Effects of nitric oxide synthase inhibition on cutaneous vasodilation during body heating in humans

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Shastry, Shubha, Niki M. Dietz, John R. Halliwill, Ann S. Reed, and Michael J. Joyner. Effects of nitric oxide synthase inhibition on cutaneous vasodilation during body heating in humans. *J. Appl. Physiol.* 84(3): 830–834, 1998.—We sought to examine further the potential role of nitric oxide (NO) in the neurally mediated cutaneous vasodilation in nonacral skin during body heating in humans. Six subjects were heated with a water-perfused suit while cutaneous blood flow was measured by using laser-Doppler flowmeters placed on both forearms. The NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) was given selectively to one forearm via a brachial artery catheter after marked cutaneous vasodilation had been established. During body heating, oral temperature increased by 1.1 ± 0.1°C while heart rate increased by 30 ± 6 beats/min. Mean arterial pressure stayed constant at 84 ± 2 mmHg. In the experimental forearm, cutaneous vascular conductance (CVC; laser-Doppler) decreased to 86 ± 5% of the peak response to heating ($P < 0.05$ vs. pre-L-NMMA values) after L-NMMA infusion. In some subjects, L-NMMA caused CVC to fall by ~30%; in others, it had little impact on the cutaneous circulation. CVC in the control arm showed a similar increase with heating, then stayed constant while L-NMMA was given to the contralateral side. These results demonstrate that NO contributes modestly, but not consistently, to cutaneous vasodilation during body heating in humans. They also indicate that NO is not the only factor responsible for the dilation.

cutaneous blood flow; thermoregulation; autonomic nervous system

IT HAS BEEN WELL ESTABLISHED that neurally mediated vasodilation occurs in nonacral skin during body heating in humans (18, 19). The neurotransmitter responsible for this dilation is unknown. However, the increase in blood flow can be prevented by local anesthetic block of the autonomic nerves that innervate the skin or by surgical sympathectomy (18, 19). Systemic or local blockade of adrenergic and cholinergic receptors cannot prevent this dilation (12, 14, 15, 18, 19). However, botulinum toxin, which acts presynaptically to block cholinergic nerve transmission, can eliminate the dilation (12). This indicates that an unknown substance coreleased by cholinergic nerves plays a crucial role in evoking neurally mediated cutaneous vasodilation during body heating in humans. Because nitric oxide (NO) can be released by autonomic nerves, and because its release can also be evoked by stimulation of the vascular endothelium by neurotransmitters, NO has emerged as a possible mediator of cutaneous vasodilation during body heating in humans and other species (1, 22, 23).

A recent study by Taylor et al. (22) showed that the infusion of N^{ω}-nitro-L-arginine, an NO synthase (NOS) inhibitor, could abolish the active vasodilation in the rabbit ear seen during body heating. This study led us to hypothesize that NO could be the neurotransmitter responsible for cutaneous vasodilation during body heating in humans as well. We had previously tested this hypothesis by infusing the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) into a brachial artery catheter before body heating and found that L-NMMA given in this manner had little impact on the cutaneous dilator responses in the treated forearm during thermal stress (4). However, there were some important methodological differences between the original human study and the study conducted in rabbits (4, 22) that continue to raise the possibility of NO playing a key role in active cutaneous vasodilation during body heating in humans. First, in the rabbit study, the NOS inhibitor was infused during heating to ensure that it reached the dilated cutaneous vessels. Second, in the human study, we relied primarily on whole forearm blood flow (FBF) measurements by using venous-occlusion plethysmography rather than by using more selective measurements of cutaneous blood flow (CBF), such as laser-Doppler measurements (4). By contrast, the rabbit study used Doppler ultrasonic flow probes placed around the arterial supply to the ear to measure the changes in blood flow (22).

Using this information as a background, the present study in humans sought to replicate many of the features of the study in rabbits, which showed that NO played a critical role in the active cutaneous vasodilation seen during body heating (22). L-NMMA was infused via a brachial catheter during body heating after marked cutaneous vasodilation had been established to increase drug delivery to the dilated cutaneous vessels. We reasoned that if L-NMMA caused CBF to fall, a role for NO in neurogenic cutaneous vasodilation during body heating in humans would be indicated. A complementary approach to these same issues was recently adopted by Kellogg and colleagues (13), who used microdialysis techniques in conjunction with laser Doppler to selectively deliver NOS inhibitors to small areas of skin.

METHODS

Subjects. The study was approved by the Institutional Review Board, and written informed consent was obtained from the subjects. Six subjects (5 men, 1 woman) between the ages of 22 and 28 yr participated in the study. The female subject had a negative serum pregnancy test within the 48-h time period before the study. None of the subjects was taking

any medications, with the possible exception of oral contraceptives. Smokers and individuals with chronic medical conditions were excluded.

Subject monitoring. During the study, arterial pressure and heart rate were monitored by using the pressure signal from the arterial catheter. A thermocouple was inserted under the tongue to estimate body temperature.

Body heating. The subjects were heated with a water-perfused suit worn under an impermeable vinyl garment (4, 11, 13). To raise core temperature and evoke an increase in CBF, the suit was perfused with warm water (42–46°C). The suit did not cover the subject's forearms, which were exposed to ambient temperature (~23°C) to ensure that the increase in CBF was through neurogenic, not local, mechanisms.

CBF and FBF. CBF was measured with laser-Doppler flowmeters (BPM 403, TSI, St. Paul, MN) that were placed over the midportion of both forearms (4, 11). FBF was measured by venous-occlusion plethysmography with mercury-in-Silastic strain gauges. FBF is expressed as ml·100 $ml^{-1}\cdot min^{-1}$ (9). Blood flow was occluded to the hand while FBF was measured in both forearms simultaneously by use of strain gauges located over the midsection of the forearm just proximal to the laser-Doppler flowmeters. This forearm site is thought to primarily reflect changes in CBF (2). Although CBF was measured continuously, only values obtained when the arm cuffs were not cycling were used in this analysis to avoid artifacts associated with cuff inflation and deflation. Cutaneous vascular conductance (CVC) calculations were made by dividing the laser-Doppler flow signals by mean arterial pressure. These values are expressed as percentages of the peak response to heating. For plethysmography, FBF values (ml·100 $ml^{-1}\cdot min^{-1}$) were divided by mean arterial pressure and expressed as arbitrary forearm vascular conductance (FVC) units.

Sweat rate. Sweat onset and the rate of sweat production were measured with an evaporative water loss unit (model Z1885, Demco R&D, Lansing, MI). Sweat capsules were placed near the strain gauges and laser-Doppler flowmeters. Dry nitrogen was passed over 5 cm^2 of skin to evaporate the sweat, and the sweat rate was calculated by measuring the difference in relative humidity between the inlet gas and the outflow gas. Whereas sweating is governed by different mechanisms from active vasodilation, its onset is neurally mediated and occurs at approximately the same time as active vasodilation of the skin. Therefore, sweat onset is used as an index of the increase in neurogenic outflow to the skin (18, 19).

Drug preparation and infusions. L-NMMA was obtained by Calbiochem (La Jolla, CA). It was administered under United States Food and Drug Administration IND Number 41,190 (3). Commercially available pharmaceutical grade acetylcholine (ACh), obtained from IOLAB (Claremont, CA), and sodium nitroprusside, obtained from Abbott Laboratories (North Chicago, IL), were also used. Drugs were dissolved in normal saline and infused through a brachial artery catheter (3). The skin of the brachial fossa was cleaned with alcohol and 10% povidone-iodine. The area over the brachial pulse was anesthetized by locally injecting 1–2 ml of 2% lidocaine. A 20-gauge 5-cm Teflon arterial catheter was inserted into the brachial artery, connected to a pressure transducer, and continuously flushed with heparinized saline at 3 ml/h. A three-port connector was placed in series with the catheter-transducer system (3). One port was used to measure arterial pressure, whereas the other two ports were used for drug infusions. L-NMMA was infused with a mechanical syringe pump at 5 mg/min. ACh was infused at a rate of 8 μg·100 $ml^{-1}\cdot min^{-1}$ of forearm volume to test the efficacy of L-NMMA in blocking NO production (23), and nitroprusside was infused at a rate of 2 μg·100 $ml^{-1}\cdot min^{-1}$ of forearm volume to document the continued ability of the forearm vessels to dilate to exogenously administered NO (24). For all drugs, the rate of infusion was ≤5 ml/min. This infusion rate has been found to have no impact on baseline FBF in our laboratory (3–5).

Protocol. The subjects abstained from caffeine for 6 h before the study. Forearm volume and circumference were measured on both arms. The subjects were then fitted with the water-perfused suit. The nondominant (experimental) forearm was instrumented with a brachial artery catheter, whereas both forearms were instrumented to measure CBF, FBF, and sweat production. After resting CBF and FBF values were obtained, ACh was administered, and changes in CBF and FBF were measured. After BF returned to baseline values, the procedure was repeated with nitroprusside. The subjects were then heated while blood flow data were collected at 5-min intervals. When oral temperature had increased by 0.8–1.0°C and a marked rise in CBF had occurred, the CBF and FBF response to ACh was again measured. After a 5-min interval, measurements were taken during nitroprusside administration. When blood flows returned to baseline, L-NMMA was infused at a rate of 5 mg/min, and FBF and CBF were measured for 10 min. After a 1-min break, L-NMMA was again infused at 5 mg/min, and blood flows were measured for 10 min. Therefore, the total dose of L-NMMA was 100 mg, given over 21 min. This is more than twice the total dose of L-NMMA given in our previous study (4). Flows were collected for another 10 min after the infusion of L-NMMA was stopped, and CBF and FBF were reassessed in response to ACh and then nitroprusside.

Statistics. Each subject was able to serve as his or her own control because of the fact that drugs were infused into only one forearm, although measurements were taken in both forearms. The forearm that did not receive the drug served as the control. Paired *t*-tests were used to compare blood flow responses before and after L-NMMA and before and after ACh and nitroprusside infusions. Repeated-measures analysis of variance tests were used to compare systemic responses across events and to compare blood flow responses throughout the study. Significance was set at $P < 0.05$ level for all comparisons. Significant effects were further analyzed by using the Student-Newman-Keuls test. Data are reported as means ± SE.

RESULTS

Systemic responses. In response to body heating, heart rate and body temperature increased significantly ($P < 0.05$). Mean arterial pressure remained constant. The time from the initiation of heating to sweat onset was variable among subjects. Systemic responses are displayed in Table 1.

Results from laser-Doppler. Figure 1 is an individual record from one subject and demonstrates a marked fall in CVC during L-NMMA infusion. At baseline, the mean CVC in the experimental forearm for all the subjects was 9 ± 2% of that achieved during heating (i.e., peak response) and 8 ± 3% in the control arm ($P > 0.05$ experimental vs. control). After L-NMMA infusion, CVC decreased to 86 ± 5% ($P < 0.05$ vs. pre-L-NMMA values) in the experimental arm but remained constant in the control arm ($P > 0.05$ vs. peak, $P < 0.05$ vs. experimental). Two of the six subjects showed only a minimal decline [change (Δ)<5% of peak response to

Table 1. *Systemic responses from baseline to post-L-NMMA*

Event	HR, beats/min	MAP, mmHg	Absolute T, °C	ΔT	Time, min
Baseline	57 ± 6	83 ± 3	36.7 ± 0.1	0	0
Sweat onset	68 ± 5	80 ± 5	36.9 ± 0.2	0.2 ± 0.1	8–28
Pre-L-NMMA	88 ± 3*	84 ± 5	37.8 ± 0.1*	1.1 ± 0.1	57–74
L-NMMA to end	84 ± 5*	88 ± 5	37.8 ± 0.1*	1.3 ± 0.2	95–107

Values are means ± SE. L-NMMA, N^G-monomethyl-L-arginine; HR, heart rate; MAP, mean arterial pressure; T, temperature; ΔT, change in T. HR and T were significantly different from baseline (repeated-measures ANOVA, $*P < 0.05$).

heating] in CVC with L-NMMA, whereas the remaining four showed reductions of 10–30% (Fig. 2).

Before heating, ACh administration inconsistently increased CVC in the experimental arm (465 ± 188%, $P = 0.1$). During heating but before L-NMMA, ACh increased CVC (12 ± 4%, $P < 0.05$ vs. baseline). After L-NMMA, administration of ACh failed to alter CVC (9 ± 11%, $P > 0.05$). Before heating, nitroprusside infusion increased CVC (549 ± 255%, $P < 0.05$) from baseline. During heating, but before L-NMMA, CVC increased from baseline in response to nitroprusside infusion (28 ± 10%, $P < 0.05$). After L-NMMA, nitroprusside administration inconsistently increased CVC in the experimental arm (16 ± 7%, $P = 0.1$ vs. baseline). There were no changes in CVC in the control forearm (i.e., contralateral side) during either ACh or nitroprusside infusions.

Results from plethysmography. At baseline (saline), FVC averaged 1.3 ± 0.2 units in the experimental forearm and 1.0 ± 0.1 units in the control arm. With heating, FVC increased significantly to 10.0 ± 2.0 units in the experimental arm and to 8.8 ± 1.2 units in the control arm ($P < 0.05$ vs. baseline in both arms, $P > 0.05$ experimental vs. control). After L-NMMA, FVC was 9.3 ± 1.4 units in the experimental arm and 9.4 ± 1.0 units in the control arm ($P > 0.05$ vs. pre-L-NMMA values in both arms, $P > 0.05$ experimental vs. control). ACh caused FVC to increase ($P < 0.05$) above baseline in the experimental arm before heating, during heating, and after L-NMMA infusion. Nitroprusside caused FVC to increase ($P < 0.05$) from baseline before heating. Nitroprusside infusion inconsistently increased FVC ($P = 0.09$) during heating. After L-NMMA, nitroprusside infusion also inconsistently increased FVC ($P = 0.08$). There were no changes in FVC in the control forearm (i.e., contralateral side) during either ACh or nitroprusside infusions.

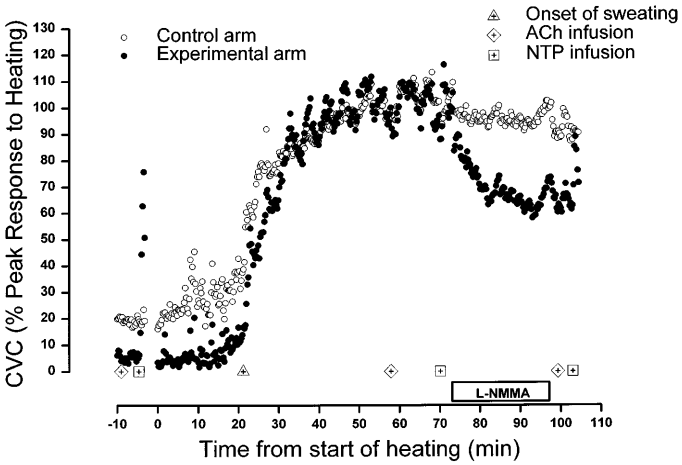

Fig. 1. Individual record of changes in cutaneous vascular conductance (CVC; %peak response to heating) throughout the study in response to drug infusions, heating, and N^G-monomethyl-L-arginine (L-NMMA). This subject showed an ~30% drop in CVC in response to L-NMMA. In this subject, there was little vasodilation seen in skin with laser-Doppler when acetylcholine (ACh) was given, but marked dilation was seen with nitroprusside (NTP). In addition to demonstrating that nitric oxide can play a role in cutaneous vasodilation during body heating, lack of response to ACh highlights the fact that ACh responses can be variable among subjects.

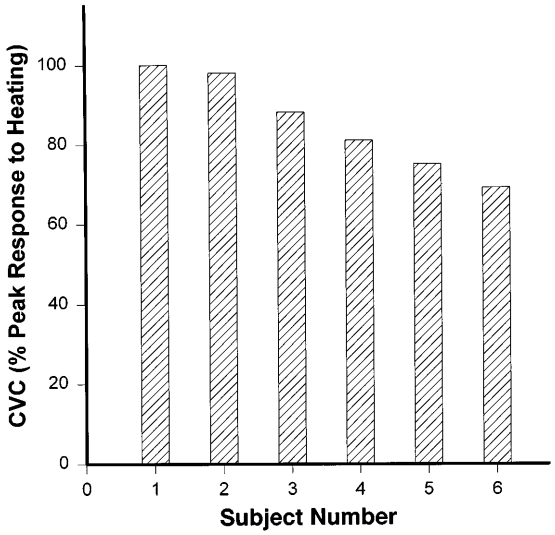

Fig. 2. Individual CVC responses in experimental arm expressed as a percentage of peak response to heating. Decreases in responses ranged from minimal to 30%.

DISCUSSION

In this study, we examined the potential role of NO in cutaneous vasodilation during body heating in humans. The principal finding was that NOS inhibitors can significantly blunt cutaneous vasodilation during body heating in some individuals but do not completely eliminate the response. These results suggest that NO contributes modestly, but not consistently, to cutaneous vasodilation during body heating in humans. The findings are consistent with observations made by Kellogg and colleagues (13), who used different techniques to address the same issue. These results indicate that NO is not the only factor responsible for cutaneous dilation during body heating in humans.

This finding contrasts with the previous conclusion that NO does not play a significant role in cutaneous vasodilation during body heating in humans (4). One reason for the conflicting findings is that in the previous study conclusions were based primarily on FBF (venous-occlusion plethysmography), whereas the present study relied on laser-Doppler measurements of CBF. The laser-Doppler method allows a more selective picture of changes in CBF, whereas plethysmography

can allow only a general picture of CBF in the forearm. However, plethysmography results obtained in the present study are consistent with those from our earlier study (4) in that a role for NO was not indicated. A second reason for the conflicting results is that the dose of L-NMMA infused was greater in the present study by at least a factor of two. More importantly, infusion of the L-NMMA during heating instead of before heating may have permitted more of the drug to reach the dilated cutaneous vessels. It is also possible that the factors that initiate the dilation may not be those which sustain it. Initiation of the vasodilation in response to body heating may be neurally mediated, whereas a flow-induced mechanism might be needed to sustain it.

However, our results continue to contrast with those found in the rabbit ear by Taylor et al. (22), who showed that administration of NOS inhibitors to the dilated rabbit ear during heating abolished the response. One obvious explanation is a species difference. It appears that in the rabbit ear the presence of NO is necessary to evoke vasodilation during body heating. However, it seems that NO alone is not responsible but plays a permissive role in causing the dilation, meaning that it must be present for another neurotransmitter or substance to cause the active vasodilation (6). In this context, it appears that the active vasodilation occurs through a cGMP-mediated pathway and that NO must be available to evoke the response (7).

Potential limitations associated with the present study are related primarily to the dose of L-NMMA we infused and the use of ACh to test NOS activity. Was NOS adequately inhibited with the dose of L-NMMA we used? This is difficult to assess based on the ACh responses. In previous studies, we have shown that there is little correlation between the ability of L-NMMA to blunt physiological vasodilator responses and the degree to which it inhibits ACh-mediated dilation (5). Those observations are consistent with data from Mugge et al. (17) in the rabbit hindlimb, which lead us to question the overall utility of ACh-mediated dilation as an index of NOS activity. It is also possible that a second dilating factor might be released by ACh (17). Additionally, there may be differences in the ability of compounds like L-NMMA to block NOS at various levels of the microcirculation, particularly in the resistance vessels (10). There is also the possibility that insufficient concentrations of L-NMMA reached the tissue of interest to block NO production in that tissue. Despite these possible limitations, it appears that the amount of L-NMMA that reached the cutaneous circulation was sufficient to reduce the cutaneous dilator response to body heating by at least 30% in some subjects.

There are several ways that NO might contribute to active cutaneous vasodilation in humans. First, there could be a direct neurogenic release of NO (23). Second, NO could also be released by endothelial cells through ACh spillover from cholinergic nerves. This possibility is attractive, since the total reduction in CVC with L-NMMA is similar to that seen when atropine is applied via either the brachial artery or with iontophoresis (12, 19). Third, it has also been proposed that, when stimulated by cholinergic nerve fibers, sweat glands can release bradykinin-forming enzymes (8). The activation of bradykinin receptors can also trigger the release of NO (20, 21). Finally, NO release could be flow induced (16, 20, 21). Some as yet unidentified vasodilating substance could increase blood flow, increasing the shear stress the endothelial cells are exposed to. This could then stimulate NO release from these cells.

How, then, do these findings contribute to the emerging information concerning the factors responsible for neurally mediated cutaneous vasodilation during body heating in humans? Recent evidence suggests that an unidentified substance coreleased with ACh from cholinergic nerves plays a major role in this vasodilation (12). It is possible that this cotransmitter could be NO, but this possibility is less likely based on observations from this study and the complementary study of Kellogg et al. (13). More likely, events leading to NO-mediated dilation with heating include ACh spillover from sudomotor nerves and/or flow-induced NO release from endothelial cells.

In summary, these results, along with those obtained with the use of microdialysis, confirm that NO contributes modestly, but not consistently, to cutaneous vasodilation during body heating in humans (13). The mechanisms by which its release is stimulated remain unclear. Further investigations are needed to elucidate both how NO is released during heating and its total contribution to the observed cutaneous vasodilation. The exact nature of the neurally mediated factor responsible for cutaneous vasodilation during body heating has yet to be determined.

We thank Lori Lawler and Tamara Eickhoff for their invaluable assistance and Janet Beckman and Cathy Nelson for their secretarial support. We also thank the subjects for their participation throughout the studies.

This study was supported by National Institutes of Health Grants HL-46493, NS-32352, RR-00585–25, RR-00585–24S2 and by the Glen L. and Lyra M. Ebling Cardiology Research Endowment.

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Received 27 October 1997; accepted in final form 1 April 1998.

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