Aging and Forearm Postjunctional α-Adrenergic Vasoconstriction in Healthy Men

Frank A. Dinello, PhD; Niki M. Dietz, MD; Michael J. Joyner, MD

Background—Muscle sympathetic vasoconstrictor nerve activity increases with age in healthy humans but does not result in an augmented forearm vasoconstrictor tone. We tested the hypothesis that this is due to a reduction in postjunctional α-adrenergic responsiveness to endogenous norepinephrine (NE) release and determined whether this was specific to α₁ or α₂-adrenergic receptors.

Methods and Results—Forearm blood flow (FBF, by strain-gauge plethysmography) responses to local intra-arterial infusions of tyramine (which evokes endogenous NE release), phenylephrine (selective α₁-agonist), and clonidine (α₂-agonist) were determined in 10 young (aged 26 ± 1 [mean ± SEM] years) and 10 older (aged 65 ± 1 years) healthy normotensive men after local β-adrenergic blockade with propranolol. Basal forearm vascular tone was not different in young men and older men. The percentage reduction in FBF in response to the highest dose of tyramine was blunted in older men compared with young men (−37 ± 3% versus −49 ± 3%, respectively; P < 0.01) despite a greater increase in deep venous NE concentration in older men (910 ± 103 versus 565 ± 69 pg/mL, respectively; P < 0.001). Maximal reductions in FBF to phenylephrine were also blunted in older men (−47 ± 2% versus −58 ± 3% in young men, P < 0.05). In contrast, the reductions in FBF (−36 ± 7% versus −40 ± 3% for older versus young men, respectively) and also in venous NE concentration (−79 ± 24 versus −84 ± 13 pg/mL for older versus young men, respectively) to clonidine were similar in the 2 groups. Finally, forearm sympathetic α-adrenergic vasoconstrictor tone (assessed via nonselective α-blockade with phentolamine) was significantly lower in older men.

Conclusions—Our results indicate that human aging is associated with a reduction in forearm postjunctional α-adrenergic responsiveness to endogenous NE release and that this might be specific to α₁-adrenergic receptors. Furthermore, the contribution of sympathetic α-adrenergic vasoconstriction to basal forearm vascular tone is reduced with age in healthy men. (Circulation. 2002;106:1349-1354.)

Key Words: aging ● blood flow ● receptors, adrenergic, alpha ● vasoconstriction

Basal muscle sympathetic vasoconstrictor nerve activity (MSNA) increases with advancing age in healthy humans. 1-3 Although resting MSNA appears to be similar in the upper and lower limbs of young adults, 4 the age-related increase in MSNA has been measured and documented in only the lower limb. However, if MSNA is increased in the upper limb with age, this elevated sympathetic drive does not result in a reduced basal forearm blood flow (FBF) or vascular conductance (vasoconstriction). 3,5 In addition to this observation under resting conditions, older adults compared with young adults demonstrate a blunted forearm vasoconstrictor response for a given increase in MSNA during lower body negative pressure. 5 Whether this blunted response with age reflects a reduction in norepinephrine release per sympathetic nerve discharge, a reduction in postjunctional α-adrenergic responsiveness to endogenous norepinephrine release, or both is not completely understood.

During sympathoexcitation, the increase in forearm venous (antecubital) norepinephrine concentration per unit increase in MSNA is not different in young and older healthy men, providing indirect evidence that forearm norepinephrine release under these conditions is not significantly altered with age. 3 In contrast, data from experimental animals and humans suggest that postjunctional α-adrenergic responsiveness might be reduced with age. 5-7 However, to date, no studies have directly determined whether human aging is associated with reduced forearm vasoconstrictor responsiveness to endogenous norepinephrine release.

With this information as a background, the purpose of the present study was to test the hypothesis that forearm vasoconstrictor responses to endogenous norepinephrine release are blunted with age in healthy humans. Because postjunctional α₁- and α₂-adrenergic receptors mediate forearm vasoconstriction, 8 a second aim was to determine whether any blunted α-adrenergic responsiveness with age is specific for the α-receptor subtypes. Finally, we determined whether the contribution of tonic sympathetic α-adrenergic vasoconstriction to basal forearm vascular tone is reduced with age.
Subjects
Ten young and 10 older healthy men participated in the present study. All subjects had normal cholesterol, hemoglobin, and hematocrit levels, were normotensive, and were free of overt cardiovascular disease. The older subjects were further evaluated for cardiopulmonary disease with a physical examination and with resting and maximal exercise ECGs. The subjects were not taking medication, were not smokers, and were sedentary. The present study was approved by the Institutional Review Board of the Mayo Clinic, and each subject gave written consent before participation.

Arterial and Venous Catheterization
Under aseptic conditions, a 5-cm 20-gauge catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (2% lidocaine). The arterial catheter was connected to a pressure transducer for determination of mean arterial blood pressure (MAP) and continuously flushed at 3 mL/h with heparinized saline (2 U/mL). A 3-cm 18-gauge catheter was also inserted into an antecubital vein and directed toward the hand so that the tip was located in a deep vein that drained the forearm muscles. Venous blood samples were obtained at selective time points for determination of plasma norepinephrine concentrations.

Body Composition and Forearm Volume
Body composition and forearm fat-free mass were determined by dual-energy x-ray absorptiometry (Lunar). Body mass index was calculated as body weight (kilograms) divided by height (meters) squared. Forearm volume was determined by the water displacement method for normalization of drug administration.

FBF and Vascular Conductance
FBF was measured by use of venous occlusion plethysmography with mercury-in-silastic strain gauges. FBF was expressed as milliliters per 100 mL tissue per minute. To account for any potential changes in blood pressure, forearm vascular conductance (FVC) was calculated as (FBF/MAP)×100 and expressed as arbitrary units.

Blood Samples
Plasma concentrations of cholesterol were determined after a 12-hour overnight fast. Venous blood samples were obtained at selective time points for determination of norepinephrine concentrations. These samples were centrifuged and stored at −70°C for later determination of plasma norepinephrine concentrations via high-performance liquid chromatography.

Experimental Protocol
All measurements were performed after a 12-hour overnight fast, and drugs were administered via the brachial artery catheter at rates of 1 to 3 mL/min with the subject supine. The experimental protocol is shown in Figure 1. After 30 minutes of rest after catheterization, baseline FBF and venous norepinephrine concentrations were determined. Propranolol was then administered (10 µg/100 mL forearm volume per minute) for 5 minutes to block β-adrenergic receptors, and a low “maintenance” dose (5 µg/min) was then infused throughout the protocol. This dose has been documented to block forearm vasodilation to isoproterenol. β-Adrenergic blockade was performed to control for any direct or indirect β-mediated vasodilatory effects of the study drugs and to eliminate any potential age-related differences in β-adrenergic responsiveness that may confound the interpretation of the data regarding α-adrenergic receptors.

Postjunctional α-Adrenergic Receptor Responsiveness
Tyramine was administered at 3, 6, and 12 µg/100 mL forearm volume per minute for 3 minutes to evoke endogenous norepinephrine release and the subsequent stimulation of both α1- and α2-adrenergic receptors. Tyramine does not have any intrinsic vasoconstrictor properties. Phenylephrine was administered at 0.0625, 0.125, and 0.5 µg/100 mL forearm volume per minute for 2 minutes to selectively stimulate α1-adrenergic receptors, and clonidine was administered at 0.15, 0.6, and 1.2 µg/100 mL forearm volume per minute for 2 minutes to stimulate α2-adrenergic receptors. The order of α-agonist administration was randomized across all subjects, and infusion trials were separated by 20 minutes of quiet rest. Because the effects of human aging on tyramine-induced norepinephrine release and on the ability of presynaptic α2-adrenergic receptors to inhibit norepinephrine release are unknown, we determined venous plasma norepinephrine concentrations at baseline and at the end of each dose during tyramine and clonidine administration. Given that we were unable to obtain venous blood samples from all subjects, the presented venous norepinephrine responses to tyramine and clonidine represent the values in 9 young men and 7 older men.

Sympathetic α-Adrenergic Vasoconstrictor Contribution to Basal Forearm Vascular Tone
After 20 minutes of quiet rest after the last α-agonist administration, another 5-minute infusion of propranolol (same dose as original) was given to ensure continuous β-adrenergic blockade. Subsequently, phentolamine (12 µg/100 mL forearm volume per minute) was administered for 10 minutes to block both α1- and α2-adrenergic receptors. Tyramine (6 µg/100 mL forearm volume per minute) was then given to document the effectiveness of nonselective α-adrenergic blockade.

Data Analysis and Statistics
Data were digitized and stored on a computer at 200 Hz and analyzed offline with signal processing software (Windaq, Datq Instruments). FBF was determined from the derivative of the forearm plethysmogram. Heart rate (HR) was determined from the ECG signal (5-lead ECG), and MAP was derived from the arterial pressure waveform. For tyramine, phenylephrine, and clonidine, the data reported represent an average of the last minute of drug infusion. For phentolamine, we observed an initial vasodilation that was sustained throughout the infusion; thus, the data reported represent an average of the last 3 minutes of drug infusion. Because MAP did not change during local infusions of the α-agonists, forearm vasoconstrictor responses are expressed as changes in FBF. During phentolamine, we observed small changes in arterial blood pressure and therefore have presented the forearm hemodynamic data as both FBF and FVC.

Group differences in subject characteristics and baseline values were assessed with 1-way ANOVA. Group differences in the forearm hemodynamic responses to the administration of study drugs were determined by repeated-measures ANOVA. All data expressed are mean±SEM. Statistical significance was set a priori at P<0.05.

Results
The mean age difference between the young and older men was 40 years. There were no significant age-group differences in body mass index, forearm fat-free mass, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, MAP, or HR (Table 1). Percent body fat was significantly
higher and fat-free mass was significantly lower in older men. Deep venous norepinephrine concentrations were higher in the older men compared with the young men, possibly reflecting an elevated sympathetic activity to the arm with age. Baseline FBF and FVC were not different in young and older men (Table 1), and these values returned to baseline before each of the selective drug infusion trials.

Aging and Postjunctional α-Adrenergic Responsiveness

The absolute changes in FBF to all 3 doses of tyramine were significantly less in older men compared with young men (Figure 2A). Similar results were found when the forearm vasoconstrictor responses were expressed as percentage changes from baseline (maximal reduction -37±3% versus -49±3% for older versus young men, respectively; \( P<0.05 \)). Tyramine evoked significant increases in deep venous norepinephrine concentrations in both groups of men, but the increases at the highest dose of tyramine (255±53% versus 368±40%) were significantly lower in older compared with young men, respectively (both \( P<0.01 \)). After removal of sympathetic \( \alpha \)-adrenergic vasoconstrictor tone, absolute levels of both FBF (7.8±0.8 versus 12.6±1.7 mL/100 mL per minute) and deep venous norepinephrine levels evoked via tyramine were not significantly different in older and young men (\( P=0.25 \) to 0.45, Figure 4B).

The absolute reductions in FBF to each dose of clonidine were not significantly different in older and young men (\( P=0.25 \) to 0.45, Figure 4B). Similarly, the maximal percentage reductions in FBF were not different in older and young men (\(-36±7\% \) versus \(-40±3\%, \) respectively; \( P=0.67 \)). All

Figure 2. Changes in FBF (A) and deep venous norepinephrine concentrations (B) to local tyramine administration. \( ^*P<0.001 \) vs young men.

Figure 3. Changes in FBF to changes in deep venous norepinephrine levels evoked via tyramine.

### Table 1. Selected Characteristics and Baseline Forearm Hemodynamics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Men</th>
<th>Older Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>25±1</td>
<td>65±1*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.3±1.2</td>
<td>26.2±1.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>21±2</td>
<td>26±2*</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>68.8±2.6</td>
<td>59.2±2.1*</td>
</tr>
<tr>
<td>Forearm fat-free mass, g</td>
<td>1248±72</td>
<td>1229±59</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.6±0.3</td>
<td>4.6±0.2</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.7±0.3</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Venous NE, pg/mL</td>
<td>163±16</td>
<td>237±24*</td>
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<tr>
<td>MAP, mm Hg</td>
<td>94±2</td>
<td>94±3</td>
</tr>
<tr>
<td>Resting HR, bpm</td>
<td>54±1</td>
<td>55±2</td>
</tr>
<tr>
<td>FBF, mL · 100 mL⁻¹ · min⁻¹</td>
<td>2.6±0.2</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>FVC, U</td>
<td>2.7±0.2</td>
<td>2.4±0.3</td>
</tr>
</tbody>
</table>

NE indicates norepinephrine.

\( ^*P<0.05 \) vs young men.
TABLE 2. Systemic Hemodynamics at Baseline and During Drug Infusions

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Older</td>
</tr>
<tr>
<td>Tyramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>93±2</td>
<td>93±3</td>
</tr>
<tr>
<td>3</td>
<td>94±2</td>
<td>93±3</td>
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<tr>
<td>6</td>
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<td>93±3</td>
</tr>
<tr>
<td>12</td>
<td>93±3</td>
<td>94±3</td>
</tr>
<tr>
<td>Phenylephrine</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>94±2</td>
<td>94±3</td>
</tr>
<tr>
<td>0.0625</td>
<td>94±2</td>
<td>93±2</td>
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<td>0.125</td>
<td>94±2</td>
<td>95±3</td>
</tr>
<tr>
<td>0.5</td>
<td>94±2</td>
<td>95±3</td>
</tr>
<tr>
<td>Clonidine</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>95±2</td>
<td>94±2</td>
</tr>
<tr>
<td>0.15</td>
<td>95±2</td>
<td>95±3</td>
</tr>
<tr>
<td>0.6</td>
<td>96±2</td>
<td>95±3</td>
</tr>
<tr>
<td>1.2</td>
<td>94±2</td>
<td>96±3</td>
</tr>
<tr>
<td>Phentolamine</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>94±2</td>
<td>94±3</td>
</tr>
<tr>
<td>After</td>
<td>91±3</td>
<td>90±3*</td>
</tr>
</tbody>
</table>

Numbers below drug are respective doses in μg/100 mL forearm volume per minute.
*P<0.05 vs baseline.

FVC (8.9±0.9 versus 14.4±2.3 U) were significantly lower in older men compared with young men, respectively (both P<0.01, Figure 5). Phentolamine slightly reduced MAP (=4 [older] and ≈3 [young] mm Hg) and increased HR (3 bpm in both groups). Forearm vasoconstrictor responses to tyramine were nearly abolished in both older (−4±2%) and young (−3±1%) men, confirming effective α-adrenergic blockade.

**Discussion**

The major new findings from the present study are as follows: First, forearm postjunctural α-adrenergic vasoconstrictor responsiveness to endogenous norepinephrine release is blunted in older healthy men. Second, the forearm vasoconstrictor responses to the α1-agonist phenylephrine are reduced with age, whereas the responses to the α2-agonist clonidine are preserved with age. Thus, it appears that the age-related reduction in α-adrenergic responsiveness might be specific to postjunctural α2-adrenergic receptors. Finally, the contribution of tonic sympathetic α-adrenergic vasoconstriction to basal forearm vascular tone is lower in older men compared with young men.

**Age and Forearm Postjunctural α-Adrenergic Responsiveness**

Previous studies in humans have provided preliminary evidence of reduced postjunctural α-adrenergic responsiveness with age. Specifically, Davy et al1 demonstrated a blunted forearm vasoconstrictor response for a given increase in MSNA in older adults during lower body negative pressure and provided indirect evidence that this does not reflect a reduced norepinephrine release with age. However, potential changes in circulating vasoactive factors can be altered during orthostatic stress (eg, epinephrine) and may limit the ability to interpret the local hemodynamic data with respect to the ability of norepinephrine, per se, to evoke vasoconstriction. In another study, forearm vasoconstrictor responses to exogenous norepinephrine administration were demonstrated to be lower in older adults.5 However, exogenous norepinephrine administration primarily stimulates luminal α-adrenergic receptors, in contrast to the abluminal receptors anatomically closer to the neuroeffector junction, which are stimulated by endogenous norepinephrine release from sympathetic nerves. Therefore, our results provide the first direct evidence of blunted postjunctural α-adrenergic responsiveness to neurally released norepinephrine (evoked via tyramine) with age in healthy humans. Furthermore, this observed difference was even greater after accounting for tyramine-induced changes in deep forearm venous norepinephrine concentrations (Figure 3).

To the best of our knowledge, this is the first study to determine the effects of aging on selective postjunctural α1- and α2-adrenergic receptor responsiveness in the arterial circulation of humans in vivo. The data from the present study indicate that the forearm vasoconstrictor responses to phenylephrine (selective α1-agonist) are blunted in older compared with young men, whereas the vasoconstrictor responses to clonidine (α2-agonist) do not differ with age. Importantly, this latter finding does not appear to be con-
that the contribution of tonic sympathetic α-adrenergic vasoconstriction to basal forearm vascular tone is reduced with age. This finding is especially interesting given the fact that tonic NO vasodilation, which has been documented to oppose sympathetic vasoconstriction, is reduced with age. Taken together, it appears that the relative contribution of local and neural factors involved in the regulation of basal forearm vascular tone are altered with age but that the net interaction does not influence overall vascular tone.

The observation of a reduced sympathetic α-adrenergic vasoconstrictor tone in the forearm with age is in stark contrast with the augmented α-adrenergic vasoconstrictor tone documented to be primarily responsible for the age-related decline in whole-leg blood flow and vascular conductance. Whether this reflects less, or possibly an absence, of an age-related reduction in α-adrenergic responsiveness in the leg circulation is currently unknown. It is also unknown whether the interaction between local and neural factors in the control of vascular tone differs with age in the arm circulation compared with the leg circulation in healthy humans. Future studies are needed to address these issues.

Previously, Hogikyan and Supiano reported that forearm vasodilation to phentolamine is not different in older and young adults. In their previous study, β-adrenergic receptors were not blocked before α-blockade, and effective (complete) α-blockade was not demonstrated, in contrast to the design of the present study. Therefore, we cannot be certain why our findings differ from those of Hogikyan and Supiano, but these experimental differences might have played a role. In the present investigation, we observed slight blood pressure changes in both groups of men during phentolamine administration. However, the absence of vasoconstriction to tyramine after phentolamine in both groups of men suggests that any baroreflex-mediated changes in sympathetic nerve activity could not exert any counterregulatory changes in forearm vascular tone and, thus, should not confound the interpretation of these findings.

**Experimental Considerations**

Recent evidence suggests that NO can blunt α-adrenergic vasoconstriction in humans and that this is specific for α2-adrenergic receptors. It is also well documented that aging is associated with a reduced tonic NO bioavailability. Therefore, if NO can blunt α-adrenergic vasoconstriction in young healthy adults and if this ability is impaired or abolished in older individuals, we might have underestimated the age-group differences in α-adrenergic responsiveness to endogenous norepinephrine release. Additionally, if aging impairs the ability of endothelial α2-adrenergic receptors to produce NO when stimulated, we might have observed a reduction in α2-adrenergic responsiveness if it had been assessed during NO inhibition (due to less blunting of the response by NO in older adults). Finally, if endothelial α2-receptors are involved in the regulation of tonic NO synthesis and release and if this is reduced with age, we may have underestimated the age-group differences in the forearm vasodilation observed during nonselective α-blockade with phentolamine.
Experimental Limitations
The mechanisms responsible for the reduction in postjunctional α-adrenergic responsiveness with age in humans are unknown. Whether this reflects a reduction in α-adrenergic receptor density, the binding of norepinephrine to postjunctional receptors, impairments in the intracellular signaling pathway, or some combination of these factors is not known. Future studies are needed to address these issues.

Potential Significance
Skeletal muscle blood flow regulation during a variety of hyperemic conditions depends on the interaction between local vasodilator and neural vasoconstrictor influences. As stated previously, recent evidence suggests that NO can blunt sympathetic α-adrenergic vasoconstriction in humans and that this is specific for α2-adrenergic receptors. Given that α2-adrenergic receptor responsiveness is preserved with age and that NO-mediated vasodilation (tonic and stimulated) is reduced with age, the net interaction among these regulators of skeletal muscle blood flow may promote an augmented vasoconstrictor state in older compared with young humans. In this context, the increases in skeletal muscle blood flow during both large muscle dynamic exercise and acute vasoconstriction with age in healthy men. Furthermore, it appears that the responsiveness to endogenous norepinephrine release is reduced with age in healthy humans (ie, vasoconstriction is augmented). Thus, perhaps the ability of local factors (eg, NO) to blunt sympathetic vasoconstriction during such hyperemic conditions is impaired with age.

Conclusions of the Study
The results from the present investigation demonstrate that forearm postjunctional α-adrenergic vasoconstrictor responsiveness to endogenous norepinephrine release is reduced with age in healthy men. Furthermore, it appears that the age-related reduction in α-adrenergic responsiveness might be specific to postjunctional α1-adrenergic receptors. Finally, tonic sympathetic α-adrenergic vasoconstriction is reduced with age in the forearms of healthy men. The potential physiological and pathophysiologic implications of these findings remain to be determined.

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