

CALL FOR PAPERS | *Physiology and Pharmacology of Temperature Regulation*

Cutaneous vasoconstrictor responses to norepinephrine are attenuated in older humans

Caitlin S. Thompson,^{1,2} Lacy A. Holowatz,² and W. Larry Kenney^{1,2}

¹Intercollege Graduate Degree Program in Physiology and ²Noll Laboratory, Department of Kinesiology, The Pennsylvania State University, University Park, Pennsylvania

Submitted 14 December 2004; accepted in final form 15 January 2005

Thompson, Caitlin S., Lacy A. Holowatz, and W. Larry Kenney. Cutaneous vasoconstrictor responses to norepinephrine are attenuated in older humans. *Am J Physiol Regul Integr Comp Physiol* 288: R1108–R1113, 2005. First published January 20, 2005; doi:10.1152/ajpregu.00839.2004.—Cutaneous vasoconstriction (VC) in response to cooling is impaired with human aging. On the basis of previous findings that older humans rely predominantly on norepinephrine (NE) for reflex VC of skin blood vessels, and that the VC effects of NE are blunted with age in many vascular beds, we tested the hypothesis that cutaneous VC responses to exogenous NE are attenuated in aged skin compared with young skin. In 11 young (18–30 yr) and 11 older (62–76 yr) men and women, skin blood flow was monitored at two forearm sites with laser Doppler (LD) flowmetry, while local skin temperature was clamped at 34°C. At one site, five doses of NE (10^{-10} to 10^{-2} M) were sequentially infused via intradermal microdialysis while the other site served as control (C; Ringer). Cutaneous vascular conductance (CVC; LD flux/mean arterial pressure) was expressed as percent change from baseline (% Δ CVC_{base}). At 10^{-10} , 10^{-8} , and 10^{-6} M NE, older VC responses were attenuated compared with young [10^{-10} : -35 (95% confidence interval: -16, -52) vs. -49 (-40, -58) % Δ CVC_{base}, $P = 0.02$; 10^{-8} : -38 (-20, -56) vs. -50 (-40, -61) % Δ CVC_{base}, $P = 0.03$; 10^{-6} : -52 (-35, -70) vs. -67 (-60, -74) % Δ CVC_{base}, $P = 0.01$]. Older maximal VC responses were also blunted compared with young [-80 (confidence interval: -73, -87) vs. -88 (confidence interval: -87, -90) % Δ CVC_{base}, $P = 0.03$]. NE-mediated cutaneous VC is blunted at both physiological and superphysiological doses in older subjects compared with young subjects. Considering that NE is the only functional neurotransmitter mediating reflex VC in aged skin, attenuated NE-mediated VC may further predispose older humans to excess heat loss in the cold.

aging; thermoregulation; skin blood flow; microdialysis; adrenergic responses

REFLEX-MEDIATED cutaneous vasoconstriction (VC) is the initial thermoregulatory response to moderate skin cooling, effectively minimizing convective heat loss to the environment. However, reflex VC is impaired with advancing age (17, 18, 25, 32), presenting a health risk for older humans by rendering them more susceptible to excessive heat loss and, potentially, hypothermia (2, 3, 16). Specifically, older humans lose functional cotransmitter-mediated VC and rely solely on norepinephrine (NE) to mediate reflex cutaneous VC, accounting for at least part of this reflex VC impairment (32). Furthermore,

aged skin may also exhibit blunted responsiveness to NE, further contributing to this age-associated attenuation of reflex VC function (32, 36).

Studies in both humans and animals have demonstrated an age-related impairment of NE-mediated VC in many vascular beds, including thermoregulatory circulations (5, 13, 22, 35). Because plasma NE concentrations are elevated with advancing age (10, 13), this age-related impairment of NE-mediated VC has often been attributed to chronic adrenoceptor desensitization. Several studies have utilized NE dose-response protocols to characterize this age-related desensitization in humans; however, the majority of these studies have examined responses to NE in whole limbs, encompassing blood flow changes not only in the cutaneous circulation, but underlying muscle and adipose tissues, as well. To date, only one study, conducted by Wilson and colleagues (36), has attempted to characterize NE responsiveness with a dose-response protocol in aged skin, finding no age difference in cutaneous VC responses to NE infusion.

However, further NE dose-response work is warranted, not only to verify or refute the unexpected findings of Wilson et al., but also to analyze the dose-response relationship to NE across a broader range of NE concentrations, as well as to more fully characterize the greater individual variability in vascular responses that often accompanies human aging. Accordingly, the purpose of this study was to characterize both individual and group mean changes in cutaneous NE responsiveness seen with advancing age in response to graded NE administration across a greater range of doses. Specifically, we tested the hypothesis that cutaneous VC responses to graded NE administration in a dose-dependent manner are attenuated in aged skin compared with young skin.

MATERIALS AND METHODS

Subjects. Eleven young (18–30 yr; 6 men, 5 women) and 11 older (62–76 yr; 5 men, 6 women) subjects participated in the present study. All young women were tested during the early follicular phase of the menstrual cycle and were not taking oral contraceptives; all older women were postmenopausal and were not taking hormone replacement therapy. All subjects underwent a standardized medical screening and were healthy, normotensive, nonobese nonsmokers. No subjects were taking any medications that might alter cardiovascular responses to cooling. They abstained from alcohol and caffeine for

Address for reprint requests and other correspondence: C. S. Thompson, The Pennsylvania State Univ., 119 Noll Laboratory, Univ. Park, PA 16802 (E-mail: cst116@psu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

12 h before coming to the laboratory for the study but were permitted to eat a modest breakfast the morning of the experiment. Approval was obtained from the Institutional Review Board of The Pennsylvania State University. Each subject gave verbal and written informed consent before participation in the study, and all procedures conformed to the standards of the Declaration of Helsinki.

Instrumentation. Subjects arrived at the laboratory between 8:00 and 9:00 AM on the morning of the experiment. Two microdialysis (MD) fibers (MD-2000, Bioanalytical Systems, West Lafayette, IN) were placed into the ventral surface of the right forearm using sterile technique. For each fiber, a 25-gauge needle was inserted into anesthetized skin and guided horizontally through the skin such that entry and exit points were ~2 cm apart. The fiber, consisting of a 10-mm membrane (320 μm OD, 20-kDa molecular mass cutoff) and connective tubing attached to either end of the membrane, was threaded through the needle. The needle was then withdrawn, leaving the membrane in the skin. After insertion of both fibers, subjects rested quietly for ~90 min to allow local hyperemia due to insertion trauma to subside. At this time, local skin temperature was clamped at 34°C at both MD sites using local heating elements (Temperature Monitor SH02, Moor Instruments).

Skin blood flow (SkBF) was measured using laser-Doppler flowmetry (LDF; MoorLAB, Moor Instruments). LDF probes were placed on the skin over each MD membrane, and LDF data were collected continuously throughout the experiment. Arterial blood pressure was monitored periodically throughout the experiment via brachial auscultation, and mean arterial pressure (MAP) was calculated as $[(1/3 \times \text{systolic blood pressure}) + (2/3 \times \text{diastolic blood pressure})]$. SkBF was converted to cutaneous vascular conductance (CVC), which was calculated as the ratio of LDF flux to MAP, and expressed as percent change from baseline values ($\% \Delta \text{CVC}_{\text{base}}$).

Protocol. After the MD fibers were in place, lactated Ringer solution was infused through all fibers at a rate of 2 $\mu\text{l}/\text{min}$ using a microinfusion pump (Harvard 22, South Natick, MA) for ~90 min. After hyperemia subsided and a steady-state baseline was established, NE (10^{-10} M) was infused for 5 min at one site. The other site remained infused only with Ringer solution and served as control (C). Following NE infusion, Ringer solution was infused at the NE site as a washout for approximately 20–30 min. Once SkBF recovered to initial baseline values, this protocol was repeated for every subsequent dose of NE (10^{-8} , 10^{-6} , 10^{-4} , and 10^{-2} M).

The NE and ascorbic acid (NE preservative; 1 mg/ml) in the present study were obtained from Sigma Chemical (St. Louis, MO) and were mixed just before usage. All NE dilutions were dissolved in lactated Ringer solution and sterilized using syringe microfilters (Acrodisc, Pall, Ann Arbor, MI).

Data collection and analysis. Data were recorded and stored as 1-min averages using computer software (LabView) and a data-acquisition system (National Instruments, Austin, TX). VC was defined as the lowest CVC 1-min average following NE infusion, verified by the subsequent onset of CVC recovery due to washout. Data were analyzed using Student's *t*-test (for subject characteristics), and repeated-measures ANOVAs with Tukey-Kramer post hoc and planned comparison tests when significant differences were detected (SAS statistical software, version 8.01). Statistical significance was set at $\alpha = 0.05$. Values are expressed as means (SD), unless otherwise noted.

RESULTS

Subject characteristics are presented in Table 1. Subjects in the two age groups were well matched for height, weight, and body mass index. Although resting MAP was higher in older subjects, there was no change in MAP over time during the protocol in either young or older subjects. No sex differences in CVC responses were detected, so data from men and women in each age group were pooled for analysis.

Table 1. *Subject characteristics*

Variable	Young	Older	<i>P</i> Value
Sex (M, F)	6, 5	5, 6	
Age, yr	23 (SD4)	69 (SD5)	<0.0001
Height, cm	171 (SD9)	169 (SD6)	0.585
Weight, kg	69 (SD9)	71 (SD9)	0.526
BMI, kg/m ²	23 (SD2)	25 (SD2)	0.196
Resting MAP, mmHg	85 (SD7)	94 (SD7)	0.008

Values are means (SD) for 11 young and 11 older men (M) and women (F). BMI, body mass index; MAP, mean arterial pressure.

Figure 1 presents mean CVC responses to five incremental doses of NE (10^{-10} , 10^{-8} , 10^{-6} , 10^{-4} , and 10^{-2} M) in young and older subjects. At doses of 10^{-10} , 10^{-8} , and 10^{-6} M NE, older subjects' CVC responses were substantially attenuated compared with young responses [10^{-10} M: -35 (SD 28) vs. -49 (SD 16) $\% \Delta \text{CVC}_{\text{base}}$, $P = 0.02$; 10^{-8} M: -38 (SD 31) vs. -50 (SD 18) $\% \Delta \text{CVC}_{\text{base}}$, $P = 0.03$; 10^{-6} M: -52 (SD 29) vs. -67 (SD 11) $\% \Delta \text{CVC}_{\text{base}}$, $P = 0.01$]. There was also a nonsignificant tendency for older subjects to exhibit blunted VC vs. young at 10^{-4} M [-75 (SD 17) vs. -84 (SD 7) $\% \Delta \text{CVC}_{\text{base}}$, $P = 0.09$].

Figure 2, A and B, illustrates the individual responses to all five NE doses in young and older subjects, respectively. The variability of VC responses in older subjects was much greater than that of young subjects. Specifically, the coefficients of variation for older subjects were greater at every NE dose compared with young subjects (10^{-10} M: 80 vs. 33%; 10^{-8} M: 82 vs. 36%; 10^{-6} M: 56 vs. 16%; 10^{-4} M: 23 vs. 8%; 10^{-2} M: 16 vs. 7%).

Figure 3, A and B, illustrates individual and mean maximal VC responses in young and older subjects, respectively. This figure differs from Fig. 2 and is presented separately because maximal VC did not always occur at the highest dose of NE. Eleven subjects (6 young, 5 older) exhibited maximal VC at 10^{-2} M NE, while maximal VC occurred at 10^{-4} M NE in the remaining 11 subjects (5 young, 6 older). Blunted maximal VC was observed in older subjects compared with young subjects [-80 (SD 11) vs. -88 (SD 2) $\% \Delta \text{CVC}_{\text{base}}$, $P = 0.028$]. Additionally, the variability in the maximal VC responses was greater in older subjects compared with young, as evidenced by the greater coefficient of variation for maximal VC values (older 14% vs. young 2%).

DISCUSSION

The primary findings of this study were that NE-mediated cutaneous VC is attenuated with advancing age across a wide range of NE doses. Older subjects exhibited both greater heterogeneity in VC responses and attenuated VC at physiological NE doses as well as attenuated maximal VC compared with their young counterparts, suggesting an age-related impairment of both VC sensitivity and maximal VC response to NE.

NE-mediated VC and adrenoceptor sensitivity. In young subjects, reflex cutaneous VC is mediated by both NE and sympathetic cotransmitters, most likely neuropeptide Y or ATP (27–29, 32). NE typically accounts for ~60% of total VC, with cotransmitters accounting for the remaining 40%. However, one of the hallmarks of human aging is the impairment of appropriate vasomotor responses to stimuli. For example, with healthy aging, reflex cutaneous VC is severely attenuated, by

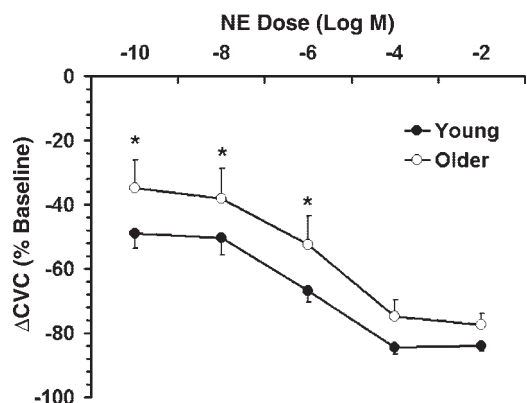


Fig. 1. Mean cutaneous vasoconstriction responses to sequential norepinephrine (NE) infusions in young ($n = 11$) and older ($n = 11$) subjects. Variability is indicated by SE bars. Δ CVC, change in cutaneous vascular conductance. $*P < 0.05$ vs. young.

as much as 50% (10, 17, 18, 32). In older subjects, the cotransmitter portion of VC is functionally abolished, leaving only NE to mediate the entirety of the reflex cutaneous VC response to cold (32). This loss of cotransmitter-mediated VC is not compensated for by an upregulated NE-mediated VC; instead, NE-mediated VC in skin may actually be blunted with age (10, 22, 31, 36). The results of the present study confirm that NE-mediated VC in aged skin is indeed impaired, not only at concentrations that reproduce physiological reflex VC responses (10, 18, 25, 32) (10^{-10} – 10^{-8} M) but also at much higher, pharmacological concentrations (10^{-4} – 10^{-2} M) that elicit maximal NE-mediated VC. If both cotransmitter- and NE-mediated portions of reflex cutaneous VC are compromised with age, older humans may be further predisposed to heat loss in the cold and subsequent hypothermia.

In addition to vasomotor dysfunction, greater variability in cutaneous vasomotor responses may also accompany healthy human aging (10, 18). Greater heterogeneity among older subjects' responses suggests that while responses from a few subjects may remain unchanged with age, the majority of responses are nonuniformly attenuated, with the degree of dysfunction varying from subject to subject (8, 9). Data from the present study support these conclusions, indicating both impaired VC and a greater variability in VC responses among older subjects (see Figs. 2 and 3). This greater heterogeneity in older subjects' VC responses did not necessarily correlate with age or sex within the older age group, suggesting that the degree of vasomotor dysfunction associated with aging is not predictable or generalizable, occurring at different rates and/or ages among older humans and may also depend on factors other than age, such as diet, exercise history, or genetics.

The majority of studies that have investigated age-associated declines in NE-mediated VC in vivo have not specifically examined changes in the cutaneous vasculature. Instead, several studies have documented desensitization to NE in using whole limb blood flow measurement techniques, such as venous occlusion plethysmography, which records blood flow changes in not only the cutaneous vasculature, but also underlying skeletal muscle and adipose tissues as well (5, 13, 30). Thus, although these studies have documented an age-related attenuation in NE-mediated VC, ascribing it to desensitization of adrenergic receptors (AR), it is not possible to discern

whether those changes in AR sensitivity occurred in the skin or in other tissues. The present study addressed this issue by assessing AR sensitivity in the cutaneous vasculature using skin-specific NE infusion (intradermal microdialysis) and blood flow measurement (LDF) techniques. The results of the present study corroborate the results of previous studies examining age-related changes in whole limbs, suggesting that cutaneous AR sensitivity declines with age.

It is likely that the impaired NE-mediated VC observed in the present study is due to a desensitization of α_1 -AR, α_2 -AR, or a combination of the two. Both in vitro and in vivo studies that have examined AR subtype desensitization report that age-associated impairment of NE-mediated VC in several vascular beds involves a reduction in α_1 -AR sensitivity to NE (5, 22, 31, 35). In the human cutaneous vasculature, α_1 -AR participate in NE-mediated VC (1, 11, 12, 19, 23), albeit to a lesser degree than α_2 -AR, suggesting that α_1 -AR desensitization may be involved in the attenuated VC response to NE observed in the present study.

Studies that have investigated the possibility of α_2 -AR desensitization with age are less conclusive, reporting conflicting results (6, 7, 15, 22, 33) and raising questions as to the degree of α_2 -AR desensitization that accompanies human aging. Under thermoneutral conditions in the human cutaneous circulation, VC in response to NE infusion/release is predominantly mediated by postjunctional α_{2A} -AR; thus, considering

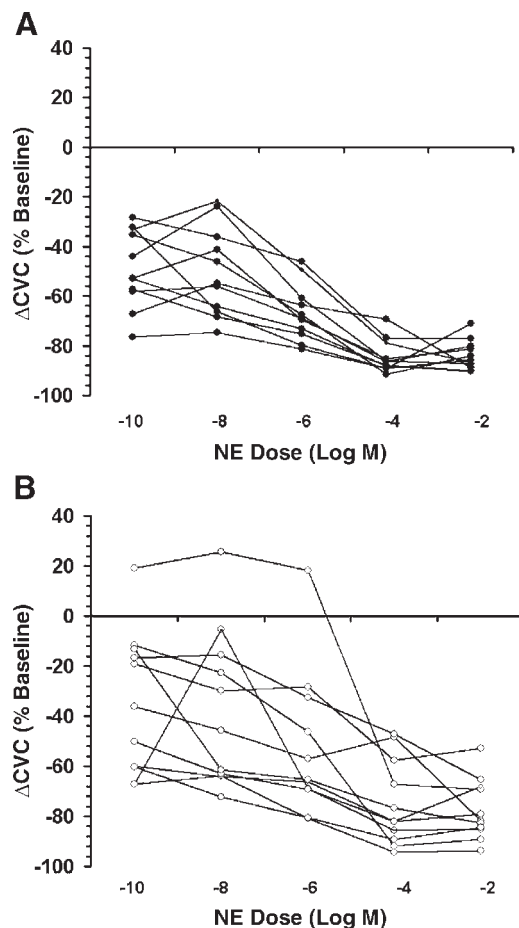


Fig. 2. Individual cutaneous vasoconstriction responses to sequential norepinephrine infusions in young (A; $n = 11$) and older (B; $n = 11$) subjects.

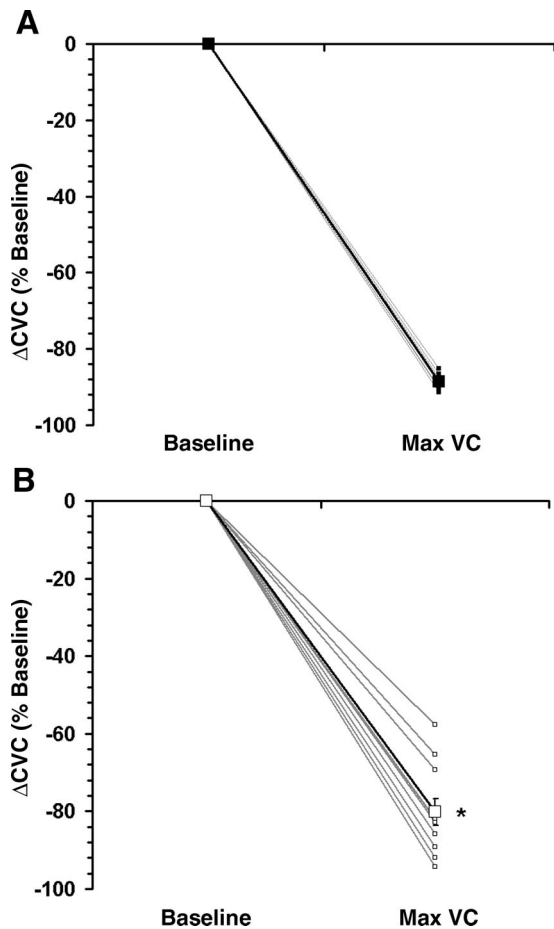


Fig. 3. Individual (thin lines) and mean (bold lines) maximal cutaneous vasoconstriction (max VC) responses to norepinephrine infusion in young (A; $n = 11$) and older (B; $n = 11$) subjects. Variability is indicated by SE bars. * $P < 0.05$ vs. young.

the important role of α_2 -AR in the regulation of skin blood flow, further studies need to be conducted to more fully address the effects of age on α_2 -AR sensitivity in human skin.

Although functional β -AR are present in the cutaneous vasculature (4), it is unlikely that NE-mediated β -AR stimulation accounts for the limited VC observed in older subjects. When β -AR in human skin are stimulated by NE, cutaneous blood vessels dilate, countering a small portion of the VC mediated by α -AR; however, β -AR-mediated vasodilation is attenuated with healthy human aging (20, 24), suggesting that stimulation of β -AR by exogenous NE does not account for the attenuated NE-mediated VC observed in the present study.

The hypotheses and overall methodology of the present study are very similar to those presented by Wilson et al. (36); both studies explore age-related changes in cutaneous postsynaptic adrenoceptor function. However, there are several differences in both conclusions and methodology that distinguish the present study from that conducted by Wilson and colleagues. First, the results of the present study indicate that there is an attenuated VC response to NE in aged skin, contrary to the study by Wilson et al., which reported no age difference in VC responses to NE at previously untreated sites, but in agreement with the majority of literature that addresses adrenoceptor function with aging. Second, the present study also documents

the heterogeneity of individual VC responses in older subjects, suggesting that this more general characteristic of human aging is applicable to the cutaneous circulation. Additionally, various elements of the experimental protocol differed between the two studies, namely that 1) the present study extended the lowest NE dose to 10^{-10} M, $100 \times$ more dilute than the lowest dose infused by Wilson et al.; and 2) the present study included a washout period after each NE infusion to allow adequate clearance of NE from the synapse before starting the next infusion. Finally, data normalization techniques and statistical analyses differed between the two studies, introducing the possibility that differences in data treatment may impact the interpretation of those results and subsequent conclusions.

There are several explanations that may account for the discrepancy in conclusions between the two studies. First, it is possible that the different methods of data normalization employed in these two studies masked the relative differences between young and older subjects. Wilson and colleagues reported that 10^{-8} M was a subthreshold dose of NE and normalized VC responses observed at all subsequent doses of NE (10^{-7} – 10^{-2} M) to the CVC values observed during 10^{-8} M NE infusion. In the present study, the weakest dose of NE, 10^{-10} M, induced significant VC in both young and older subjects; thus data were not normalized to the CVC values observed during the infusion of the first dose of NE but were instead normalized to pre-NE baseline CVC values. When data from the present study were normalized to the CVC values observed during the first NE infusion, age differences in VC responses disappeared, and the dose-response curves for young and older subjects were virtually superimposed. Thus it is plausible that the choice of normalization techniques utilized in the Wilson study may have masked significant age differences in the VC response to NE.

Second, it is unclear why the initial dose of 10^{-10} M in the present study induced significant VC, yet the initial dose of 10^{-8} M in the study conducted by Wilson and colleagues failed to induce any VC. However, it is possible that NE mixing and dilution techniques differed between the two studies. Wilson et al. did not report that any preservative was added to the NE solutions to prolong NE half-life, raising the possibility that NE degradation may have occurred before the solutions were sequentially infused. In the present study, ascorbic acid was added to the NE dilutions (1 mg/ml) to act as a preservative, extending NE half-life from 8.5 min to over 180 min (14). In pilot studies, ascorbic acid alone did not induce VC, so it is unlikely that the addition of ascorbic acid in this study contributed to the marked VC observed at initial NE doses.

Finally, it is possible that the presence or absence of a washout period between NE doses may have been a determinant of VC responses. In the study conducted by Wilson and colleagues, the investigators did not report the inclusion of a washout period between NE infusions, whereas a minimum washout period of 20–30 min was included between NE doses in the present study, allowing CVC to return to near-baseline values before infusing the next dose. Because NE clearance declines with age (7, 21), it is likely that a greater concentration of NE remains in the synaptic cleft after each infusion in older subjects compared with their young counterparts. If a washout period is included, the NE remaining in the synapse can be slowly taken up in older subjects, permitting VC to abate between infusions. However, in the absence of a washout

period, it is possible that the concentration of NE remaining in the synapse may be much greater in older subjects because of impaired NE clearance mechanisms. As sequential doses are continually infused without break, the accumulation of NE that cannot be cleared from the synapse may continue to stimulate α -AR on cutaneous vessels, functionally increasing the NE dose in older subjects during a given infusion. If this is the case, the similarity in VC responses between age groups in the Wilson study may be due to the fact that more NE was present in older subjects at each NE dose, augmenting VC to the extent that older subjects' responses were no longer different from those of young subjects.

Limitations. One of the limitations of the present study was that it was not possible to quantifiably compare NE microdialysis infusion concentrations with previously reported plasma NE spillover values in response to cooling to define physiological vs. superphysiological (i.e., pharmacological) NE doses. This is because the rate and degree of drug diffusion between membrane and tissue vary greatly, depending not only on the original drug and concentration, but also on membrane length and pore size, infusion rate, and tissue temperature and physical properties (26, 34). Thus it was not possible to assume that the infused NE dose was in fact the same NE concentration that entered the skin and acted on cutaneous blood vessels.

Instead, VC responses to sequential graded NE infusions from the present study were compared with reflex VC induced by whole body cooling in previous studies (10, 18, 25, 32), indirectly comparing observed responses rather than directly comparing NE concentrations in the skin. When NE-mediated VC responses from the present study were compared with reflex VC induced by cooling in both age groups, it was determined that VC in response to 10^{-10} – 10^{-8} M NE approximated physiological reflex cutaneous VC observed during whole body cooling. These comparisons also suggest that NE doses of 10^{-4} – 10^{-2} M are superphysiological (i.e., pharmacological), as they exceed the NE concentration necessary to evoke VC comparable to reflex-mediated VC by 10,000–10,000,000 \times .

A second limitation to the present study was that the NE dose-response curves could not be analyzed using nonlinear regression curve analysis. Nonlinear regression analysis using a standard sigmoidal curve equation is usually performed to obtain an EC_{50} and/or an E_{max} value, indicators of VC sensitivity and capacity, respectively. However, the curves generated in the present study departed substantially from the traditional "dose-response" sigmoidal curve, most obviously exhibiting a significant response at a dose that was thought to be subthreshold. Although previous studies in the literature and pilot work for this study indicated that 10^{-10} M was a subthreshold dose of NE, data collected during the study exhibited significant VC in response to 10^{-10} M NE, such that an EC_{50} value would be estimated at a NE dose less concentrated than even 10^{-10} M. Instead, one- and three-way ANOVAs were utilized to evaluate VC responses and possible differences between young and older subjects, yielding the results that 1) VC responses in older subjects at 10^{-10} , 10^{-8} , and 10^{-6} M NE were attenuated compared with young subjects (see Fig. 1), and 2) maximal VC responses in older subjects (at either 10^{-4} or 10^{-2} M) were attenuated compared with their young counterparts (see Fig. 3). Thus these results were still interpreted to

suggest that both cutaneous AR sensitivity and NE-mediated VC capacity are impaired with healthy human aging.

In summary, this study presents evidence that NE-mediated cutaneous VC is attenuated in aged skin across a wide range of graded NE doses, suggesting that both impaired cutaneous VC sensitivity to NE and attenuated maximal VC capacity accompany healthy aging. This age-associated impaired VC response was observed at NE doses that induced a physiological degree of VC, as well as at much higher doses. Although this study did not employ AR subtype-specific agonists, it is likely that the attenuated VC response observed in older subjects is due to α_1 -AR desensitization, and possibly α_2 -AR desensitization, as well. However, further studies are needed to clarify the relative changes in α_1 -AR vs. α_2 -AR sensitivity with age. Conclusions from the present study also help to clarify the vascular mechanisms that underlie age-associated reflex cutaneous VC impairment.

ACKNOWLEDGMENTS

We especially thank Dr. M. Chow for statistical advice, J. Pierzga for research assistance, the General Clinical Research Center for providing medical consultation and screenings, and the research subjects for their participation.

GRANTS

This study was supported by National Institutes of Health Grants RO1 AG-07004–14 (W. L. Kenney) and M01-RR-10732 (General Clinical Research Center).

REFERENCES

- Borbujo J, Garcia-Villalon AL, Valle J, Gomez B, and Dieguez G. Postjunctional alpha-1 and alpha-2 adrenoceptors in human skin arteries. An in vitro study. *J Pharmacol Exp Ther* 249: 284–287, 1989.
- Budd GM, Brotherhood JR, Hendrie AL, and Jeffery SE. Effects of fitness, fatness, and age on men's responses to whole body cooling in air. *J Appl Physiol* 71: 2387–2393, 1991.
- Collins KJ, Dore C, Exton-Smith AN, Fox RH, MacDonald IC, and Woodward PM. Accidental hypothermia and impaired temperature homeostasis in the elderly. *Br Med J* 1: 353–356, 1977.
- Crandall CG, Etzel RA, and Johnson JM. Evidence of functional beta-adrenoceptors in the cutaneous vasculature. *Am J Physiol Heart Circ Physiol* 273: H1038–H1043, 1997.
- Dineno FA, Dietz NM, and Joyner MJ. Aging and forearm postjunctional α -adrenergic vasoconstriction in healthy men. *Circulation* 106: 1349–1354, 2002.
- Docherty JR. Aging and vasoconstrictor responses mediated by alpha 2-adrenoceptors and 5-HT1 and 5-HT2 receptors. *Ann NY Acad Sci* 30: 282–292, 1994.
- Featherstone JA, Veith RC, Flatness D, Murburg MM, Villacres EC, and Halter JB. Age and alpha-2 adrenergic regulation of plasma norepinephrine kinetics in humans. *J Gerontol* 42: 271–276, 1987.
- Ferrari AU. Age-related modifications in neural cardiovascular control. *Aging* 4: 183–195, 1992.
- Ferrari AU, Radaelli A, and Centola M. Aging and the cardiovascular system. *J Appl Physiol* 95: 2591–2597, 2003.
- Frank SM, Raja SN, Bulcao C, and Goldstein DS. Age-related thermoregulatory differences during core cooling in humans. *Am J Physiol Regul Integr Comp Physiol* 279: R349–R354, 2000.
- Freedman RR, Sabharwal SC, Moten M, and Migaly P. Local temperature modulates α_1 - and α_2 -adrenergic vasoconstriction in men. *Am J Physiol Heart Circ Physiol* 263: H1197–H1200, 1992.
- Gomez B, Borbujo J, Garcia-Villalon AL, Nava-Hernandez E, Valle J, Garcia JL, and Dieguez G. Alpha₁- and alpha₂-adrenergic response in human isolated skin arteries during cooling. *Gen Pharmacol* 22: 341–346, 1991.
- Hogikyan RV and Supiano MA. Arterial α -adrenergic responsiveness is decreased and SNS activity is increased in older humans. *Am J Physiol Endocrinol Metab* 266: E717–E724, 1994.
- Hughes IE and Smith JA. The stability of noradrenaline in physiological saline solution. *J Pharm Pharmacol* 30: 124–126, 1978.

15. **Hyland L and Docherty JR.** An investigation of age-related changes in pre- and postjunctional α -adrenoceptors in human saphenous vein. *Eur J Pharmacol* 114: 361–364, 1985.
16. **Inoue Y, Nakao M, Araki T, and Ueda H.** Thermoregulatory responses of young and older men to cold exposure. *Eur J Appl Physiol* 65: 492–498, 1992.
17. **Kenney WL and Armstrong CG.** Reflex peripheral vasoconstriction is diminished in older men. *J Appl Physiol* 80: 512–515, 1996.
18. **Khan F, Spence VA, and Belch JJJ.** Cutaneous vascular responses and thermoregulation in relation to age. *Clin Sci* 82: 521–528, 1992.
19. **Lindblad LE and Ekenvall L.** Alpha-adrenoceptors in the vessels of human finger skin. *Acta Physiol Scand* 128: 219–222, 1986.
20. **Marin J and Rodriguez-Martinez MA.** Age-related changes in vascular responses. *Exp Gerontol* 34: 503–512, 1999.
21. **Morrow LA, Linares OA, Hill TJ, Sanfield JA, Supiano MA, Rosen SG, and Halter JB.** Age differences in the plasma clearance mechanisms for epinephrine and norepinephrine in humans. *J Clin Endocrinol Metab* 65: 508–511, 1987.
22. **Nielsen H, Hasenkam JM, Pilegaard HK, Aalkjaer C, and Mortensen FV.** Age-dependent changes in α -adrenoceptor-mediated contractility of isolated human resistance arteries. *Am J Physiol Heart Circ Physiol* 263: H1190–H1196, 1992.
23. **Nielsen H, Mortensen FV, and Mulvany MJ.** Responses to noradrenaline in human subcutaneous resistance arteries are mediated by both α_1 - and α_2 -adrenoceptors. *Br J Pharmacol* 99: 31–34, 1990.
24. **Pan HY, Hoffman BB, Pershe RA, and Blaschke TF.** Decline in beta adrenergic receptor-mediated vascular relaxation with aging in man. *J Pharmacol Exp Ther* 239: 802–807, 1986.
25. **Richardson D, Tyra J, and McCray A.** Attenuation of the cutaneous vasoconstrictor response to cold in elderly men. *J Gerontol* 47: M211–M214, 1992.
26. **Schnetz E and Fartasch M.** Microdialysis for the evaluation of penetration through the human skin barrier—a promising tool for future research? *Eur J Pharmacol Sci* 12: 165–174, 2001.
27. **Stephens DP, Aoki K, Kosiba WA, and Johnson JM.** Nonnoradrenergic mechanism of reflex cutaneous vasoconstriction in men. *Am J Physiol Heart Circ Physiol* 280: H1496–H1504, 2001.
28. **Stephens DP, Bennett LAT, Aoki K, Kosiba WA, Charkoudian N, and Johnson JM.** Sympathetic nonnoradrenergic cutaneous vasoconstriction in women is associated with reproductive hormone status. *Am J Physiol Heart Circ Physiol* 282: H264–H272, 2002.
29. **Stephens DP, Saad AR, Bennett LAT, Kosiba WA, and Johnson JM.** Neuropeptide Y antagonists reduced reflex cutaneous vasoconstriction in humans. *Am J Physiol Heart Circ Physiol* 287: H1404–H1409, 2004.
30. **Supiano MA, Hogikyan RV, Sidani MA, Galecki AT, and Krueger JL.** Sympathetic nervous system activity and α -adrenergic responsiveness in older hypertensive humans. *Am J Physiol Endocrinol Metab* 276: E519–E528, 1999.
31. **Supiano MA, Hogikyan RV, Stoltz AM, Orstan N, and Halter JB.** Regulation of venous α -adrenergic responses in older humans. *Am J Physiol Endocrinol Metab* 260: E599–E607, 1991.
32. **Thompson CS and Kenney WL.** Altered neurotransmitter control of reflex vasoconstriction in aged human skin. *J Physiol* 558: 697–704, 2004.
33. **Tsai H, Buchholz J, and Duckles SP.** Postjunctional alpha 2-adrenoceptors in blood vessels: effect of age. *Eur J Pharmacol* 237: 311–316, 1993.
34. **Ungerstedt U.** Microdialysis-principles and applications for studies in animals and man. *J Internal Med* 230: 365–373, 1991.
35. **Vila E, Vivas NM, Taberno A, Giraldo J, and Arribas SM.** α_1 -Adrenoceptor vasoconstriction in the tail artery during ageing. *Br J Pharmacol* 121: 1017–1023, 1997.
36. **Wilson TE, Monahan KD, Short DS, and Ray CA.** Effect of age on cutaneous vasoconstrictor responses to norepinephrine in humans. *Am J Physiol Regul Integr Comp Physiol* 287: R1230–R1234, 2004.

