Role of nitric oxide in the vascular effects of local warming of the skin in humans

D. L. KELLOGG, JR., Y. LIU, I. F. KOSIBA, AND D. O’DONNELL

Role of nitric oxide in the vascular effects of local warming of the skin in humans. J. Appl. Physiol. 86(4): 1185–1190, 1999.—Local warming of skin induces vasodilation by unknown mechanisms. To test whether nitric oxide (NO) is involved, we examined effects of NO synthase (NOS) inhibition with G-nitro-L-arginine methyl ester (L-NAME) on vasodilation induced by local warming of skin in six subjects. Two adjacent sites on the forearm were instrumented with intradermal microdialysis probes for delivery of L-NAME and sodium nitroprusside. Skin blood flow was monitored by laser-Doppler flowmetry (LDF) at microdialysis sites. Local temperature (Tloc) of the skin at both sites was controlled with special LDF probe holders. Mean arterial pressure (MAP; Finapres) was measured and cutaneous vascular conductance calculated (CVC = LDF/MAP = mmHg/mMg). Data collection began with a control period (Tloc at both sites = 34°C). One site was then warmed to 41°C while the second was maintained at 34°C. Local warming increased CVC from 1.44 ± 0.41 to 4.28 ± 0.60 mV/mMgHg (P < 0.05). Subsequent L-NAME administration reduced CVC to 2.28 ± 0.47 mV/mMgHg (P < 0.05 vs. heating), despite the continued elevation of Tloc. At a Tloc of 34°C, L-NAME reduced CVC from 1.17 ± 0.23 to 0.75 ± 0.11 mV/mMgHg (P < 0.05). Administration of sodium nitroprusside increased CVC to levels no different from those induced by local warming. Thus NOS inhibition attenuated, and sodium nitroprusside restored, the cutaneous vasodilation induced by elevation of Tloc; therefore, the mechanism of cutaneous vasodilation by local warming requires NOS generation of NO.

skin blood flow; skin temperature; vasodilation; laser-Doppler flowmetry; microdialysis

IN HUMANS, CUTANEOUS BLOOD VESSELS are controlled by both neurogenic reflexes and local factors (11, 17). In nonglabrous, or “hairy,” skin, reflex control of the cutaneous vasculature is mediated by two sympathetic pathways: a noradrenergic vasconstrictor system and an active vasodilator system that involves cholinergic cotransmission (14). Nitric oxide (NO) production is also involved in cutaneous active vasodilation (13, 20). A powerful local factor that controls the cutaneous vasculature is the local temperature (Tloc) of skin blood vessels (17). Local cooling of the skin causes a localized vasoconstriction that can reduce skin blood flow (SkBF) to minimal levels (17). This vasoconstriction is mediated by an axon reflex mechanism and requires norepinephrine release from vasconstrictor nerve terminals (17). Local warming of the skin causes a localized vasodilation that is graded with Tloc that can reach maximal levels if skin temperature is increased to 42°C for 20–40 min (12, 21). Thus Tloc control mechanisms can augment vasodilator effects of the neurogenic active vasconstrictor system during exposure to high ambient temperatures. This will facilitate thermal homeostasis.

The degree of vasodilation induced by local warming of the skin has been proposed as a clinical tool for evaluation of vasomotor dysfunction in diabetes and other disease states (3, 18), despite the fact that the mechanism that mediates the effects of local warming on the cutaneous vasculature is not fully defined. The initial increase in SkBF during local warming may be caused by a reduced effectiveness of noradrenergic vasoconstriction through decreased affinity of α2-receptors for norepinephrine (5). This mechanism appears to contribute no more than 10% of the maximal skin vasodilation caused by prolonged local warming to 42°C (17). Given these observations, and that local warming of forearm causes a vasodilation far greater than that caused by blockade of noradrenergic vasoconstrictor nerves alone, mechanisms other than alteration of noradrenergic vasoconstriction must be involved (7, 17, 23). For example, it was postulated that the effects of local warming could be mediated through axon reflex mechanisms and that local warming elicits vasodilation through activation of the same nerves and neurotransmitters that cause active thermoregulatory vasodilation (17). This appears not to be the case on the basis of the observation that botulinum toxin abolishes neurogenic active vasodilation during heat stress but does not alter the vasodilation induced by local skin warming (14). Furthermore, the observation that the vasodilation caused by local warming is preserved in skin denervated by burns and in skin grafts suggests that the process might not be neurally mediated at all (2, 9). Alternatively, endothelial factors such as NO may be involved in the vasodilation.

Recent work showed that NO synthase (NOS) production of NO is required for full expression of active vasodilation of the skin during hyperthermia (13, 20). Other work in humans showed that NO donors can increase SkBF directly (22) and that NO contributes to a basal dilator tone in forearm and finger skin (4). Furthermore, Goldsmith et al. (10) found that intradermal injection of either G-nitro-L-arginine-methyl ester (L-NAME) or G-monomethyl-L-arginine (L-NMMA) at-

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tenuates the cutaneous vasodilation produced by 30 s of forearm immersion in 45°C water. The observations that NOS production of NO plays a significant role in the cutaneous vasculature suggests that NO could be involved in the cutaneous vasodilation caused by increases in the temperature of the skin. We sought to examine this possibility by testing the hypothesis that the cutaneous vasodilation effected by local warming of the skin requires NOS production of NO.

**METHODS**

To test our hypothesis, we locally administered a NOS inhibitor to forearm skin while monitoring SkBF. The approach we chose was to combine NOS inhibition by local administration of L-NAME by intradermal microdialysis with local SkBF measurements with laser-Doppler flowmetry (LDF) from a small volume of skin (~1 mm²). Intradermal microdialysis permitted local administration of L-NAME directly into the interstitial space of a small area of dermis. Monitoring LDF over a site instrumented with an intradermal microdialysis probe permitted monitoring of local drug effects with high local concentrations without risking confounding systemic effects. Because there is no question about the locus of measurement or the locus of drug delivery, the combination of LDF with local administration of L-NAME provided a direct approach to study the role of NO in the local control of SkBF.

Six subjects (5 men and 1 woman) participated in this study. Their average age was 31 ± 4 (SE) yr, average weight was 66 ± 5 kg, and average height was 173 ± 3 cm. All subjects were normotensive, in good health, and taking no medications. All subjects gave their informed consent to participate in these institutionally approved studies. There was no caffeine intake on the day of the study, and all subjects were nonsmokers. The menstrual phase was not assessed in the female subject.

On arrival in the laboratory, each subject had two intradermal microdialysis probes placed on the ventral aspect of one forearm. The probes are made in our laboratory from borosilicate glass tubing and a 1-cm length of capillary microdialysis membrane (200-µm diameter, molecular weight cutoff 20) reinforced by a 51-µm-diameter coated stainless steel wire placed in the lumen of the membrane and tubing (Fig. 1). Microdialysis probes were placed at least 5 cm apart on the forearm so that manipulations at one site did not influence the other. Probes were inserted at each site as follows. A 25-gauge needle was inserted through the dermis by using sterile technique. Entry and exit points were ~2–3 cm apart. The microdialysis probe was threaded through the internal lumen of the needle, the needle was then withdrawn, and the probe was left in place. The microdialysis membrane was entirely within the dermis, with entry and exit through the skin via the borosilicate tubing. Ultrasound measurements showed that probes were placed 0.3–1.0 mm under the epidermal surface and thus were within the dermis. After probe insertion, subjects waited for ~2 h or more to permit resolution of needle insertion trauma before additional instrumentation was placed. Anderson et al. (1) reported that the injury caused by insertion of the needle required to place an intradermal microdialysis probe resolves during a period of 90–135 min, thus permitting in vivo studies without the confounding effects of trauma.

After needle trauma had resolved, subjects were placed in the supine position and instrumented to measure LDF from skin at the two microdialysis sites (MBF-3D dual-channel flowmeter, Moor Instruments, Devon, UK). Cutaneous LDF measurements are a reliable index of SkBF and are uninfluenced by blood flow in the underlying skeletal muscle (19). Mean arterial pressure (MAP) was recorded continuously from a finger (Finapres blood pressure monitor, Ohmeda, Madison, WI). LDF probes were held in place by special probe holders that permit LDF measurements and control of Tloc (14). After placement of the LDF probes, both microdialysis probes were perfused with Ringer solution at a rate of 2 µl/min by using a microinfusion pump (Harvard 22 syringe pump, Harvard Apparatus, South Natick, MA).

As illustrated in Fig. 2, data collection began with a 10-min control period with Tloc held constant at 34°C. Tloc was then increased at one of the LDF microdialysis sites to 41°C. This temperature was chosen to avoid any activation of pain fibers as occasionally happens with local warming to 42°C. After LDF measurements had increased and stabilized, both microdialysis probes were perfused with a 5 mM solution of L-NAME (Sigma Chemical) in Ringer solution for 20–40 min to effect L-arginine methyl ester (L-NAME) in Ringer solution. Finally, both microdialysis probes were perfused with 28 mM sodium nitroprusside (SNP) in Ringer solution.
SNP. A dose of 28 mM was found to reproducibly cause a maximal vasodilation, indistinguishable from that caused by local warming of the skin to 41°C.

On a separate day, subjects returned to the laboratory and the above protocol was repeated except that no l-NAME was administered: microdialysis probes were perfused with Ringer solution only. This series of studies tested for any effects of microdialysis in responses to local warming of the skin to 41°C.

Data are presented as means ± SE. For data analysis, CVC was indexed as LDF (in mV) divided by MAP (in mmHg) to control for any changes in blood pressure during data collection. Vasomotor responses were analyzed by comparing the average levels of CVC over the last 5 min of each period. CVC responses were analyzed by repeated-measures ANOVA followed by specific means comparisons.

RESULTS

Figure 3 summarizes the results of the study with l-NAME administration. During the initial control period, before any local warming or l-NAME perfusion, CVC values did not differ significantly between the two microdialysis sites (1.44 ± 0.41 and 1.17 ± 0.23 mV/mmHg; P > 0.05 between sites). During local warming to 41°C, CVC increased to 4.28 ± 0.60 mV/mmHg (P < 0.05 vs. initial control). Local warming of one site did not influence the normothermic site.

Perfusion with l-NAME significantly reduced CVC at both the normothermic and the locally warmed sites. At a Tloc of 34°C, l-NAME perfusion reduced CVC to 0.75 ± 0.11 mV/mmHg (P < 0.05). At a Tloc of 41°C, CVC fell from 4.28 ± 0.60 to 2.28 ± 0.47 mV/mmHg (P < 0.05) during perfusion with l-NAME.

Perfusion with SNP increased CVC at both the normothermic and locally warmed sites. At a Tloc of 34°C, SNP perfusion increased CVC to 4.05 ± 0.79 mV/mmHg (P < 0.05 vs. initial control and l-NAME at Tloc = 34°C). At a Tloc of 41°C, CVC increased to 4.02 ± 0.63 mV/mmHg (P < 0.05 vs. initial control and l-NAME at Tloc = 41°C; P > 0.05 vs. 41°C alone) during perfusion with SNP. Overall, the levels of CVC achieved with SNP perfusion did not differ between sites with Tloc values of 34 and 41°C (P > 0.05).

Figure 4 summarizes the results of the study without l-NAME administration, i.e., with perfusion of the microdialysis probes with Ringer solution only. After 30 min of local warming to 41°C, CVC had increased significantly (P < 0.05 vs. initial control) and remained unchanged for a total of 90 min of warming. Subsequent perfusion with SNP did not change CVC from levels achieved with local warming alone. At a Tloc of 34°C, perfusion with Ringer solution alone for 90 min did not alter CVC from initial levels (P > 0.05); however, SNP perfusion increased CVC significantly (P < 0.05, Ringer vs. SNP).

DISCUSSION

The results of this study show that NOS production of NO participates in the vasodilation induced by local warming of the skin. This conclusion is based on the observation that blockade of NOS by l-NAME greatly attenuates the local increase in CVC as effected by local warming of the skin to 41°C. No such changes in CVC occurred during perfusion of microdialysis fibers with Ringer solution alone; thus effects of microdialysis are not responsible for the observed changes in CVC with l-NAME perfusion. The abolition is not likely due to
any antimuscarinic effects of L-NAME (3) because the local vasodilation is not altered by atropine blockade of muscarinic receptors or blockade of cholinergic nerves by botulinum toxin (14).

When L-NAME was infused at the microdialysis site with a $T_{loc}$ of 34°C, there was a significant reduction in CVC, showing that NO generation by NOS contributes to a tonic vasodilator tone to cutaneous blood vessels under normothermic conditions. No such changes occurred during administration of Ringer solution alone. A similar finding was reported by Coffman (4), who recorded reductions in LDF during intra-arterial infusion of L-NMMA under normothermic conditions. Dietz et al. (6) also reported that forearm blood flow (FBF) was reduced during infusion of L-NMMA into the brachial artery under normothermic conditions. Although FBF measurements cannot distinguish between blood flow to skin and skeletal muscle, their results lend support to a tonic vasodilator effect of NO. In contrast, Noon et al. (16) reported that intra-arterial infusion of L-NMMA at doses of 1, 2, and 4 µM/min 1 reduced FBF, 2 reduced LDF from skin on the glabrous skin of the finger, and 3 had no effect on LDF from skin on the dorsum of the finger. These different findings may be attributable to cooler thermal conditions in the study by Noon et al. because NOS inhibition does not reduce SkBF under cool conditions (4). Overall, results from our study favor a contribution by NO generated by NOS in the basal tone in nonglabrous skin in normothermia.

In response to the initial warming of the skin, during the perfusion with Ringer solution only, CVC increased greatly. This demonstrates that the ability of the cutaneous vessels to respond to increases in $T_{loc}$ persisted after microdialysis probe placement. Thus trauma associated with placement of the probes did not render the vessels incapable of responding to local thermal factors.

After CVC had increased and stabilized at a high level with $T_{loc}$ held at 41°C, L-NAME blockade on NOS activity produced a significant reduction in CVC to levels not statistically different from those with a $T_{loc}$ of 34°C. Subsequent perfusion with SNP, an endothelium-independent NO donor, restored CVC to the levels  

Fig. 4. Summary of CVC responses to Ringer solution perfusion with and without local warming of the skin to 41°C. A: summary of CVC responses to Ringer solution perfusion without local warming. With a $T_{loc}$ of 34°C, perfusion with Ringer solution did not change CVC ($P > 0.05$ vs. initial control). Subsequent perfusion with SNP significantly increased CVC (*$P < 0.05$, SNP vs. control and SNP vs. Ringer). These results show that microdialysis per se did not alter basal skin blood flow. B: summary of CVC responses to Ringer solution perfusion with local warming. Local warming of the skin to 41°C increased CVC significantly (*$P < 0.05$ vs. control). CVC remained elevated throughout local warming ($P > 0.05$). CVC remained elevated during SNP infusion, at a level no different from that achieved by local warming alone ($P > 0.05$ vs. $T_{loc} = 41°C$). These results show that microdialysis per se did not alter vasodilation induced by local warming of the skin.

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Fig. 5. CVC from a single subject after reporting pain for 10 s during local warming to 42°C. $T_{loc}$ was lowered to 41°C immediately after pain was reported and maintained at that level. Note that despite subsequent L-NAME perfusion, CVC did not fall. Perfusion with 10 mM norepinephrine (NE) did reduce CVC, showing that blood vessels were still reactive to vasomotor influences. Similar results occurred in 2 other subjects after reporting brief periods of pain during local warming. This suggests that activation of nociceptors either greatly accelerates nitric oxide synthase generation of nitric oxide or that nociceptor activation elicits a nitric oxide-independent mechanism to maintain vasodilation.
achieved with a $T_{loc}$ of 41°C before L-NAME perfusion. These results show that NOS activity is required for full expression of cutaneous vasodilation as it is for full expression of neurogenic active vasodilation during hyperthermia (13, 20). However, in the case of local warming, NOS activity, and presumably generation of NO, appears required for most of the vasodilation to occur. In the case of neurogenic reflex vasodilation, mediated by cutaneous cholinergic cotransmission, only ~30% of the vasodilation appears to be NO dependent (13, 20).

Although our results demonstrate that NO generation by NOS plays a major role in the vasodilation induced by prolonged local warming, this may not be the sole vasodilator mechanism involved that can be activated by local application of heat, particularly when pain fibers are activated. In our preliminary studies, $T_{loc}$ was increased to 42°C to maximally dilate the skin vessels (21); however, three subjects reported pain at the locally warmed sites as $T_{loc}$ approached 42°C. $T_{loc}$ was immediately reduced to 41°C, with prompt resolution of pain in all subjects. The overall duration of the sensation of pain was very brief, varying from ~5 to 20 s. All subjects described the pain as “mild.” Subsequent perfusion with l-NAME at doses up to 20 mM failed to reduce CVC; however, CVC did fall in response to subsequent perfusion with a 10 mM norepinephrine in Ringer solution. This verified that the vessels had not been “damaged” by the painful temperature and were still capable of vasoconstriction. Figure 5 illustrates this phenomenon in one subject. The failure of l-NAME to reduce CVC at locally warmed sites after pain had been reported, even for the briefest of moments, suggests that another mechanism could be involved in cutaneous vasodilation after nociceptors have been activated. Some evidence suggests that prostaglandins could be generated by nociceptor activation and could effect an NO-independent vasodilation (22). Alternatively, it is possible that activation of pain fibers increased NOS activity greatly, perhaps by release of substance P. In our studies, in which the subjects experienced pain, relatively high doses of l-NAME (up to 20 mM) did not reduce CVC. It is possible that NOS activity had increased to such a level that even this relatively high dose did not give complete NOS blockade; thus we are not able to say whether the mechanism related to pain is an NO-dependent process or whether NO-independent pathways are involved.

The foregoing observations suggest a number of possible roles for NO in the vasodilation induced by local warming, although current data do not distinguish among those possibilities. For example, studies in the rabbit ear suggest that NO produced by endothelial cells acts in conjunction with a neurotransmitter released during hyperthermia to effect increases in ear blood flow (8). According to this scheme, tonic generation of NO by endothelial cells mediates production of a tonic level of cGMP, which is required for neurogenic reflex active vasodilation. Farrell and Bishop (8) speculated that humans may possess mechanisms other than NO that maintain the level of cGMP so that, even with complete NOS blockade, the level of cGMP would be sufficient to permit vasodilation. Our observation that the vasodilation induced by local warming alone can be altered by NOS blockade, but that the vasodilation appears to be resistant to NOS blockade after pain fiber activation, is consistent with this proposal.

In summary, we found that blockade of NOS with L-NAME reduces SkBF under normothermic conditions, demonstrating that this system contributes a basal vasodilator tone to cutaneous blood vessels. In addition, blockade of NOS abolishes the cutaneous vasodilation induced by local warming of the skin in humans. Thus NO generation by NOS is involved in the mechanism of vasodilation induced by local warming of the skin in humans.

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Address for reprint requests: D. L. Kellogg, Jr., Divs. of Geriatrics and Gerontology and of Clinical Pharmacology, Dept. of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284 (E-mail: kellogg@uthscsa.edu).

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