Nitric oxide and attenuated reflex cutaneous vasodilation in aged skin

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Thermoregulatory cutaneous vasodilation is diminished in the elderly. The goal of this study was to test the hypothesis that a reduction in nitric oxide (NO)-dependent mechanisms contributes to the attenuated reflex cutaneous vasodilation in older subjects. Seven young (23 ± 2 yr) and seven older (71 ± 6 yr) men were instrumented with two microdialysis fibers in the forearm skin. One site served as control (Ringer infusion), and the second site was perfused with 10 mM NG-nitro-L-arginine methyl ester to inhibit NO synthase (NOS) throughout the protocol. Water-perfused suits were used to raise core temperature 1.0°C. Red blood cell (RBC) flux was measured with laser-Doppler flowmetry over each microdialysis fiber. Cutaneous vascular conductance (CVC) was calculated as RBC flux per mean arterial pressure, with values expressed as a percentage of maximal vasodilation (infusion of 28 mM sodium nitroprusside). NOS inhibition reduced CVC from 75 ± 6% maximal CVC (CVCmax) to 53 ± 3% CVCmax in the young subjects and from 64 ± 5% CVCmax to 29 ± 2% CVCmax in the older subjects by a 1.0°C rise in core temperature. Thus the relative NO-dependent portion of cutaneous active vasodilation (AVD) accounted for ~23% of vasodilation in the young subjects and 60% of the vasodilation in the older subjects at this level of hyperthermia (P < 0.001). In summary, NO-mediated pathways contributed more to the total vasodilatory response of the older subjects at high core temperatures. This suggests that attenuated cutaneous vasodilation with age may be due to a reduction in, or decreased vascular responsiveness to, the unknown neurotransmitter(s) mediating AVD.

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taking medications that had the potential to impact the cardiovascular or thermoregulatory variables of interest. One older subject was taking 81 mg of aspirin, and one older subject was taking Pamelor. The subject taking aspirin abstained from this medication for 72 h before participation in the study. These data were included in the older aspirin sub-group data because there was no difference between their individual data and the group data. Older subjects underwent a physician-supervised maximal graded exercise test at least 1 wk before participating in the study to ensure that they did not have any underlying cardiovascular disease. Subject characteristics are summarized in Table 1.

**Subject characteristics**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>Body Mass Index</th>
<th>Resting MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older</td>
<td>71.3 ± 2.2*</td>
<td>1.79 ± 0.07</td>
<td>83.7 ± 3.2*</td>
<td>26.1 ± 0.8*</td>
</tr>
<tr>
<td>Younger</td>
<td>22.6 ± 1.6</td>
<td>1.79 ± 0.03</td>
<td>74.0 ± 3.1</td>
<td>23.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 in each group. MAP, mean arterial pressure. *Significant difference from younger subjects (P < 0.05).

**Protocol.** After placement of the microdialysis fibers, RBC flux over each microdialysis site was monitored to ensure that the initial hyperemia caused by the insertion trauma had resolved before the study started. One microdialysis site was randomly assigned to receive 10 mM N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME; Calbiochem, San Diego, CA) dissolved in lactated Ringer solution to inhibit NO production by nitric oxide synthase (NOS), and the other site received only lactated Ringer solution. These infusions were maintained throughout the baseline and heating periods. Our laboratory previously showed (16) that this dose of L-NAME is sufficient to maximally inhibit NO production in both subject groups. The microdialysis fibers were perfused at a rate of 2.0 μl/min for at least 30 min to ensure adequate NOS inhibition before starting data collection. A 10-min baseline period of measuring RBC flux by laser-Doppler flowmetry was obtained. After baseline measurements, 50°C water was circulated through the water-perfused suit to raise T\textsubscript{or}. All subjects were heated until T\textsubscript{or} had increased by 1.0°C. The subjects were then cooled to their baseline T\textsubscript{or} by circulating 32°C water though the water-perfused suit. After cooling, the NO donor sodium nitroprusside (SNP, 28 mM; Nitropres, Ciba Pharmaceuticals) was infused through both microdialysis fibers for 30 min at a rate of 4.0 μl/min to obtain maximal CVC at both sites. We previously determined (16) that 28 mM SNP maximally vasodilates the skin of both groups of subjects.

**Data acquisition and analysis.** Data were digitized and stored on a computer at 150 Hz. Data were analyzed offline with signal processing software (Windaq; Data Instruments, Akron, OH). CVC values were determined by averaging values over a stable 2-min period for a given rise in T\textsubscript{or}. In general, the change in T\textsubscript{or} from baseline (ΔT\textsubscript{or}) was used to compare the groups and for graphic display. However, we also analyzed the absolute T\textsubscript{or} thresholds for reflex vasodilation. A reviewer blinded to the age of the subjects visually identified the absolute T\textsubscript{or} at which the threshold for reflex cutaneous vasodilation was initiated in both microdialysis sites. All data are presented as a percentage of maximal CVC (%CVC\textsubscript{max}). The relative percent NO contribution to CVC for a given rise in T\textsubscript{or} was calculated as [(control CVC − NO-inhibited CVC)/control CVC] × 100.

**Statistical analyses.** Student’s t-tests were used to determine significant differences between the young and older groups for physical characteristics, baseline T\textsubscript{or}, baseline T\textsubscript{sk}, absolute T\textsubscript{or} at the threshold for reflex vasodilation, and relative percent NO contribution. Two-way repeated-measures analysis of variance (age × ΔT\textsubscript{or}) was performed for CVC on the control site (Ringer infusion) and the experimental site (L-NAME infusion) from baseline throughout heating. Tukey’s post hoc analyses were performed when significance was achieved. The level of significance was set at P < 0.05. Values are means ± SE.

**RESULTS**

The physical characteristics of the subjects are presented in Table 1. Older (O) and younger (Y) men differed in age by ~50 yr. The older men had a significantly higher body mass index than the younger men (O: 26.1 ± 0.7, Y: 23.1 ± 0.6 kg/m²; P < 0.05). There was no significant statistical difference in resting MAP.
between the groups, although the older subjects tended to have higher resting MAP.

The older subjects began the protocol at a significantly lower $T_{or}$ (O: 36.2 ± 0.1, Y: 36.5 ± 0.1°C) and lower $T_{sk}$ (O: 33.8 ± 0.3, Y: 35.3 ± 0.9°C) (both $P < 0.05$). A 1.0°C increase in $T_{or}$ from baseline was achieved during heating in all subjects. Representative tracings of a young subject and an older subject at the control site and the NOS-inhibited site are presented in Fig. 1, A and B, respectively.

Group mean data for the control and the NOS-inhibited sites for the subject groups are depicted in Fig. 2, A (young) and B (older). In the young subjects, CVC at the control site was significantly elevated from baseline after $T_{or}$ had risen 0.3°C ($P < 0.05$). However, CVC in the young subjects at the NOS-inhibited site did not increase significantly from baseline until $T_{or}$ had risen 0.6°C ($P < 0.05$; Fig. 2A). The threshold for onset of reflex cutaneous vasodilation in the young subjects was 36.5 ± 0.2°C in the control site and 36.7 ± 0.1°C in the NOS inhibited site ($P = 0.45$). In the older subjects, CVC at the control site did not significantly differ from baseline until $T_{or}$ had risen 0.6°C ($P < 0.05$) whereas at the NOS-inhibited site CVC did not become significantly elevated above baseline until $T_{or}$ had risen 0.9°C ($P < 0.05$) (Fig. 2B). The threshold for onset of reflex cutaneous vasodilation in the older subjects was 36.6 ± 0.1°C and 36.8 ± 0.1°C for the control site and the NOS-inhibited site, respectively ($P = 0.21$). There was no difference between the groups for absolute temperature thresholds for the onset of reflex cutaneous vasodilation in either site ($P = 0.67$ control site, $P = 0.44$ NOS-inhibited site).

Group mean data for the control site of both groups are shown in Fig. 3A. Baseline CVC at the control site was similar between groups. There were significant main effects in CVC for age ($P < 0.001$) and for the change in $T_{or}$ at the control site ($P < 0.001$). Post hoc analyses on the interaction effect revealed that significant differences in CVC across age and $\Delta T_{or}$ occurred with a $T_{or}$ rise from 0.3°C and 0.9°C above baseline. CVC at the control site rose to $75 \pm 6\% CVC_{max}$ in the young subjects and $64 \pm 5\% CVC_{max}$ in the older subjects with a 1.0°C rise in $T_{or}$.

Group mean data for the NOS-inhibited site for both groups are presented in Fig. 3B. There was no difference in CVC between the groups at baseline in the NOS-inhibited site. There was a significant main effect in CVC for age ($P < 0.001$) and for the change
in $T_{or}$ at the NOS-inhibited site ($P < 0.001$). Post hoc analyses on the interaction effect revealed that differences in CVC across age and $\Delta T_{or}$ occurred after $T_{or}$ had risen 0.5°C above baseline. When $T_{or}$ had risen 1.0°C, the younger subjects’ CVC was 53 ± 3%CVC$max and the older subjects’ CVC was 29 ± 2%CVC$max ($P < 0.001$).

The relative percent NO contribution to CVC in the control site for a given rise in $T_{or}$ above baseline is presented in Fig. 4. With a 0.3°C rise in $T_{or}$, younger subjects had significantly greater relative percent NO contribution to CVC compared with older subjects (O: 20 ± 8%, Y: 40 ± 6%; $P < 0.05$). There was no significant difference in the relative percent NO contribution to CVC between the older and younger subjects with a 0.5°C rise in $T_{or}$. In terms of percent maximal vasodilation, NOS inhibition decreased CVC by 35%CVC$max in the older subjects and by 22%CVC$max in the young subjects. The relative percent NO contribution to CVC was greater in the older subjects with a 1.0°C rise in core temperature (O: 60 ± 3%, Y: 23 ± 4%; $P < 0.05$).

DISCUSSION

It has been well established that older individuals have an attenuated rise in skin blood flow during whole body heating (9, 10, 17, 21). The major finding of the present study is that NO-mediated pathways contributed more to the total vasodilatory response of the older men at high $T_{or}$. In fact, there was very little cutaneous vasodilation in the absence of functional NO in older subjects. Therefore, the attenuated reflex vasodilation in older subjects is primarily due to diminished sympathetic mediated vasodilation by an unknown active vasodilator(s).

These findings were unexpected in light of previous studies suggesting that advanced age impacts NO-mediated vasodilation (16, 18). Reckelhoff and colleagues (18) reported that there is an age-related reduction in l-arginine, the NO precursor, and its metabolites nitrate and nitrite. Furthermore, our laboratory recently demonstrated (15, 16) an attenuated NO-dependent vasodilation during local heating of the skin in older subjects. In light of the present data, a better explanation for the age-related decline in reflex cutaneous vasodilation directly involves the unidentified neurotransmitter(s) and/or the cutaneous vascular response to this activated sympathetic pathway.

There are a number of mechanisms by which the relationship between the neural thermoregulatory stimulus and AVD may be altered with aging. First, attenuated reflex cutaneous vasodilation in the older subjects might be explained by an age-related reduction in efferent cutaneous neural outflow for a given rise in $T_{or}$. This would suggest a centrally mediated alteration in AVD with advanced age. Second, there may be less neurotransmitter released for a given neural outflow. If this were true, it would take a greater stimulus (i.e., a greater increase in $T_{or}$) for the onset of reflex cutaneous vasodilation. Finally, the efferent signal may not be altered but vascular sensitiv-

Fig. 3. A: mean ± SE subject responses by age group for young (18–26 yr) and older (65–81 yr) men during passive whole body heating in the control microdialysis site (Ringer infusion only). B: mean ± SE subject responses by age group for young (18–26 yr) and older (65–81 yr) men during passive whole body heating in the NOS-inhibited microdialysis site (l-NAME infusion throughout). *Significant difference between young and older subject groups.

Fig. 4. Mean ± SE relative % contribution of NO to CVC [(control CVC – NOS-inhibited CVC)/control CVC] × 100) for a given rise in core temperature ($T_{or}$). Young subjects showed a significantly greater relative % contribution of NO to CVC with a 0.3°C rise in $T_{or}$, with a 0.5°C rise in $T_{or}$ there was no difference between the age groups, and when $T_{or}$ was increased 1.0°C older individuals had a greater NO contribution to skin blood flow. *Significant difference between young and older subject groups.
ity to the unknown neurotransmitter may be decreased, such as a reduction or desensitization of the receptors or secondary messengers mediating AVD. Although these possibilities could explain attenuated reflex cutaneous vasodilation in older subjects, we are unable to distinguish between these possibilities with the present data and our limited understanding of the cutaneous active vasodilator system.

One problem in interpreting age-related changes in reflex cutaneous vasodilation stems from the fact that the role of NO in this response is poorly understood. Studies in the rabbit ear suggest that NO may play a permissive role with the unknown neurotransmitter(s) to mediate AVD (4, 5). That is, there is a convergence in the molecular pathways between NO and the unknown neurotransmitter(s) that neurotransmitter-mediated vasodilation is dependent on the presence of NO. In this model, NO maintains basal levels of cGMP and the unknown neurotransmitter(s) mediate vasodilation via a cAMP pathway that is dependent on phosphodiesterase inhibition by cGMP (5). A study by Cran dall and McLean (3) supports this role for NO in humans, because they were not able to measure an increase in metabolites of NO during whole body heating. In this context, older subjects may have a diminished ability to produce or respond to NO but may be able to maintain a sufficient basal level of NO during whole body heating. However, we (26) and others (7) have preliminary data to argue against a purely permissive role for NO in AVD.

An alternative to the foregoing hypotheses regarding the role of NO during reflex vasodilation is that NO may be released via flow-mediated mechanisms. In this construct, the unknown active vasodilator substance(s) increase flow to a sufficient degree to stimulate NO release, possibly through an increase in shear stress. Consistent with this hypothesis, the contribution of NO to reflex cutaneous vasodilation was greater with a smaller rise in $T_{or}$ in the young subjects, whereas the contribution of NO to reflex cutaneous vasodilation was greater in the older subjects once the unknown vasodilator(s) had increased skin blood flow significantly above baseline (Figs. 3A and 4). That is, in older subjects it takes a greater rise in $T_{or}$ for reflex cutaneous vasodilation to develop, so little NO-dependent vasodilation is seen with small increases in $T_{or}$ ($\Delta T_{or}$ 0.0–0.5°C). However, once CVC is significantly elevated by the unknown vasodilator(s), the contribution of NO is critical for full expression of reflex cutaneous vasodilation. It is important to note that although the percent contribution of NO to reflex cutaneous vasodilation is greater in older subjects with large increases in $T_{or}$, the overall skin blood flow at these elevated $T_{or}$ values is significantly attenuated in older subjects (10, 16, 20). That is, NO contributes more to a lesser overall vasodilation.

Shibasaki and colleagues (25) recently reported that the initial rise in skin blood flow during reflex cutaneous vasodilation, but not the sustained rise in skin blood flow, is mediated by acetylcholine-stimulated NO production. This finding is consistent with other studies showing that the initial rise in skin blood flow during reflex vasodilation is diminished with atropine (12), but atropine has little effect once vasodilation has been established in hyperthermia (24). Diminished acetylcholine-mediated NO release early in heating with aging may partially explain the rightward shift to a greater rise in $T_{or}$ for the onset of reflex cutaneous vasodilation in older subjects, as observed by Kenney et al. (10) and shown in Fig. 3A. Consistent with this hypothesis, the rise in skin blood flow during acetylcholine iontophoresis is diminished in older subjects (1). However, it is unknown whether the NO-mediated portion of dilation to acetylcholine iontophoresis is attenuated with advanced age.

The rightward shift in reflex vasodilation with age brings to light questions regarding baseline $T_{or}$ and threshold for thermoregulatory reflexes. In our study, the older subjects’ baseline $T_{or}$ was lower than the young subjects’, but absolute $T_{or}$ at threshold for vasodilation was similar between groups. Kenney and colleagues (10, 11) also showed that the thresholds for reflex vasodilation do not change with age. This is different from what is observed during classic “resetting” of thermoregulatory responses, such as that observed with oral contraceptive use. During the course of oral contraceptive use, baseline $T_{or}$ is higher during the active pill phase than during the placebo pill phase. However, the threshold for vasodilation is likewise shifted to a higher temperature during active pill phase (2). This suggests that a central shift in overall thermoregulatory responses occurs along with a shift in baseline $T_{or}$ during oral contraceptive use. In contrast, our data suggest that thermoregulatory reflexes are not shifted with age but advanced age results in only a lower baseline $T_{or}$. This baseline temperature shift without concomitant shifts in reflex vasodilation thresholds may be due to nonthermoregulatory influences on reflex vasodilation thresholds such as changes in plasma volume and blood volume with advanced age (10, 11). More studies are needed to determine whether “classic” resetting occurs with advanced age but the shifts in reflex vasodilation are blunted because of nonthermoregulatory reflexes.

In summary, we found that the attenuated reflex cutaneous vasodilation in older subjects is likely due to diminished release of, or vascular responsiveness to, the unknown vasodilator(s) mediating active vasodilation. Furthermore, our data suggest that NO is critical for the increase in skin blood flow in older subjects during heat stress. More research is needed to understand the mechanisms of AVD in humans and how they may be altered with advanced age.

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