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Nitric oxide concentration increases in the cutaneous interstitial space during heat stress in humans

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Kellogg, D. L., Jr., J. L. Zhao, C. Friel, and L. J. Roman. Nitric oxide concentration increases in the cutaneous interstitial space during heat stress in humans. *J Appl Physiol* 94: 1971–1977, 2003; 10.1152/japplphysiol.00826.2002.—To examine the role of nitric oxide (NO) in cutaneous active vasodilation, we measured the NO concentration from skin before and during whole body heat stress in nine healthy subjects. A forearm site was instrumented with a NO-selective, amperometric electrode and an adjacent intradermal microdialysis probe. Skin blood flow (SkBF) was monitored by laser-Doppler flowmetry (LDF). NO concentrations and LDF were measured in normothermia and heat stress. After heat stress, a solution of ACh was perfused through the microdialysis probe to pharmacologically generate NO and verify the electrode's function. During whole body warming, both SkBF and NO concentrations began to increase at the same internal temperature. Both SkBF and NO concentrations increased during heat stress (402 ± 76% change from LDF baseline, $P < 0.05$; 22 ± 5% change from NO baseline, $P < 0.05$). During a second baseline condition after heat stress, ACh perfusion led to increases in both SkBF and NO concentrations (496 ± 119% change from LDF baseline, $P < 0.05$; 16 ± 10% change from NO baseline, $P < 0.05$). We conclude that NO does increase in skin during heat stress in humans, attendant to active vasodilation. This result suggests that NO has a role beyond that of a permissive factor in the process; rather, NO may well be an effector of cutaneous vasodilation during heat stress.

skin blood flow; vasodilation; amperometric electrode; thermoregulation

THE HUMAN CUTANEOUS CIRCULATION is a major effector of thermoregulatory homeostasis. During periods of whole body heat stress, this circulation undergoes a profound reflex vasodilation to deliver blood, and hence heat, to the body's surface for removal to the environment. During periods of cold stress, the cutaneous blood vessels are intensely vasoconstricted to reduce loss of heat from the body to the environment. These reflex changes in skin blood flow (SkBF) occur in response to changes in internal temperature and skin temperature (T_{sk}) and are mediated by two branches of the sympathetic nervous system: an active vasodilator system and an active vasoconstrictor system (17).

The mechanisms by which the active vasodilator system causes increases in SkBF during heat stress have been the subject of numerous investigations since the system was first described over 70 years ago (18). A number of these studies demonstrated that muscarinic-receptor blockade with atropine could slightly delay (10, 17, 19, 22) and attenuate (17, 19) the reflex increase in SkBF during heat stress. This finding of partial reductions in the active vasodilator response despite complete muscarinic-receptor blockade established a role for cholinergic nerves in the process of active vasodilation, but it left open to question the exact nature of this involvement. In 1995, our laboratory showed that thermoregulatory active vasodilation involves cholinergic nerve cotransmission on the basis of the observation that, in contrast to the relatively minor effects of atropine, pretreatment of skin with botulinum toxin blocked release of all neurotransmitters from cholinergic nerves and completely abolished SkBF increases during heat stress (17).

The failure of atropine to abolish cutaneous vasodilation has generally been accepted as evidence against a role for ACh as the neurotransmitter involved. However, atropine does delay and attenuate the process, albeit to small extents, suggesting some role for ACh (12, 23). This also implies a mechanistic role for NO, because ACh causes vasodilation at least in part through NO-dependent pathways (12, 23).

Several studies have examined the role of NO in cutaneous active vasodilation. The first was conducted in the rabbit by Taylor and Bishop (25). They used the rabbit ear as a model of the human cutaneous vasculature because rabbits actively vasodilate the skin of their ears as a reflex response to increased body temperature. In this model, Taylor and Bishop found that thermoregulatory active vasodilation could be completely abolished by NO synthase (NOS) inhibition. They concluded that NO is mechanistically involved in the ear blood flow response of the rabbit to heat stress. Their results provided rationale for further studies of

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the role of NO in human cutaneous active vasodilation, although there are some significant differences between the skin of the rabbit ear and human skin. Rabbits have numerous arteriovenous anastomoses in the skin of their ears (25); arteriovenous anastomoses are not numerous in nonapical human skin where active vasodilation occurs in humans.

Subsequent work by Farrell and Bishop (9) addressed the question of how NO functions as a vasodilator in the rabbit ear. They noted that, after NOS blockade, infusion of a small dose of the NO donor nitroprusside did not increase ear blood flow in normothermic rabbits. However, they also found that the same small dose would restore the vasodilation abolished by prior NOS blockade in the heat-stressed rabbit. The implication was that active vasodilation required both NO and activation of the vasodilator nerves by heat stress. Furthermore, Farrell and Bishop proposed that these two elements were not arranged in series (vasodilator activity did not increase NO production) as had been supposed. Instead, their studies led them to the provocative conclusion that NO served a "permissive" role in active vasodilation in the rabbit ear. According to their hypothesis, NO maintained a basal level of cGMP-mediated phosphodiesterase inhibition that was required for an unknown neurotransmitter to effect cutaneous vasodilation by a cAMP-dependent mechanism (9). This implied that NO had to be present for vasodilation to be effected by the neurotransmitter of active vasodilation but that the absolute concentration of NO need not increase in heat stress in the rabbit ear.

In humans, a role for NO in active vasodilation was first investigated in studies based on observing the vascular effects of NOS antagonists, such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME) (8, 12, 16, 23). Studies with these agents showed that the extent of active vasodilation during heat stress was significantly attenuated when NOS was inhibited. These studies demonstrated that NOS activity, and therefore the presence of NO, was required during heat stress for complete cutaneous active vasodilation. They did not distinguish whether concentrations of NO increased (suggesting an active effector role for NO) or remained unchanged [suggesting a role as a permissive factor (9, 12, 23)].

Crandall and MacLean (7) sought to test which of these two possibilities was correct in humans. They used the chemiluminescence technique to measure nitrite and nitrate, the oxidation products of NO, as an index of NO concentrations. They found no increase in their NO index in response to heat stress. Crandall and MacLean concluded that "whole body heating does not increase cutaneous interstitial NO concentration" and suggested "that a basal pool of NO acts permissively with an unknown neurotransmitter to elicit active cutaneous vasodilation." However, recent findings that early in heat stress, elevation of mean body temperature leads to an ACh-mediated, NO-dependent increase in SkBF conflicts with this suggestion (24).

Crandall and MacLean (7) used an indirect approach to estimate NO concentrations. We chose a direct approach to measure cutaneous interstitial NO concentrations, in vivo, during whole body heating rather than index NO concentrations through its oxidation products. We measured diffusible NO concentrations directly by amperometric, NO-selective electrodes in normothermia and heat stress. We used this technique to reexamine the finding that cutaneous interstitial NO concentrations do not increase during whole body heat stress and thus to reexamine the hypothesis that NO acts permissively in cutaneous active vasodilation.

METHODS

The approach we chose to test our hypothesis was direct: we measured diffusible NO from the cutaneous interstitial space and SkBF in human subjects. These measurements were made under both normothermic and heat stress conditions. We measured diffusible NO by a selective-membrane, amperometric electrode technique (ISO-NO Mark II, World Precision Instruments, Sarasota, FL). The ISO-NO Mark II system is based on the diffusion of NO through a NO-selective membrane that coats the electrode's surface. The diffusible NO is then oxidized at the working surface of the electrode, resulting in the generation of an electrical current. The amount of current produced is directly proportional to the concentration of diffusible NO (2, 3, 26). For our study, NO-selective electrodes were placed directly into the cutaneous interstitial space for measurement of diffusible NO.

We combined our measurements of NO with monitoring of SkBF by laser-Doppler flowmetry (LDF) from forearm skin. LDF and NO measurements were made simultaneously from skin sites no more than 1–2 mm apart to allow us to detect concurrent changes in both variables under any thermal conditions. We also verified our ability to detect changes in diffusible NO and SkBF by simultaneously examining alterations of NO concentrations and LDF in response to administration of exogenous ACh by local intradermal microdialysis. Intradermal microdialysis permitted local administration of ACh directly into the interstitial space of the dermis adjacent to the NO-sensing electrode and the LDF probe. This verification technique permitted monitoring of local effects of ACh without risking confounding systemic effects of the agent.

Nine subjects (5 men and 4 women) participated in this study. Their average age was 30 ± 4 (SE) yr, average weight was 63 ± 3 kg, and average height was 167 ± 3 cm. All subjects were in good health and were taking no medications. All subjects gave their informed consent to participate in these institutionally approved studies.

After arriving in the laboratory on the morning of the study, subjects had an intradermal microdialysis probe placed at a site on the ventral aspect of one forearm. The probe was of our own manufacture. It was made from polyimide tubing with a 1-cm length of capillary microdialysis membrane (200-μm diameter, molecular mass cutoff 20 kDa) and was reinforced by a stainless steel wire inserted through the lumen of the membrane and tubing. To place the microdialysis probe, a 25-gauge needle was inserted through the dermis by using sterile technique. The microdialysis probe was threaded through the lumen of the needle that was then withdrawn, leaving the microdialysis probe in place (12, 15, 16). The microdialysis probe was perfused with Ringer solution at a rate of 5 μl/min by using a microinfusion pump.

After insertion of the microdialysis probe, a NO-selective electrode was inserted into the dermis adjacent to the microdialysis probe. The electrode was inserted by making a small puncture through the epidermis and into the dermis with a 25-gauge needle. The needle was withdrawn, and the electrode was inserted down the track left by the needle. The NO-selective electrode was taped in place. Subjects then waited 140 min or more to allow for insertion trauma to resolve before additional instrumentation (1).

After insertion trauma resolved, subjects were placed in the supine position and instrumented to measure LDF from skin at the microdialysis-NO measurement site (MBF3D dual-channel flowmeter, Moor Instruments, Devon, UK). LDF measurements are specific to skin and are not influenced by blood flow in the underlying tissues (21).

Thermoregulatory reflexes were induced as follows. Subjects wore a tube-lined suit used to control T_{sk} by perfusion with water of different temperatures (11–13, 17). Over the suit, subjects wore a water-impermeable plastic garment to insulate them from the room environment and prevent evaporation of sweat. The suit and garment covered the entire body except for the head, the arm from which the measurements were made, the hands, and the feet. The suit was perfused with warm water to raise T_{sk} to 38–39°C during heating periods.

Internal temperature was monitored with a thermocouple placed in the sublingual sulcus (T_{or}). T_{sk} was recorded as the weighted electrical average from six thermocouples taped on the skin surface (11, 20). Pulse rate (PR) and mean arterial pressure (MAP) were recorded continuously from a finger (Finapres BP Monitor, Ohmeda, Madison, WI).

Data collection began with a 10- to 20-min normothermic control period, with T_{sk} maintained at a level of 34°C. T_{sk} was then raised to 38–39°C and maintained at that level for 35–50 min to induce heat stress and thus activate the vasodilator system. After heat stress, subjects were cooled for 30–60 min. They were then returned to a normothermic T_{sk} of 34°C for 10–15 min, and the perfusate through the probe was changed from Ringer solution to 160 mM ACh (Sigma Chemical, St. Louis, MO) in Ringer solution. ACh was perfused for 30–40 min at a rate of 5 μl/min to effect simultaneous increases in LDF and diffusible NO to verify our ability to detect changes in both variables. The protocol is illustrated in Fig. 1.

After each subject had left the laboratory, the NO electrode was calibrated for both NO concentration and temperature effects. NO concentrations were calculated from standard curves by using the protocol given by the manufacturer. Final calculated NO concentrations were derived from these standard curves. Standard curves for temperature effects over a range of 30–36°C were made and used to correct NO values for changes in the local temperature recorded at the NO measurement site over the course of a study.

Data are presented as means ± SE. For data analysis, cutaneous vascular conductance (CVC) was indexed as LDF (in mV) divided by MAP (in mmHg). The vasomotor and NO responses to heat stress were analyzed by comparing the internal temperature thresholds for increases in both variables. The internal temperature threshold for the onset of vasodilation was defined as the level of T_{or} at which a sustained increase in CVC began, after T_{sk} had been increased to 38°C. The internal temperature threshold for increased concentrations of NO was defined as the level of T_{or} at which a sustained increase in NO concentrations began after T_{sk} had been increased to 38°C. Both T_{or} thresholds were chosen from individual graphs of CVC or NO concentrations vs. time by an investigator blinded as to the conditions, subjects, and measured variable of the graph. Pre-heat stress levels of CVC were compared with the levels achieved during the last minute of heat stress. A separate comparison of the pre-heat stress NO concentration was made with the concentration measured during the final minute of heat stress. The vascular and NO responses to exogenous ACh were analyzed by comparing the levels obtained during perfusion with Ringer solution alone, with values obtained during perfusion of 160 mM ACh in Ringer solution. Responses were analyzed by paired *t*-tests.

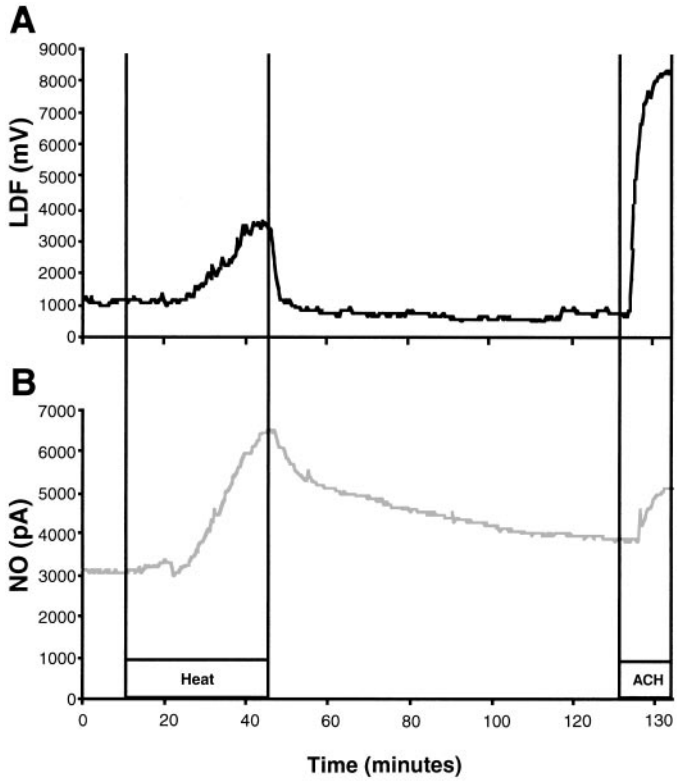

Fig. 1. Illustration of protocol and results for laser-Doppler flowmetry (LDF) and amperometric nitic oxide (NO) electrode measurements from 1 subject. The protocol began with a normothermic control period, which was followed by whole body heating to induce heat stress and thus activate the cutaneous vasodilator system. After heat stress, subjects were cooled and ACh was administered by intradermal microdialysis to confirm the ability of the amperometric electrode to measure NO concentrations.

RESULTS

Under normothermic conditions, with T_{sk} maintained at 34°C, subjects' T_{or} averaged 36.74 ± 0.05°C, and PR averaged 58 ± 3. After T_{sk} had been increased to effect heat stress, T_{or} progressively increased to a threshold for the onset of vasodilation of 36.96 ± 0.08°C. The concentration of NO began to increase at a T_{or} threshold of 37.01 ± 0.10°C. There was no statistical difference between these threshold temperatures. These results are illustrated in Fig. 2.

During the initial period of normothermia, CVC averaged 12 ± 3 mV/mmHg and NO concentrations averaged 548 ± 108 nM. Both of these values rose during heat stress. By the peak of heat stress, T_{or} had increased to 37.50 ± 0.10°C ($P < 0.01$) and PR had

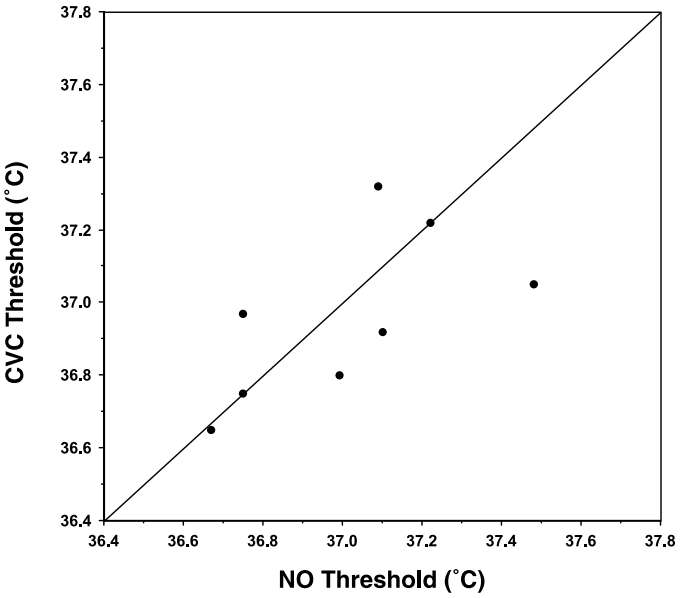

Fig. 2. Identity plot illustrating the similarity of oral temperature threshold values for the onset of cutaneous vasodilation and increases in cutaneous interstitial NO concentrations during body heating. Note that points fall along the line of identity (solid line). Overall, no statistically significant difference was found between threshold values of oral temperature for increases on skin blood flow [cutaneous vascular conductance (CVC)] and NO concentrations during the early phase of heat stress.

increased to 90 ± 3 ($P < 0.01$). CVC had increased to an average of 48 ± 5 mV/mmHg ($P < 0.01$, normothermia vs. heat stress), and NO concentrations had increased to an average of 663 ± 126 nM ($P < 0.01$, normothermia vs. heat stress). From normothermic levels, CVC increased 402 ± 76% and NO concentrations increased 22 ± 5% by the peak of heat stress. These results are summarized in Fig. 3.

After heat stress, when subjects had been cooled and returned to normothermia, T_{or} averaged 36.88 ± 0.05°C and PR averaged 58 ± 3. T_{or} and PR remained at these levels throughout the remainder of the study. During this post-heat stress period, when the microdialysis fibers were perfused with Ringer solution, CVC averaged 15 ± 3 mV/mmHg and NO concentrations averaged 596 ± 121 nM. Both of these values rose when the perfusate was changed from Ringer solution alone to 160 mM ACh in Ringer solution. By the last minute of ACh perfusion, CVC had increased to an average of 50 ± 10 mV/mmHg ($P < 0.01$, Ringer vs. ACh) and NO concentrations had increased to an average of 735 ± 148 nM ($P < 0.01$, Ringer vs. ACh). Perfusion with ACh increased CVC by 496 ± 119% and NO concentrations by 24 ± 3%. These results are summarized in Fig. 4.

DISCUSSION

The results of our study demonstrate that the cutaneous interstitial concentration of NO increases with SkBF during heat stress in humans. Both SkBF and the concentration of NO began to increase at similar internal temperature thresholds. From normothermic levels, T_{or} increased by ~0.8°C over the course of heat stress. When compared with normothermic values, diffusible NO concentrations and CVC in the skin both increased significantly during heat stress with the increase in T_{or}. Our findings that cutaneous interstitial NO concentrations increase with SkBF during heat stress in humans suggest that an increased concentration of NO is mechanistically related to the increase in SkBF that occurs during heat stress. Such a role is also consistent with observations of attenuated cutaneous vasodilation during hyperthermia with NOS antagonists (12, 23).

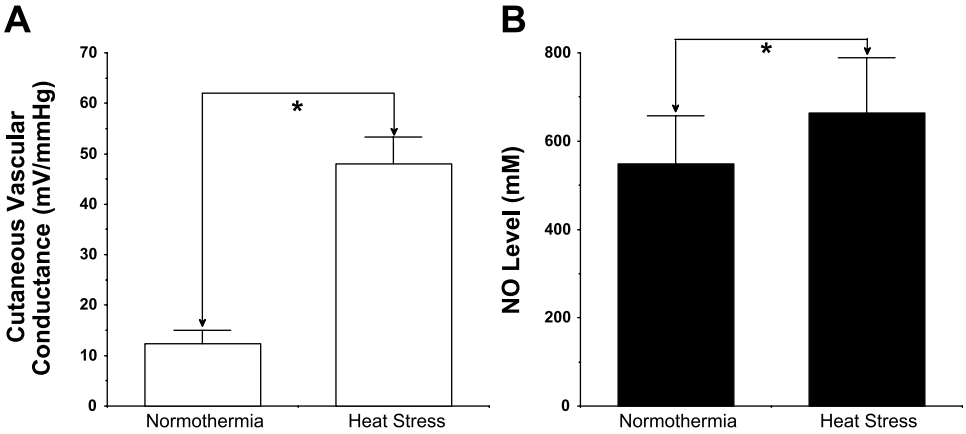

Fig. 3. Summary of CVC (*A*) and diffusible NO levels (*B*) in normothermia and during heat stress. Values are means ± SE. CVC was indexed as LDF (mV)/mean arterial pressure (mmHg). NO levels in the cutaneous interstitial space were measured by a selective-membrane, amperometric electrode technique. From normothermic levels to the peak on heat stress, both CVC and diffusible NO levels increased significantly (*$P < 0.01$, CVC and NO, normothermia vs. heat stress). These data demonstrate that the cutaneous interstitial concentration of diffusible NO increases with whole body heating as does skin blood flow. In addition, these findings suggest that the role of NO in cutaneous active vasodilation may not be that of a permissive factor that must be present for vasodilation to occur; rather they suggest that the absolute level of NO plays an active role in cutaneous active vasodilation.

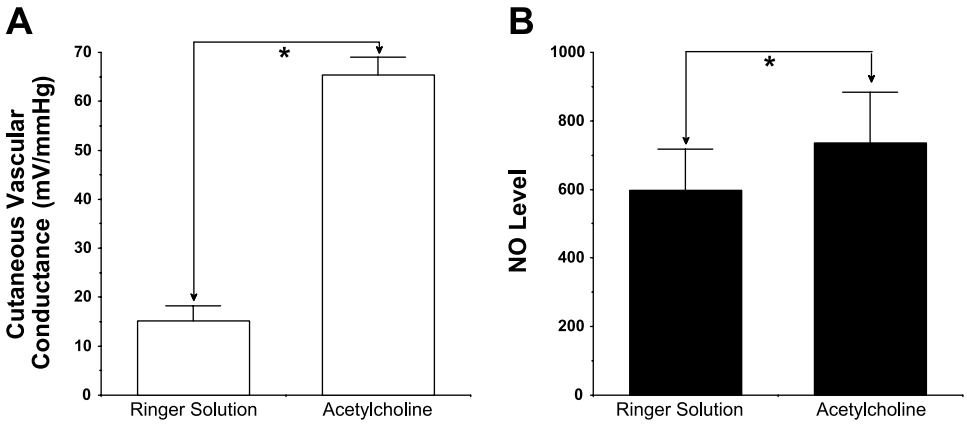

Fig. 4. Summary of CVC (*A*) and diffusible NO levels (*B*) in response to exogenous ACh during normothermia. Values are means ± SE. CVC was indexed as LFD (mV)/mean arterial pressure (mmHg). NO levels in the cutaneous interstitial space were measured by a selective membrane, amperometric electrode technique. To verify the ability of our combination technique to detect simultaneous changes in CVC and NO, an intradermal microdialysis fiber was placed adjacent to the CVC and NO measurement sites. Basal levels of CVC and diffusible NO were monitored during perfusion of the intradermal microdialysis fiber with Ringer solution. The perfusate was then changed to a 160 mM solution of ACh in Ringer solution. When compared with Ringer solution alone, ACh perfusion increased both CVC and diffusible NO levels significantly (*$P < 0.01$, CVC and NO, normothermia vs. ACh). These data demonstrate that both CVC and the cutaneous interstitial concentration of diffusible NO increase in response to exogenous ACh and thus verify that our combination technique detects simultaneous changes in SkBF and diffusible NO levels.

The role of NO in cutaneous active vasodilation has been studied by a number of researchers using a variety of techniques over the last decade. The first question addressed was whether NO had any role in cutaneous active vasodilation during heat stress. The initial approaches to this issue examined the effects of NOS antagonists on the extent of cutaneous vasodilation during hyperthermia (8, 12, 23).

The first of these studies was done by Dietz et al. (8), who infused L-NMMA (a NOS antagonist) intra-arterially in the forearm and examined the effect on forearm vasodilation during body heating. They found no attenuation of the reflex vasodilator response by L-NMMA and concluded that the NO system had little or no role in cutaneous active vasodilation. However, some concern arose about the adequacy of the L-NMMA blockade because six of the eight subjects had a full vasodilator response to intra-arterial ACh at the end of the study. This suggested that the L-NMMA blockade may have been incomplete during the latter part of their studies.

Several subsequent studies clarified the role of the NO system in cutaneous active vasodilation in humans (12, 23). Our laboratory tested the role of NO in this process by continuous administration of the NOS inhibitors, L-NAME and L-NMMA by intradermal microdialysis probes (12, 14). It was found that active vasodilation during heat stress was attenuated, but not abolished, by NOS inhibition (12, 14). Shastry et al. (23) also investigated this issue with LDF from forearm skin in combination with intra-arterial infusion of the NOS inhibitor, L-NMMA. This agent was infused intra-arterially into one forearm at higher doses than those used by Dietz et al. (8). LDF was simultaneously monitored from that arm and from the contralateral forearm that served as an untreated control. L-NMMA was delivered after cutaneous vasodilation had been established by whole body heating. In the arm that received L-NMMA, CVC decreased modestly to 86 ± 5% of the peak response to hyperthermia. CVC remained constantly elevated in the untreated, control arm. These studies demonstrated that NOS activity, and therefore the presence of NO, were required for complete cutaneous active vasodilation (12, 23).

Although the foregoing studies showed a role for NO in human cutaneous active vasodilation, there remained two possible mechanisms: *1*) levels of diffusible NO could increase to effect cutaneous vasodilation or *2*) NO levels could remain unchanged so that NO acted only as a permissive factor as in the rabbit ear (9, 12, 23). The latter mechanism was initially suggested by Farrell and Bishop (9), who proposed that NO acted as a "permissive factor" that need not vary in concentration, but had to be present for vasodilation to occur.

As previously noted, Crandall and MacLean (7) failed to find any increase in NO breakdown products from the cutaneous interstitial space during heat stress and suggested that NO acted as a permissive factor for active vasodilation. In contrast, we used a direct measurement of diffusible NO. We found that SkBF and NO concentrations both began to increase at similar internal temperatures during body heating. We found concomitant increases in SkBF and NO concentrations during heat stress. Although our results do not exclude a permissive role for NO, our finding that NO concentrations increase at the onset of active vasodilation and continue to increase during heat stress suggest that NO may be an effector of the process. Such a role for NO is consistent with the finding of attenuated cutaneous active vasodilation after inhibition of NO generation by NOS antagonists during heat stress (12, 23).

The increase in diffusible NO in skin during heat stress that we report could be generated by several mechanisms. These mechanisms include the following: *1*) stimulation of NO production by ACh released from cutaneous nerves, *2*) stimulation of NO production by cholinergic cotransmitter release, *3*) increased generation of NO by endothelial NOS (eNOS) due to increased shear stress on endothelial cells, and/or *4*) enhanced release of NO from neuronal NOS (nNOS) in cutaneous nerves.

Recent work by Shastry et al. (22) suggests that cholinergic nerve stimulation of NO generation by ACh during heat stress is not the mechanism involved. Shastry and coworkers performed intra-arterial infusions and microdialysis administration of atropine and L-NAME during established whole body heat stress. They found that atropine did not blunt the effects of L-NAME given during established hyperthermia. They were thus able to confirm that NO contributed to active vasodilation; however, they also found that ACh activation of muscarinic receptors was not responsible for generation of the requisite NO.

A second possibility is that, during heat stress, a neurotransmitter other than ACh is released that causes an increase in NO production. Cutaneous active vasodilation is mediated by a cholinergic cotransmitter system (17); however, the exact identity of the cotransmitter is unknown. According to this possibility, the release of the neurotransmitter, presumably the cotransmitter, during heat stress would stimulate NO production and thus increase the concentration of NO.

A third means by which cutaneous interstitial NO concentrations could increase during heat stress is by increased shear stress (22). It is possible that during heat stress, release of neurotransmitters from the cholinergic cotransmitter system increases SkBF (17). The increase in SkBF could then increase shear stress on the endothelial cells that in turn generate more NO through activation of eNOS. The fact that eNOS is constituitively expressed in human skin is consistent with this possibility (4).

A fourth possible mechanism for increased cutaneous interstitial NO concentrations during heat stress is by increased release of NO from neuronal sources (22). According to this possibility, the mechanisms that effect cutaneous active vasodilation would involve nitroxidergic neurotransmission in which increased release of NO generated by nNOS would function as a neurotransmitter. This possibility would require that the nerves that elaborate NO be sensitive to botulinum toxin as cutaneous active vasodilation is abolished by this agent (17). This possibility is supported by the observation that nNOS is constituitively expressed in human skin (4).

The amperometric, NO-selective electrode technique allowed us to make direct measurements of diffusible NO. Our direct measurements of NO with the amperometric electrode showed that basal diffusible NO concentration was 548 ± 108 nM, similar to those values reported by Clough et al. on the basis of NO breakdown products [i.e., 490 ± 60 nM (6) and 600 ± 140 nM (5)]. In contrast, both our values and those of Clough et al. differ markedly from those published by Crandall and MacLean (7). Using a chemiluminescent technique, to estimate actual NO concentrations from nitrite and nitrate levels, Crandall and MacLean estimated the basal, interstitial concentration in skin to be 7,600 ± 700 nM. This value is 10-fold greater than values found by Clough et al. (5, 6) or in the present study. The reasons for this large difference are unclear; however, the present study and those published by Clough et al. employed amperometric techniques for NO measurement. Our study measured diffusible NO directly from human skin, whereas the studies by Clough et al. measured NO generated by chemical reduction of the NO breakdown products nitrite and nitrate. Crandall and MacLean also measured NO regenerated from NO breakdown products, but they made their actual NO measurements by the chemiluminescent technique. The fact that both of these groups used a similar approach to indexing NO concentrations from breakdown products, but obtained vastly disparate results with different techniques, suggests that the techniques employed may be the source of the differences in reported NO concentrations. Indeed, the fact that we were able to detect an increase in NO with the amperometric electrode technique that was undetected by the chemiluminescent approach suggests that the former approach may be more sensitive than the latter.

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