The ability to raise skin blood flow (SkBF) in response to environmental heat stress diminishes with advanced age (8, 9, 13, 17), particularly in those over the age of 65 yr. This attenuated SkBF response may contribute to the much higher incidence of heat-related illness and death in the elderly (5). Recent evidence has shown that the diminished ability to reflexively increase SkBF in the elderly is due to either an alteration in the active vasodilator mechanism or the skin vascular responsiveness to a given level of stimulation (9). A common tool used to evaluate changes in microvascular or endothelial function is the direct application of heat to an area of skin while evaluating the SkBF response. Locally heating an area of skin causes an increase in SkBF that is partially mediated by, or dependent on, nitric oxide (NO) (7, 12).

Several investigators have shown that maximal SkBF to prolonged local heating is significantly reduced in older vs. young subjects (4, 11, 15, 16, 22). It has been suggested that both functional and structural changes occur in the skin vasculature with aging that may account for the reduced SkBF to this stimulus (1, 11, 22). However, no studies have investigated the specific mechanisms that underlie changes in SkBF during local heating with aging, because until recently the mechanisms that mediate the SkBF response to local heating were unknown. Our laboratory recently reported that there are at least two independent mechanisms that contribute to the rise in SkBF during submaximal local heating: a fast-responding vasodilator primarily mediated by the axon reflexes and a more slowly responding vasodilator system that relies on local production of NO (12). There is evidence to suggest that NO-dependent mechanisms may be reduced with advanced age, and this may contribute to the attenuated SkBF responses. It has been reported that levels of the NO precursor L-arginine and the metabolites of NO, nitrite and nitrate, are reduced with aging (14). Furthermore, the SkBF response to iontophoresis of sodium nitroprusside, a NO donor, is reduced in older subjects (1). These data suggest that the ability to produce NO or the vascular responsiveness to NO may be attenuated with advanced age. However, depending on the local heating protocol, a number of different vasodilator mechanisms may be activated. For example, a very rapid rise in skin temperature, a prolonged period of heating, or a slight
sensation of thermal pain during heating can cause the cutaneous vasodilation to be insensitive to NO synthase (NOS) inhibition (7, 10, 12). The specific mechanisms activated under these conditions are not known. Because our goal in the present study was to examine the role of NO in the attenuated SkBF response to local heating with age, we employed a local heating protocol that our laboratory previously determined would allow us to examine the contribution of NO and the axon reflexes to the SkBF response to local heating (12). We hypothesized that the sustained NO-dependent vasodilation, but not the initial rise in SkBF mediated by the axon reflex, would be diminished with advanced age during local heating.

METHODS

Subjects. We recruited 10 young (18–24 yr old; 5 men and 5 women) and 10 older subjects (69–84 yr old; 5 men and 5 women) to participate in this study. All young women were studied during menstruation (early follicular phase of the menstrual cycle) because the menstrual cycle is known to alter the SkBF response to local heating (2). All older women were postmenopausal and were not on hormone replacement therapy. All subjects were healthy, normotensive, nonsmokers, and not taking any medications. Institutional Review Board approval was obtained, and each subject gave informed consent before participation.

Instrumentation. Subjects wore a water-perfused suit to clamp whole body skin temperature between 32 and 33°C. The water-perfused suit did not cover the face or the forearm being studied, and it was used to minimize the influence of whole body skin temperature on reflex changes in SkBF at the local skin sites. Respiration and electrocardiogram were continuously measured throughout the study.

Two microdialysis fibers (MD 2000, Bioanalytical Systems) with a membrane length of 10 mm and a 20-kDa cutoff were placed in the skin at least 5 cm apart in the ventral aspect of the nondominant forearm of each subject. The ventral aspect of the forearm was used to minimize the effects of chronic sun exposure on the results of the study and thereby isolate the effects of aging on the cutaneous vascular responses to local heating. Insertion of the microdialysis fibers was performed by first placing a 25-gauge needle just under the surface of the skin with entry and exit points ~3 cm apart. The microdialysis fiber was then threaded through the needle so that the microdialysis membrane was 1 cm from the lumen of the needle. The needle was then partially withdrawn as the microdialysis membrane was pulled into place in the skin. Once the membrane was in place between the insertion and exit points, the needle was completely withdrawn. The microdialysis fibers were taped in place, and Ringer solution was perfused through the fibers at a rate of 2 µl/min.

To obtain an index of SkBF, cutaneous red blood cell (RBC) flux (in mV) was measured over the two microdialysis sites using a Moor laser-Doppler flowmetry (LDF) system (DRT-4). Skin temperature was controlled at the two microdialysis sites with Moor local heating units (SH02) each covering ~100 mm² of tissue. RBC flux was measured directly over the microdialysis membrane. To ensure that blood pressure was stable throughout the experimental protocols, blood pressure was measured at 10-min intervals by brachial auscultation in the dominant arm.

Protocol. After placement of the microdialysis fibers, RBC flux over the microdialysis sites was monitored to ensure that insertion trauma had resolved before the studies were started (between 60 and 130 min). The temperature of the local heating units at the microdialysis sites was kept constant at 33°C during the baseline period. One of the microdialysis sites was randomly assigned to receive 10 mM N⁶-nitro-L-arginine methyl ester (L-NAME; Calbiochem) dissolved in Ringer solution. We performed pilot studies in young and older subjects to determine that doses of L-NAME above 10 mM did not result in a greater inhibition of NO production by NOS. The microdialysis fibers were perfused at a rate of 2 µl/min for at least 30 min before local heating to inhibit NOS. Infusion of L-NAME in this site was continued throughout local heating. After L-NAME infusion and baseline measurements, the local heating protocols were performed as follows. Temperature of the local heating units was increased at a rate of 0.5°C every 5 s to a temperature of 42°C. This results in an increase in skin temperature to ~40°C at the heating probe-skin surface interface (12). Subjects did not feel any sensations of pain during the rise in temperature at this rate or with prolonged heating at this temperature. The local heating units were held constant at 42°C throughout the entire protocol. After RBC flux in both sites had reached a stable plateau (~30–40 min), 10 mM L-NAME was infused through the second microdialysis site (previously infused with Ringer solution only) at a rate of 2 µl/min. Local heating was continued in both sites until RBC flux at this site decreased to a new stable plateau for at least 10 min. The local heaters were then returned to 33°C, and 28 mM sodium nitroprusside (Nitropres, Ciba Pharmaceutilicals) was infused through the microdialysis fibers for 20–30 min to maximally vasodilate the skin at both sites. We performed pilot studies in young and older subjects to determine that 28 mM sodium nitroprusside was a sufficient dose to maximally vasodilate the skin at the microdialysis sites. Cutaneous vascular conductance (CVC) was calculated as LDF (mV²/m²/min)–mean arterial pressure (MAP; mmHg) to account for any differences in blood pressure between the groups. Data are expressed as percentage of maximal CVC obtained during nitroprusside infusion (%CVCmax).

Data analysis. Data were digitized and stored on a computer at 100 Hz. Data were analyzed offline with signal-processing software (Windaq, Dataq Instruments, Akron, OH). Baseline, plateau, and the reduction in CVC with NOS inhibition (post-NOS inhibition drop) were calculated by averaging values over a stable 10-min period. Initial peak and nadir CVC values were calculated by averaging the highest and lowest values, respectively, over a stable 30-s period. The phases of the SkBF response to local heating are presented in Fig. 1.

Statistical analysis. All data are presented as means ± SE. Group characteristics, the contribution of NO to the initial peak and plateau phase of the SkBF response to local heating, and maximal CVC values to 28 mM sodium nitroprusside were compared by using t-tests. CVC during baseline, initial peak, nadir, plateau, and the drop in SkBF with NOS inhibition were analyzed by two-way ANOVA (age group × local heating phase) with repeated measures. When a significant interaction effect was observed, Tukey’s post hoc analysis was used to identify significant differences in the pairwise comparisons. The level of significance was set at P < 0.05.

RESULTS

Subject characteristics are presented in Table 1. No gender differences in CVC were observed in either age group. Thus the data from men and women were combined for each group. Baseline systolic blood pressure
and MAP were significantly higher in the older subjects (both P < 0.05).

At the initiation of heating, skin temperature at the heating probe-skin surface interface rapidly increased to ~40°C and remained stable throughout the heating protocol. No subject reported feeling any sensation of pain during local heating of either site. Figure 1A is a representative tracing of the CVC responses to the local heating protocol when NOS was inhibited after 40 min of heating in a young and older subject. Figure 1B displays the group data (means ± SE) during baseline, initial peak, nadir, plateau, and the decline in cutaneous vascular conductance with NOS inhibition (post-NOS inhibition). *Significant difference from the young group, P < 0.05.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>174 ± 3</td>
<td>67.3 ± 4.0</td>
<td>23.6 ± 1.1</td>
<td>120 ± 4</td>
<td>71 ± 3</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>Older</td>
<td>169 ± 3</td>
<td>72.7 ± 5.3</td>
<td>23.8 ± 0.8</td>
<td>137 ± 6*</td>
<td>79 ± 4</td>
<td>99 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *Significant difference from the young group, P < 0.05.

was no significant main effect of age on the CVC response to local heating (P = 0.155). This was not unexpected because the general pattern of the SkBF response to local heating was similar in the two groups. There was a significant main effect for phase of local heating (P < 0.001). There was a significant interaction (age × local heating phase; P = 0.002) with significant differences by pairwise comparisons observed during the initial peak (young: 61 ± 2%CVCmax vs. older: 46 ± 4%CVCmax; P < 0.05) and plateau (young: 93 ± 2%CVCmax vs. older: 82 ± 5%CVCmax; P < 0.05) phases, displaying diminished responses in the older group. No significant differences in CVC were observed between the groups during baseline, nadir, or after NOS inhibition.

Figure 2 displays the contribution of NO to the plateau phase of the SkBF response to local heating. Values were calculated by subtracting CVC values after NOS inhibition from CVC during the plateau phase in the site in which NOS was inhibited during the stable plateau phase. The contribution of NO to the plateau phase during local heating was significantly less in the older subjects (young: 75.0 ± 2.3%CVCmax vs. older: 61.1 ± 4.5%CVCmax; P < 0.05).

Figure 3A is a representative tracing of the CVC responses to the local heating protocol when NOS was inhibited before and throughout the local heating protocol in a young and older subject. Figure 3B displays the group data (means ± SE) during baseline, initial peak, nadir, and plateau phase when NOS was inhibited throughout the protocol. There was no significant main effect for age on the CVC response to local heating (P = 0.160). There was a significant main effect for local heating phase (P < 0.001), and for the interaction...
age group × local heating phase; \( P = 0.04 \), with a significant difference by a pairwise comparison only observed during the initial peak (young: 52 ± 4\( \% \)CVC\(_{\text{max}} \) vs. older: 38 ± 5\( \% \)CVC\(_{\text{max}} \); \( P = 0.004 \)). No significant differences were observed between the groups during baseline, nadir, or between 30 and 40 min of local heating in this site.

To examine the contribution of NO to the initial peak in both groups of subjects, the difference in peak CVC values from the two sites (initial peak before NOS inhibition - initial peak with NOS inhibition; expressed as Δ\( \% \)CVC\(_{\text{max}} \)) were compared. No difference between the groups was observed (young: 8.6 ± 3.6 Δ\( \% \)CVC\(_{\text{max}} \) vs. older: 8.0 ± 3.5 Δ\( \% \)CVC\(_{\text{max}} \); \( P = 0.90 \)). This demonstrates that the attenuated initial peak in the older subjects was not due to diminished NO-dependent vasodilation. Maximal CVC obtained by infusion of 28 mM sodium nitroprusside at the end of local heating (expressed as mV/100 mmHg) was significantly less in the older subjects (young: 192 ± 12 mV/100 mmHg vs. older: 156 ± 15 mV/100 mmHg; \( P = 0.03 \)).

**DISCUSSION**

The goal of this study was to investigate the mechanisms that underlie the observed age-related attenuation of cutaneous vasodilator responses in humans. We performed two local heating trials in young and older subjects to examine the role of NO and the axon reflex in the SkBF response to a local heating protocol. We found that the initial rise in SkBF and the sustained vasodilation to local heating were significantly diminished in the older subjects. This finding suggests that healthy aging impacts the nerves that mediate the axon reflex or vascular responsiveness to the neurotransmitters released from these nerves. The smaller sustained rise in SkBF during prolonged local heating in the older subjects further suggests the ability to either produce or respond to NO is diminished with advanced age. Importantly, our findings cannot simply be explained by an age-related reduction in maximal SkBF, because the responses were evaluated as a percentage of each individual subject’s maximal vasodilation obtained at each experimental site.

The initial rise in SkBF during local heating appears to be predominantly mediated by an axon-reflex mechanism that remains robust when NOS is inhibited (12). In older subjects, we observed an attenuated initial peak response that was not due to diminished NO-dependent vasodilation. NO only contributes modestly to the initial peak response, and the magnitude of the reduction in the initial peak was similar in the two groups with NOS inhibition. Presently, we can only speculate on the neurotransmitters involved in the initial response to local heating and how it is reduced with advanced age. Recent evidence suggests that calcitonin gene-related peptide is the most likely neurotransmitter involved in the axon reflex (18). However, substance P cannot be ruled out because of the difficulty in measuring this peptide. Calcitonin gene-related peptide is one of the most abundant neuropeptides in the skin, and it is found alone or colocalized with substance P (21). Thus it appears likely that aging results in either a diminished release of these neurotransmitters or a diminished responsiveness of the vasculature to these neurotransmitters. This finding was in contrast to our hypothesis that only the NO-dependent portion of the local heating response would be diminished with advanced age. All of our older subjects were healthy, and none of them reported having decreased sensations to thermal or painful stimuli. Importantly, the rapid rise in SkBF in response to local heating is a vital step in protecting the skin from acute trauma. For example, the increase in blood flow will minimize the heat transferred to the tissues to protect the skin from damage. A diminished ability to rapidly increase SkBF in response to directly applied heat may make the elderly more susceptible to local tissue damage. Thus studies are needed to further explore the exact mechanisms involved in the initial rise of SkBF during local heating and to determine how aging impacts these mechanisms.
The plateau phase of the SkBF response to local heating is primarily, but not entirely, mediated by NO (12). When NOS is inhibited during the sustained plateau in SkBF, SkBF decreases to a value that remains significantly elevated above baseline levels even when the cutaneous vasoconstrictor nerves are blocked (12). This suggests that an unknown vasodilator is present and mediates a portion of the plateau-phase dilation. Our finding of a diminished plateau phase (Fig. 1) and a significantly smaller contribution of NO to the plateau phase (Fig. 2) in the older subjects suggest that NO-dependent vasodilator mechanisms in the skin are diminished with advanced age. If plateau CVC values were similar in the two groups of subjects, then the decline in CVC with NOS inhibition would be similar and we would not be able to conclude that NO-mediated dilation was diminished with age. Alternatively, if post-NOS inhibition values were significantly less in the older subjects, then the lower plateau CVC values would be due to diminished vasodilation to the unknown vasodilator and not due to attenuated NO-mediated vasodilation. Taken together, we interpret our findings to suggest that the cutaneous vasculature either produces less NO or is less responsive to NO with age.

The exact mechanism by which NO causes vasodilation in the skin during local heating is not known. It seems likely that the locally applied heat directly causes increased NO production by endothelial cells. However, there are other potential mechanisms by which NO may mediate cutaneous vasodilation. For example, NO is known to contribute ~30% to the rise in SkBF during whole body heating, even though skin temperature at the measurement site does not increase (6, 19, 20). Crandall and MacLean (3) were unable to measure a rise in NO concentration in the skin during whole body heating, suggesting that NO may play a “permissive role” in active vasodilation. That is, during whole body heating, NO may only need to be present to allow the activity of another vasodilator substance to achieve full expression. It is possible that NO is playing a similar role in mediating vasodilation to local heating, but this remains to be tested.

Attenuated NO-dependent vasodilation in the elderly could be due to diminished levels of the NO precursor L-arginine. In support of this concept, Rckelhoff et al. (14) reported that levels of L-arginine and the metabolites of NO, nitrite and nitrate, are reduced with age. Alternatively, another possibility could be that the transduction of the NO signal in the smooth muscle of the skin could be diminished with age, causing reduced vascular responsiveness. In agreement with this concept, others have reported that the rise in SkBF to sodium nitroprusside iontophoresis is diminished with advanced age (1). Sodium nitroprusside is an endothelium-independent vasodilator acting through NO, so a reduced SkBF response to sodium nitroprusside iontophoresis in older subjects suggests the smooth muscle does not dilate to the same extent as in younger subjects for a given level of NO.

We found that the rise in SkBF to the infusion of 28 mM sodium nitroprusside (sufficient to cause maximal vasodilation in both young and older subjects) was significantly lower in the older subjects. Although this finding is consistent with our concept of reduced vascular responsiveness to NO with age, we believe that this finding needs to be interpreted with caution. Site-to-site variability of the laser-Doppler technique makes a comparison of data expressed in arbitrary units of RBC flux between subjects, or even from one area of skin to another within a subject, tenuous at best. We used the infusion of 28 mM sodium nitroprusside as a tool to obtain the maximal vasodilator capacity of each site to allow comparisons between the groups of subjects. However, our finding of a reduced maximal SkBF with age by using these techniques is consistent with studies that maximally vasodilated the skin by prolonged local heating (4, 11, 15, 16, 22). These studies found that the increase in SkBF during local heating sufficient to maximally vasodilate the skin is diminished in the older subjects (4, 11, 15, 16, 22). It was suggested that this represents a structural limitation in the skin of older individuals (1, 11, 22). However, maximally vasodilating the skin by local heating may activate a number of mechanisms that could mediate the vasodilation (7, 10, 12). Kellogg et al. (7) first reported that even a very brief sensation of mild pain (<5–10 s) during local heating rendered the vasodilation insensitive to NOS inhibition. We confirmed these findings and further found that a very rapid rise in local heating temperature altered the mechanisms of vasodilation and eliminated the bimodal increase in SkBF (12). Magerl and Treede (10) reported that a sustained skin temperature of >41°C may activate specific nociceptive neurons with or without the sensation of pain. Thus we chose to use a submaximal local heating protocol that we knew would allow us to specifically examine the role of NO and the axon reflex in our young and older subjects. Although we cannot rule out the possibility that differences in maximal cutaneous vasodilation may be due to structural limitations of the skin with age, our data may also suggest that there are functional changes in the nerves, neurotransmitters, or vascular responsiveness to locally applied heat with advanced age.

In summary, the initial rise in SkBF and the maintained high SkBF during local heating of the skin are diminished with advanced age. These results suggest that axon reflex- and NO-dependent vasodilatation are reduced in the skin of healthy older individuals. Future studies are needed to further investigate the exact mechanisms that underlie the increase in SkBF with local heating and to develop possible strategies to minimize the impact of age on these mechanisms.

The considerable time and effort of the subjects are greatly appreciated.

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