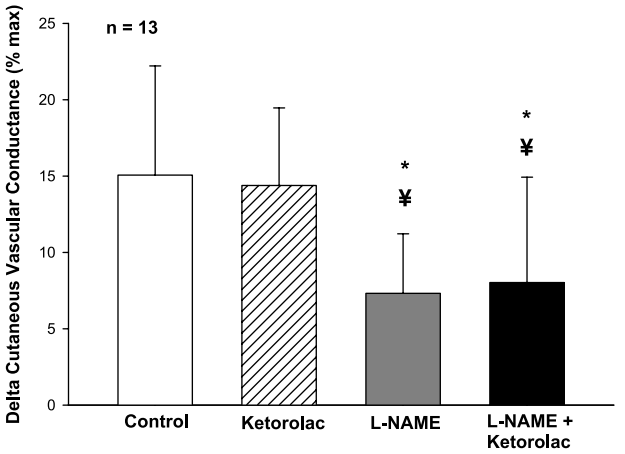

FIG. 2. Skin blood flow results. Mean (±SD) peak increases in cutaneous vascular conductance from baseline during 13 hot flash episodes are shown. Whereas nitric oxide synthase inhibition (L-NAME) attenuated the magnitude of cutaneous vasodilation, prostaglandin synthesis inhibition did not alter cutaneous vasodilation during the hot flash episodes. *Significant difference from control ($P < 0.001$). ¥Significant difference from ketorolac ($P < 0.004$). L-NAME, N^G-L-arginine methyl ester.

RESULTS

Data from 13 hot flash episodes were analyzed for SkBF responses. Participant characteristics and hot flash incidence for each participant are shown in Table 1. There were no differences in mean pre–hot flash baseline CVC at any of the

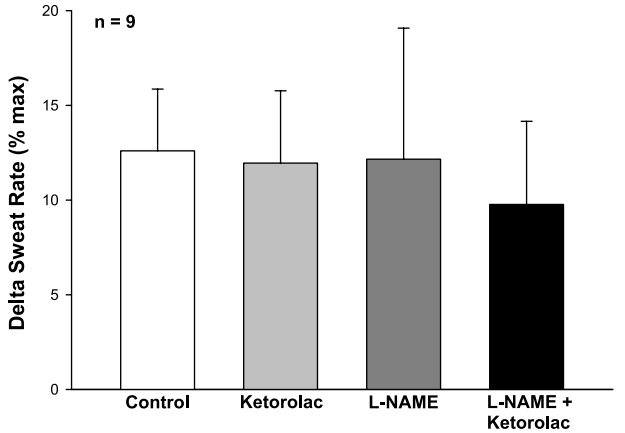

FIG. 3. Sweat rate results. Mean (±SD) peak increase in sweat rate from baseline during nine hot flash episodes. The increase in sweat rate during the hot flash episodes was not affected by the inhibition of nitric oxide or prostaglandin synthesis ($P = 0.24$). L-NAME, N^G-L-arginine methyl ester.

sites ($P = 0.34$). At the control site, the average peak increase in CVC during hot flash episodes was 15.5% ± 6% CVC_{max} units (Fig. 2). This value was not different relative to the peak increase in CVC at the Keto site (13.0% ± 5% CVC_{max} units; $P = 0.09$). However, the peak increase in CVC during hot flash episodes was attenuated at the L-NAME and L-NAME + Keto sites (7.4% ± 4% and 8.7% ± 7% CVC_{max} units, respectively) relative to both the control site ($P < 0.001$ for both) and the Keto-only site ($P < 0.004$ for both; Fig. 2).

Data from nine hot flash episodes were analyzed for sweating responses. Four fewer hot flash episodes were analyzed for this variable relative to SkBF because of the fact that some hot flashes were not strong enough to elicit a forearm sweating response at the control site, despite increases in SkBF and sternal sweat rate during those flashes. There were no significant differences in the peak increases in sweat rate between any of the four sites ($P = 0.24$; Fig. 3). The mean peak increase in sweat rate was 12.6% ± 3.3% SR_{max} at the control site, 12% ± 3.8% SR_{max} at the Keto site, 12.2% ± 6.9% SR_{max} at the L-NAME site, and 9.8% ± 4.4% SR_{max} at the L-NAME + Keto site.

DISCUSSION

The primary finding of this study is that cutaneous vasodilation during hot flash episodes is mediated in part by NO mechanisms given that inhibition of NO synthase via L-NAME attenuated increases in CVC during the hot flash episodes. In contrast, normal increases in CVC at the Keto site suggest that prostaglandins do not contribute to cutaneous vasodilation during hot flash episodes. The absence of differences in sweat rate during hot flash episodes at the sites where NO and prostaglandin synthesis was inhibited suggest that these mechanisms do not contribute to the sweating response during hot flash episodes.

Previously, a number of investigators hypothesized that SkBF increases during hot flash episodes.[10-13] This hypothesis was recently confirmed upon assessment of cutaneous vascular responses via laser-Doppler flowmetry.[9] Both data sets demonstrate that increases in SkBF during hot flash episodes occur quite rapidly, even before measurable increases in sweat rate or indices of sweat rate (eg, galvanic skin response) are identifiable.

The primary mechanism by which SkBF increases during a hot flash episode is unknown. Freedman et al[11] conducted a study in which the nerves of one hand were blocked with a local injection of lidocaine in postmenopausal women. Significant increases in finger temperature and blood flow occurred during hot flash episodes in both the nerve-blocked and non–nerve-blocked fingers. Based upon these findings, it was proposed that a circulating vasodilating substance may cause vasodilation during hot flash episodes. Counter to the hypothesis of Freedman et al, the rapid rise in SkBF observed in this study and others[9] strongly suggests that this response may be neurally mediated.

NO and prostaglandins contribute to cutaneous vasodilation during a number of perturbations. For example, both have been reported to contribute to cutaneous vasodilation during exogenous administration of acetylcholine.[22-24] Likewise, studies have reported clear NO and prostaglandin contributions to heat stress–induced cutaneous vasodilation.[15,16,25] Sympathetic cholinergic nerves recognized to cause vasodilation during heat stress release acetylcholine as well as other cotransmitters.[18] Given these observations, if cutaneous vasodilation during a hot flash episode occurs through engagement of the sympathetic cholinergic system, then one may likewise expect an NO and prostaglandin component in mediating vasodilation during a hot flash episode. However, the absence of an effect of prostaglandin inhibition on vasodilation during a hot flash episode raises the possibility of differing mechanisms of cutaneous vasodilation relative to that which occurs during heat stress.

With the present data, we are unable to identify the source of the NO contributing to the cutaneous vasodilation. One possibility could be secondary from acetylcholine binding to muscarinic receptors on the endothelium, thereby stimulating the release of NO. Alternatively, hot flash–induced cutaneous vasodilation would be expected to cause an increase in shear stress within the cutaneous vasculature, resulting in an increase in NO release and facilitated vasodilation. Finally, it has been reported that neuronally released NO contributes to vasodilation during heat stress,[26] and thus, neuronally released NO may directly cause vasodilation during a hot flash episode.

It is intriguing to note that neither NO nor prostaglandin inhibition altered the sweating response. Recent findings have demonstrated that NO is capable of modulating sweat rate to exogenous acetylcholine administration as well as during pronounced heat stress.[19,27] However, other data suggest that sweat rate during more moderate heat stress is not affected by inhibition of NO synthesis.[16,28] Taken together, it may be that NO affects sweating only after sweat rate has reached a sufficiently high level. Consequently, the modest sweat rates observed in the present study were apparently insufficient to be alterable by inhibition of NO synthase. One may speculate that NO synthase inhibition may attenuate the sweat response during a hot flash episode in which the individual sweats profusely.

A limitation to the interpretation of these results is the low number of hot flash episodes experienced by the participants from which the data could be analyzed. Despite the application of a mild heat stress, most participants exhibited only one or two hot flash episodes during the observation period. For the SkBF assessment, this resulted in 13 hot flash episodes analyzed, which were clearly sufficient to identify an effect of NO in mediating a component of the cutaneous vasodilator response (Fig. 2). However, four of these hot flash episodes were not of significant magnitude to evoke a forearm sweating response, despite clearly cutaneous vasodilation and sternal sweating, resulting in only nine hot flash episodes being analyzed for sweat rate. This low number of hot flash episodes, coupled with the absence of a significant difference between drugs sites for sweat rate (Fig. 3), raises the issue of whether the analysis was sufficiently

powered to test the proposed hypothesis. For this reason, a power analysis was performed, which confirmed that the statistical analysis for sweat rate was underpowered. However, to achieve sufficient power (0.80) to confirm the absence of a statistical difference between the control site and the L-NAME + Keto site using the obtained data (Fig. 3), more than 400 hot flash episodes would be necessary. Thus, given the recognized underpowered analysis for sweat rate, these data must be viewed with the understanding of a remote possibility of a type II error.

CONCLUSIONS

In conclusion, the main finding of this study was that NO contributes to cutaneous vasodilation that occurs during a hot flash episode, whereas inhibition of prostaglandin synthesis did not attenuate cutaneous vasodilation. In addition, inhibition of NO and prostaglandin synthesis did not significantly alter sweating. These data provide valuable insight into the mechanisms responsible for cutaneous vasodilation and sweating during hot flash episodes.

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