Effect of age on cutaneous vasoconstrictor responses to norepinephrine in humans

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Wilson, Thad E., Kevin D. Monahan, Daniel S. Short, and Chester A. Ray. Effect of age on cutaneous vasoconstrictor responses to norepinephrine in humans. Am J Physiol Regul Integr Comp Physiol 287: R1230–R1234, 2004—To test the hypothesis that cutaneous vasoconstrictor responsiveness to exogenous norepinephrine is reduced in older compared with younger subjects, we used laser-Doppler flowmetry (Moor Instruments, Devon, UK) to index changes in cutaneous vascular conductance (laser-Doppler flux). Local skin temperature was maintained at 34°C throughout the protocol. Dose-response relations were established at each temperature. Seven doses of norepinephrine (1 × 10⁻⁸ to 1 × 10⁻² M) were perfused (2 µl/min) intradermally (4 min/dose) using cutaneous microdialysis (2 probes/site). To account for possible differences in endogenous norepinephrine between groups, one microdialysis probe was perfused with bretylium tosylate to locally block noradrenergic vesicle release before establishing the norepinephrine dose-response relations. Skin blood flow was indexed via laser-Doppler flowmetry directly over both microdialysis probe sites and is expressed as cutaneous vascular conductance (laser-Doppler flux/mean arterial blood pressure). Local skin temperature was maintained at 34°C at both sites throughout the protocol. Dose-response relation between norepinephrine and cutaneous vascular conductance was similar between control and bretylium-pretreated sites in young subjects (EC₅₀ = −5.18 ± 0.27 and −5.03 ± 0.27 log M, respectively). In contrast, the dose-response relation was significantly shifted to the right (i.e., a higher dose of norepinephrine was needed to produce the same vasoconstrictor response) in the bretylium-pretreated site in older subjects (EC₅₀ = −5.46 ± 0.23 and −4.53 ± 0.23 log M, respectively). Significant increases in EC₅₀ were observed in older compared with young subjects at the bretylium-pretreated but not the control sites. These data indicate that cutaneous vasoconstrictor responsiveness is decreased in older subjects when endogenous release of norepinephrine is antagonized. Furthermore, these findings suggest that differences in presynaptic norepinephrine release between older and younger subjects are profound enough to affect dose-response relations between norepinephrine and cutaneous vascular conductance.

AGING modulates autonomic thermoregulatory responses during environmental stress (14, 34, 35). Reduced cutaneous vasodilation and sweating during heat stress are well established in older subjects (10, 12, 17, 19, 24). However, the effects of age on autonomic responses to cold stress are less clear. This lack of clarity is especially apparent when assessing age-related cutaneous vasoconstrictor responses.

Kenney and Armstrong (13) have identified impaired rate and extent of forearm vasoconstriction in older subjects during a 120-min cold air stress. Although the mechanisms responsible for this vasoconstrictor deficit were not identified in this study, one possible mechanism for this impaired vasoconstric-

METHODS

Subjects. Eight young (18–35 yr of age) and eight older (55–75 yr of age) subjects were studied (Table 1). All subjects were normotensive (resting arterial blood pressure <140/90 mmHg), nonobese (body mass index <30 kg/m²) nonsmokers, in good health (assessed by medical examination) and not taking any medications. Subjects abstained from caffeine and alcohol for 12 h before the experimental session. Each subject gave written informed consent before participating in this study approved by The Pennsylvania State University College of Medicine and Milton S. Hershey Medical Center Institutional Review Board.

Protocol and measurements. Skin blood flow was indexed via laser-Doppler flowmetry (Moor Instruments, Devon, UK). Doppler probes were housed in local heaters (surface area = 0.385 cm²) allowing for the control of local skin temperature at 34°C. Arterial...
Table 1. Selected subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (n = 8)</th>
<th>Older (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25 ± 1</td>
<td>63 ± 2*</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>3/5</td>
<td>4/4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177 ± 3</td>
<td>170 ± 2*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>78 ± 6</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>118 ± 5</td>
<td>124 ± 5</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>64 ± 2</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Mean BP, mmHg</td>
<td>82 ± 3</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>57 ± 1</td>
<td>61 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; M, male; F, female. *P < 0.05 vs. Young.

Blood pressure was measured using automated sphygmomanometry (Critikon, Tampa, FL). Cutaneous vascular conductance was calculated by dividing laser-Doppler flux by mean arterial blood pressure and expressed as arbitrary units.

Two intradermal microdialysis probes, consisting of two reinforced sections of polyimide tubing connected by a 1-cm 20-kDa-cutoff dialysis membrane (Bioanalytical Systems, West Lafayette, IN) were inserted into dorsal forearm skin. This was accomplished by advancing a 25-gauge needle 15–20 mm through the dermal layer, followed by threading the microdialysis probe through the lumen of the needle and withdrawing the needle, keeping the membrane centered within the skin.

One microdialysis site was perfused with lactated Ringer solution (Baxter, Deerfield, IL), and the other site was perfused with bretylium tosylate (Abbott Laboratories, North Chicago, IL; 10 mM) in lactated Ringer solution at a rate of 2 μl/min via a microdialysis perfusion pump (CMA Microdialysis, Stockholm, Sweden). These solutions were perfused until hyperemia associated with injection trauma subsided (60–90 min). The abolition of sympathetic vasoconstrictor responses in the bretylium tosylate site was verified via cold stress. Cold stress consisted of convective fan cooling for 2–5 min. To augment evaporative heat loss, exposed skin areas (arms, legs, and neck) were wetted with a water-soaked washcloth during cooling. Dose-response trials did not begin until after the skin was dried and local heaters were engaged to increase local skin temperature to 34°C directly over the microdialysis membranes (duration ~ 15 min).

A dose-response protocol that involved both unobservable and saturated responses was used to assess the relations between norepinephrine and cutaneous vascular conductance (32, 33). This involved perfusing seven increasing doses of norepinephrine (Abbott Laboratories, North Chicago, IL; 1 × 10^{-8} to 1 × 10^{-2} log M) for 4 min/dose at a perfusion rate of 2 μl/min at both sites. Arterial blood pressure was measured in the final 2 min of each norepinephrine dose in the contralateral arm.

Data analysis. Data were collected at 40 Hz by a data-acquisition system (ADInstruments, Castle Hill, Australia). Measures of laser-Doppler flux and cutaneous vascular conductance were obtained in the final minute of each norepinephrine dose. Cutaneous vascular conductance values were normalized to the initial norepinephrine dose. Responsiveness to norepinephrine was determined by the effective concentration causing 50% of the maximal response (EC_{50}) derived from logistic regression modeling of the dose-response curve (GraphPad, San Diego, CA). Data were analyzed by repeated-measures ANOVA. Statistical significance was accepted at P < 0.05. Data are expressed as means ± SE.

RESULTS

During cold stress significant decreases in cutaneous vascular conductance in young subjects were observed in the control site (10.5 ± 2.2 to 7.2 ± 2.5 arbitrary units) but not the bretylium-pretreated site (11.1 ± 0.6 to 10.8 ± 1.5 arbitrary units). Similar to young subjects, during cold stress cutaneous vascular conductance in older subjects was significantly decreased in the control site (6.1 ± 1.0 to 4.4 ± 0.6 arbitrary units) but not the bretylium-pretreated site (6.2 ± 1.5 to 5.8 ± 1.4 arbitrary units).

The dose-response relation between skin blood flow and norepinephrine was established in young and older subjects. In both subject populations, dosages encompassed both sub-threshold and saturated responses. The dose-response relation for norepinephrine was similar between control and bretylium-pretreated sites in young subjects (EC_{50} = −5.18 ± 0.27 and −5.03 ± 0.27 log M, respectively; Fig. 1). Dose-response modeling had high goodness of fit in young subjects in both the control (R = 0.93 ± 0.02) and bretylium-pretreated (R = 0.96 ± 0.01) sites.

In contrast to those of young subjects, older subjects’ dose-response relation was significantly shifted to the right (i.e., a higher dose of norepinephrine was needed to cause the same vasoconstrictor response) in the bretylium-pretreated site compared with control (Fig. 2). The EC_{50} increased from −5.46 ± 0.23 log M in the control site to −4.53 ± 0.23 log M in the bretylium-pretreated site (Fig. 1). Dose-response modeling of older subjects also had high goodness of fit in both control (R = 0.97 ± 0.01) and bretylium-pretreated (R = 0.98 ± 0.01) sites.
No significant differences were observed in the comparison of age groups in the control site. However, a rightward shift in the dose-response relation of skin blood flow to norepinephrine was observed in older compared with young subjects in the bretylium-pretreated site (Fig. 2). This indicates that a greater amount of norepinephrine is needed to cause a similar cutaneous vasoconstriction in older compared with young subjects after bretylium pretreatment. Despite this ∼0.5 log M difference in the EC_{S0} observed in the bretylium-pretreated site between age groups, there were no significant differences observed in the vasoconstrictor response to the maximum dose of norepinephrine between age groups or treatment sites.

**DISCUSSION**

The major finding of this study is that cutaneous vasoconstrictor responsiveness to norepinephrine is reduced in older compared with young subjects. Alterations in cutaneous vasoconstrictor responsiveness were not observed using a standard norepinephrine dose-response protocol, but rather only when the dose-response protocol was performed after presynaptic noradrenergic vesicle release was inhibited by bretylium tosylate. This decrease in norepinephrine responsiveness in the skin indicates that larger amounts of norepinephrine are required in older subjects to cause the same amount of cutaneous vasoconstriction observed in young subjects. Additionally, these data suggest that there may be differences in synaptic norepinephrine levels or presynaptic release characteristics in the skin between older and young subjects and that these differences affect norepinephrine-vasoconstrictor dose-response relations.

Dose-response relations of norepinephrine to skin blood flow of young subjects were similar to those described previously (32, 33). Neither 14-day bed rest nor exercise during bed rest altered the dose-response relation between norepinephrine and skin blood flow (33). In contrast, a rightward shift in norepinephrine dose-response relations was reported during both whole body and local heating (32). To our knowledge, this is the first report comparing dose-response relation of a vasoconstrictor agonist in the skin between young and older subjects.

We observed no statistical differences in norepinephrine dose-response relations using a standard dose-response protocol between young and older subjects. These data could be interpreted as evidence for unaltered cutaneous vasoconstrictor responsiveness with aging. This result is in contrast to forearm data (comprising muscle and skin blood flow) demonstrating decreased vasoconstrictor responsiveness in older subjects to norepinephrine (9). Plasma norepinephrine levels increase with advancing age in humans (36). These increases may be due to regional changes in release, uptake, clearance, and spillover of norepinephrine (25). Presently it is unknown if norepinephrine release from the whole forearm or from skin differs between young and older subjects. Accordingly, we also performed the same norepinephrine dose-response protocol after presynaptically blocking noradrenergic release of neurotransmitter with bretylium tosylate. In these bretylium-pretreated areas, we observed a rightward shift in the dose-response relation in older compared with young subjects.

This finding of impaired cutaneous vasoconstrictor ability of skin of older adults in sites pretreated with bretylium tosylate could be related to a number of mechanisms. First, an alteration in postsynaptic α-adrenergic receptor responsiveness could lead to a reduced cutaneous vasoconstrictor response in older subjects. Frank et al. (7), using an α-adrenergic blockade protocol during cold stress, observed a dose-dependent vasoconstrictor impairment in older compared with young subjects. However, the lack of an effect in our standard norepinephrine dose-response relation combined with rightward shift after bretylium pretreatment in older subjects suggests that the mechanism may be more complex than solely a reduction in postsynaptic α-adrenergic receptor responsiveness.

A second possible mechanism for the reduction in cutaneous vasoconstrictor responses is a decrease in both pre- and postsynaptic α2-adrenergic receptor responsiveness. A reduction in postsynaptic α2-adrenergic receptor responsiveness is similar to the argument presented above. Reduction in presynaptic α2-adrenergic receptor responsiveness has been previously observed in the peripheral vasculature of animals (5). Presynaptic α2-adrenergic receptors serve as negative-feedback modulators of noradrenergic vesicle release (30). It could be possible that in the young subjects, exogenous norepinephrine infusion inhibits noradrenergic vesicle release via presynaptic α2-adrenergic receptors, but in older subjects this mechanism is attenuated, thereby allowing for more endogenous norepinephrine to be released in the older subjects. If this hypothesis is correct it could allow for greater vasoconstriction in older subjects per dose of exogenous norepinephrine perfused. However, this is unlikely because forearm experiments in older subjects demonstrate decreases in α1-adrenergic but not in α2-adrenergic vasoconstrictor responses (4).

A third possibility is an alteration in baseline sympathetic input to the cutaneous vasculature with aging. Grassi et al. (8) observed lower skin sympathetic nerve activity in older compared with young subjects at rest and attenuated responses during a cold air stress. This is in contrast to sympathetic outflow to muscle, which is augmented in older subjects during baseline conditions (20, 23). A novel protocol by Khan et al. (16) measured both muscle sympathetic nerve activity and interstitial norepinephrine, identifying strong correlations between the two. If this relation is similar in the skin (i.e., skin sympathetic nerve activity corresponding to intradermal norepinephrine levels), this would indicate that there are lower levels of norepinephrine in the skin in older subjects. However, our data do not support this concept. We observed no differences in EC_{S0} values during standard norepinephrine dose-response protocols between age groups but increases in EC_{S0}
values at bretylium pretreatment sites in older subjects when endogenous norepinephrine levels would be reduced or eliminated.

Another interpretation of these data is that the vasoconstrictor dysfunction observed in older subjects cannot be observed without bretylium pretreatment. Bretylium tosylate blocks release of vesicles of noradrenergic nerve terminals in the skin (11). This blockade has been well demonstrated by showing that a site pretreated with bretylium does not vasoconstrict to cold stress, while an adjacent untreated site vasoconstricts to near maximal levels (11, 21, 22). Once noradrenergic vesicles are blocked, endogenous norepinephrine levels should be similar between young and older subjects. This permits exogenously administered norepinephrine to be uninfluenced by endogenous norepinephrine levels, which as described above could differ between age groups. Therefore, without bretylium pretreatment a reduced cutaneous vasoconstrictor response in older subjects is masked.

Cutaneous vasoconstrictor responses are mediated directly via norepinephrine but also by coreleased neuromodulators (26, 27). Recently, Stephens et al. (28) have identified that up to 40% of the cutaneous vasoconstrictor response may be mediated via neuropeptide Y in young subjects during cold stress. Thompson and Kenney (29) have extended this work and identified that neuropeptide Y mediated cutaneous vasoconstriction is dramatically reduced in older compared with young subjects. Our data combined with previous reports suggest multiple mechanisms are likely responsible for the reduced cutaneous vasoconstrictor responses observed in older subjects. These reductions in the ability to reduce skin blood flow could contribute to problems in the maintenance of thermal homeostasis during cold stress, which predisposes older individuals to health-related problems in thermal extremes (2).

Limitations. We cannot exclude the possibility that the selective presynaptic noradrenergic release inhibitor bretylium tosylate could be interfering with postsynaptic vasoconstrictor mechanisms differently in older compared with young subjects. Pergola et al. (22) observed that vasoconstriction during local cooling was altered in bretylium-pretreated vs. control sites. We did not observe an alteration in vasoconstrictor responses in young subjects with bretylium pretreatment. Recent work indicates that vasoconstrictor response to a single near maximal dose of norepinephrine is attenuated in older compared with young subjects after bretylium pretreatment (29). Observations from the current study identify a significant increase in the EC50 in older subjects with bretylium pretreatment. Although it is possible that there may be postsynaptic effects of bretylium tosylate to the above data, we are unaware of data that would propose a mechanism whereby the effects of bretylium tosylate would differ between young and older subjects. Therefore, we believe it is more likely that the vasoconstrictor dysfunction observed in older subjects is due to a decrease in vasoconstrictor responsiveness to norepinephrine that cannot be observed without a noradrenergic vesicle release inhibitor to normalize endogenous norepinephrine levels between age groups.

Summary. The novel finding of this study is that cutaneous norepinephrine responsiveness is decreased in older compared with young subjects when endogenous norepinephrine is controlled for between age groups. These data indicate that in this condition a larger amount of norepinephrine is required in older subjects to cause the same amount of cutaneous vasoconstriction observed in young subjects. These findings also suggest that there may be differences in presynaptic norepinephrine release between older and young subjects and that these differences are profound enough to affect dose-response relations between norepinephrine and cutaneous vascular conductance.

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GRANTS

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