Augmented Cardiopulmonary and Integrative Sympathetic Baroreflexes but Attenuated Peripheral Vasoconstriction With Age

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Abstract—Based on observations of smaller increases in limb vascular resistance during acute incremental hypovolemia in older adults, cardiopulmonary and integrative (combined cardiopulmonary and arterial) baroreflex control of sympatho-circulatory function is thought to be impaired with aging in humans. We tested this hypothesis directly by making intraneural measurements of skeletal muscle sympathetic nerve activity (MSNA; peroneal microneurography) in groups of young (23±1 years; n=11) and older (64±1 years; n=12) healthy adult men during progressive hypovolemia produced by graded (−5 to −40 mm Hg) lower body negative pressure (LBNP). Baseline levels of MSNA and arterial blood pressure were higher and heart rate was lower in the older subjects (P<0.05). Lower levels of LBNP (−5 to −20 mm Hg) did not affect arterial blood pressure or heart rate in either group; systolic and pulse pressures declined during higher levels of LBNP (−30 and −40 mm Hg) but only in the young subjects (P<0.05). Graded LBNP evoked progressive, linear reductions in peripheral venous pressure (PVP) and increases in MSNA, plasma norepinephrine concentration (PNE), and forearm vascular resistance (FVR) in both groups (all P<0.05). ΔMSNA/ΔPVP was ≈150% greater in the older versus young men during both lower and higher levels of hypovolemia (P<0.01); however, ΔFVR/ΔPVP was ≈50% smaller in the older men (P<0.05). There was no difference in the MSNA-PNE relation with age, but ΔFVR/ΔMSNA was ≈65% to 70% smaller in the older subjects (P<0.05). Our findings indicate that cardiopulmonary and integrative baroreflex control of central sympathetic outflow during hypovolemia is augmented, not impaired, with age in healthy humans. However, the reflex-mediated increases in limb vascular resistance during hypovolemia are smaller in older adults because of attenuated vasoconstrictor responsiveness to sympathetic stimulation. (Hypertension. 1998;32:298-304.)

Key Words: hypovolemia ■ aging ■ blood pressure

The cardiopulmonary and arterial baroreflexes play a critical role in maintaining circulatory homeostasis, in large part through their tonic inhibition of SNA.1,2 In humans, baroreflex control of SNA often has been studied using graded hypovolemia.1,2 Low levels of hypovolemia at which heart rate and arterial blood pressure are unaffected are thought to preferentially deactivate cardiopulmonary baroreflexes and evoke increases in skeletal MSNA, PNE concentration, and limb vascular resistance.1,7 More severe levels of hypovolemia that cause tachycardia and, at least in healthy young adults, reductions in arterial blood pressure are thought to unload both cardiopulmonary and arterial baroreceptors, resulting in a greater and more systemic “integrative” baroreflex stimulation of SNA.1,2,4,5 Primary aging produces a number of changes in cardiovascular structure and function in humans.5,9 It has been proposed that among these changes is an impairment in cardiopulmonary and integrative baroreflex sympatho-circulatory control.10,11 This idea is based largely on observations of smaller reflex increases in FVR in older compared with young adult subjects during both low and more severe levels of hypovolemia.10,11 In a prior investigation of this issue,12 our laboratory found that the increases in PNE concentration during graded hypovolemia were essentially identical in normotensive young and older adult males but that the increases in FVR tended to be smaller in the older subjects. In a more recent study,13 we found similar reflex increases in MSNA in young and older adult men and women in response to a modest postural challenge (sitting up). Taken together, our previous observations are more consistent with the hypothesis that cardiopulmonary and integrative baroreflex control of SNA is well maintained with advancing age in healthy humans but that there may be an attenuated peripheral vasoconstrictor response to sympathetic activation.

Accordingly, in the present study we prospectively tested this hypothesis. To do so, in a series of experiments we performed direct (intraneural) recordings of MSNA and...
determined PNE and FVR in groups of healthy young and older adult men in the supine position under control conditions and during progressively greater levels of hypovolemia induced by graded LBNP.

Methods

Subjects
Eleven young and 12 older healthy nonobese men participated in the present investigation. All subjects were normotensive and free from overt cardiovascular disease, as assessed from casual blood pressure measurements and medical history. Older subjects were further evaluated for clinical evidence of cardiopulmonary disease with a physical examination and resting and maximal exercise ECG. All subjects were nonsmokers, and none took medications that could affect autonomic-circulatory function. The nature, purpose, and risks of the study were explained to each subject before written informed consent was obtained. The study was approved by the Human Research Committee at the University of Colorado.

Experimental Procedures

Multiunit recordings of MSNA were obtained from the right peroneal nerve using the microneurographic technique as previously described.11-14 The neural activity was amplified, filtered (700 to 200 Hz), full-wave rectified, and integrated (time constant, 0.1 second). Recordings of efferent MSNA were deemed acceptable according to previously described criteria.16 Heart rate was obtained from an ECG, and beat-to-beat arterial pressure was measured continuously during baseline control and LBNP with subjects in a supine position (Table). Venous blood samples for subsequent determination of PNE concentration were obtained during the last minute of the control period and through the −20 mm Hg level of LBNP. Problems with blood sampling were encountered in several subjects at −30 and −40 mm Hg LBNP; thus, data for PNE are reported only for the initial 4 levels of hypovolemia. Eleven young and 12 older subjects initiated this protocol. Because of presyncopal symptoms or nonspecific discomfort, the number of subjects completing various levels of LBNP were 9 young and 11 older men through −20 mm Hg; 8 young and 9 older men through −30 mm Hg; and 7 young and 9 older men through −40 mm Hg.

Protocol 2: PVP Responses to Graded Hypovolemia
PVP (measure of the hypovolemic stimulus), heart rate, and arterial blood pressure were measured continuously during baseline control and LBNP. Subjects were in the right lateral decubitus position with the right arm extended downward as described previously.15 The numbers of subjects completing the various levels of LBNP in this protocol and protocol 3 below were similar to those described above for protocol 1.

Protocol 3: FVR Responses to Graded Hypovolemia
FVF, heart rate, and arterial blood pressure were measured during baseline control and LBNP with subjects in the supine position. FVR was calculated from mean arterial blood pressure/FBF.

Data Analysis
MSNA, heart rate, and arterial blood pressure were recorded continuously on a Gould ES1000 recorder (Gould Instruments) and were stored on videocassette (A.R. Vetter) for subsequent computer analysis. PVP and FBF were calculated manually from chart recorder tracings. The bursts of MSNA, PVP waves, and FBF curves were measured by the same investigator (H.T.), who was blinded to the identity of the subjects. MSNA was expressed as total minute activity (arbitrary units) and quantified by computer measurements of area under each burst of neural activity.15 The forearm vasoconstrictor responses to LBNP also were analyzed as changes in vascular conductance (ie, FBF/mean arterial blood pressure).

Statistical Analysis
A one-way ANOVA was used to test for differences in each subject characteristic. Main effects for group and time for each dependent variable were assessed by repeated-measures ANOVA. A Newman-Kuels post hoc procedure was used to test for differences between groups at a particular time point or within groups across time. Simple linear regression and univariate correlational analyses were used to determine the relations between MSNA and PVP and between MSNA and FVR in the young and older men. ANCOVA was used as a complementary approach to the ratio method for examining possible group differences in the ΔMSNA/ΔPVP and the ΔFVR/ΔPVP.
MSNA responses to LBNP. The significance level was set at $P<0.05$. All values are presented as mean±SE.

Results

Subject Characteristics

The older men were ≈40 years older than the young adult controls (64±1 versus 23±1 years; $P<0.05$). However, the 2 groups did not differ significantly in height (176±2 versus 179±2 cm), body mass (77.1±2.2 versus 76.7±2.0 kg), or body mass index (25.0±0.6 versus 23.9±0.6). Although well within the normotensive range, resting brachial systolic arterial blood pressure was higher in the older (124±3 mm Hg) compared with the younger (113±4 mm Hg) men ($P<0.05$); there were no group differences in resting brachial diastolic arterial blood pressure (76±2 versus 73±3, older versus younger).

Protocol 1: MSNA Responses to Graded Hypovolemia

Baseline heart rate was lower (Figure 1) and Finapres-measured systolic and mean arterial blood pressures were higher (Figure 2) in the older subjects. MSNA burst frequency (42±3 versus 21±2 bursts per minute) and total activity (Figure 1) were higher in the older men during supine rest ($P<0.05$), but PNE concentration (Figure 1) did not differ with age.

Heart rate and arterial blood pressure did not change significantly from baseline levels during LBNP at −5 to −20 mm Hg in either group (Figures 1 and 2). In the young controls, LBNP at −30 and −40 mm Hg elicited reductions in systolic and pulse pressures but no significant changes in diastolic or mean pressures. In contrast, the older men demonstrated unchanged systolic and pulse pressures but increased diastolic and mean pressures during these higher levels of LBNP. Heart rate increased above baseline levels in both groups during −30 and −40 mm Hg of LBNP ($P<0.05$); the magnitudes of the increases were smaller in the older men ($P<0.05$).

MSNA increased linearly from baseline levels with graded LBNP in both groups ($P<0.05$; Figure 1). The older men tended to demonstrate greater increases in MSNA compared with the young adult controls ($P=0.06$). PNE concentration increased linearly from baseline levels during graded LBNP ($P<0.05$), with no group differences in the magnitudes of the increases (Figure 1). The relation between PNE concentration and MSNA during progressive hypovolemia did not differ with age ($P>0.3$).

Protocol 2: PVP Responses to Graded Hypovolemia

The arterial blood pressure and heart rate responses to graded hypovolemia were not different during this protocol compared with those observed in protocol 1. Baseline PVP was not different in the young and older subjects (Figure 1). PVP decreased linearly throughout LBNP in both groups ($P<0.01$; Figure 1); the reductions tended to be smaller in the older

![Figure 1](http://hyper.ahajournals.org/)

![Figure 2](http://hyper.ahajournals.org/)
men, but the differences were not statistically significant ($P=0.15$).

Figure 3 illustrates the mean increases in MSNA per millimeter of mercury mean reduction in PVP ($\Delta$MSNA/$\Delta$PVP) during graded hypovolemia in the young and older men. The slope of the line was greater in the older subjects ($P<0.05$). The corresponding mean $\Delta$MSNA/$\Delta$PVP was $\approx$150% greater in the older men than in the young adults controls during the lower ($-5$ through $-20$ mm Hg LBNP), the higher ($-30$ and $-40$ mm Hg LBNP), and the overall ($-5$ through $-40$ mm Hg LBNP) levels of hypovolemia ($P<0.05$). ANCOVA with PVP as the covariate confirmed that the MSNA response to graded hypovolemia was greater ($P<0.05$) in the older subjects.

Protocol 3: FVR Responses to Graded Hypovolemia
The arterial blood pressure and heart rate responses to graded hypovolemia were not different during this protocol compared with those observed in protocols 1 and 2. Supine resting baseline levels of FBF and FVR (Figure 4) and forearm vascular conductance (0.038 $\pm$ 0.005 versus 0.038 $\pm$ 0.006 U) were not significantly different in the young and older men.

The FBF and FVR responses to progressive hypovolemia are illustrated in Figure 4. FBF decreased and FVR increased with graded LBNP in both groups ($P<0.05$). The reductions in FBF were not significantly different in the 2 groups but tended to be smaller in the older men. However, the increases in FVR were smaller in the older men for the $\approx 10$ mm Hg levels of LBNP ($P<0.05$ to $P<0.01$). Moreover, mean $\Delta$FVR/$\Delta$PVP was $\approx$50% smaller in the older men compared with the young adult subjects during the lower ($1.9 \pm 0.6$ versus $3.9 \pm 0.6$), the higher ($2.5 \pm 0.5$ versus $5.1 \pm 0.7$), and the overall ($1.9 \pm 0.4$ versus $4.1 \pm 0.6$) levels of hypovolemia (all $P<0.05$).

The relation between the mean increases in FVR and MSNA during graded hypovolemia is shown in Figure 5. The slope of the line was smaller in the older subjects ($P<0.05$). The mean $\Delta$FVR/MSNA was $\approx$65% to 70% smaller in the older than in the young adult subjects during the lower (0.007 $\pm$ 0.005 versus 0.024 $\pm$ 0.005), the higher (0.006 $\pm$ 0.005 versus 0.019 $\pm$ 0.003), and the overall (0.007 $\pm$ 0.005 versus 0.020 $\pm$ 0.004) levels of hypovolemia (all $P<0.05$). ANCOVA with MSNA as the covariate confirmed that the FVR response to graded hypovolemia was attenuated in the older subjects ($P<0.05$).

For all comparisons, the same age group–related differences were obtained when decreases in forearm vascular conductance were used to express the limb vasoconstrictor responses to LBNP as when increases in FVR were used.

Discussion
To our knowledge, this is the first investigation of the effects of aging on cardiopulmonary and integrative baroreflex regulation of directly measured SNA in humans. Moreover, this is the first study in humans to determine age-related changes in the relation between SNA and peripheral vasoconstrictor responsiveness.

There are 2 primary new findings from the present investigation related to our working hypothesis. First, it appears that cardiopulmonary and integrative (ie, combined cardiopulmonary and arterial) baroreflex control of MSNA during hypovolemia is augmented, rather than impaired, with advancing age in healthy adult humans. Second, the smaller increase in FVR during hypovolemia-induced baroreflex deactivation reported previously in older adults,$^{10,11}$ likely is due to an attenuated vasoconstrictor response to sympathetic stimulation.
Based largely on observations of smaller increases in FVR in response to graded hypovolemia in older compared with young adults, it has been concluded that cardiopulmonary and integrative baroreflex sympathetic-circulatory control becomes impaired with advancing age in humans.\(^1\) The present findings (Figure 4) confirm these previous observations of an age-related attenuation in the FVR response to hypovolemia. Our results (Figure 5), however, demonstrate that this likely is the result of nonneural changes affecting vasoconstrictor responsiveness rather than an impairment in baroreflex control of SNA. In fact, the latter actually appears to be enhanced in healthy older adults (Figure 3). This apparent augmented sympathetic baroreflex sensitivity may have functional importance in acting to counter the apparent age-related reduction in vascular responsiveness to sympathetic neural activation.

The present results are consistent with our previous observations of similar increases in PNE concentration but a tendency for smaller increases in FVR in older compared with young adult males during graded hypovolemia.\(^2\) Our findings also agree with recent studies in humans reporting attenuated FVR responses to norepinephrine infusion\(^3\) and delayed pressor responses to breathing-induced increases in MSNA\(^4\) in older adult humans. An age-related decline in sympathetic baroreflex sensitivity may have functional importance in acting to counter the apparent age-related reduction in vascular responsiveness to sympathetic neural activation. In healthy older adults (Figure 3). This apparent augmented sympathetic baroreflex sensitivity may have functional importance in acting to counter the apparent age-related reduction in vascular responsiveness to sympathetic neural activation.

One likely explanation for the greater FVR response in older compared with young adults to orthostatic stress is that greater vasoconstriction was evoked in other (ie, nonskeletal muscle) regional circulations in the older subjects than in the young adult controls. A smaller challenge to arterial blood pressure maintenance may also explain the smaller heart rate response to the higher levels of LBNP in this (Figure 1) and our previous studies. That is, less of an arterial baroreflex-mediated tachycardia would be required to maintain arterial pressure under these circumstances. The lack of decline in arterial systolic and pulse pressures in the older males during these higher levels of LBNP was associated with less of a decline in left ventricular end-diastolic volume (and presumably stroke volume) in older compared with young adult humans. This might result in less translocation of central blood volume to the periphery and therefore a smaller challenge to arterial blood pressure control in the older males. Another possibility is that greater vasoconstriction was evoked in other (ie, nonskeletal muscle) regional circulations in the older subjects than in the young adult controls.

In an earlier report, Iwase and colleagues\(^5\) suggested that stroke volume and cardiac output declined less during the more severe levels of hypovolemia, as we observed in our previous study,\(^6\) due in part to age-related increases in ventricular and vascular stiffness.\(^7\) In this context, Cleroux and colleagues\(^8\) found that the reduction in central venous pressure during \(-40\) mm Hg LBNP was associated with less of a decline in left ventricular end-diastolic volume (and presumably stroke volume) in older compared with young adult humans. This might result in less translocation of central blood volume to the periphery\(^9\) and therefore a smaller challenge to arterial blood pressure control in the older males. Another possibility is that greater vasoconstriction was evoked in other (ie, nonskeletal muscle) regional circulations in the older subjects than in the young adult controls.

In an earlier related investigation,\(^1\) we found that arterial blood pressure was regulated as well or better in healthy normotensive older humans compared with young adult controls during acute hypovolemia. The present study confirms these prior observations. Specifically, arterial blood pressure was well maintained in both age groups during low-moderate levels of LBNP (Figure 2). At the 2 highest levels of LBNP (\(-30\) and \(-40\) mm Hg), however, systolic and pulse pressures fell significantly below baseline levels in the young but not in the older men.

Precise regulation of arterial pressure in the face of an attenuated peripheral vasoconstriction in the older subjects deserves comment. The most likely explanation is that stroke volume and cardiac output declined less during the more severe levels of hypovolemia, as we observed in our previous study,\(^1\) due in part to age-related increases in ventricular and vascular stiffness.\(^1\) In this context, Cleroux and colleagues\(^8\) found that the reduction in central venous pressure during \(-40\) mm Hg LBNP was associated with less of a decline in left ventricular end-diastolic volume (and presumably stroke volume) in older compared with young adult humans. This might result in less translocation of central blood volume to the periphery and therefore a smaller challenge to arterial blood pressure control in the older males. Another possibility is that greater vasoconstriction was evoked in other (ie, nonskeletal muscle) regional circulations in the older subjects than in the young adult controls.
our previous studies on upright sitting and other forms of laboratory stress\textsuperscript{11,15} demonstrate that older adults evoke as great or greater absolute increases in MSNA in response to sympatho-excitatory stimuli as young adults. Moreover, the present results are consistent with recent findings that integrative baroreflex-mediated increases in MSNA in response to intravenous administration of vasodilator drugs (which produce changes in both systemic arterial and central venous pressures) are not impaired in middle-aged and older humans despite their elevated resting levels of MSNA.\textsuperscript{23,25} There are at least 4 important caveats associated with the present study that should be mentioned. First, the results of recent studies in humans suggest that changes in aortic\textsuperscript{26} and carotid\textsuperscript{27,28} artery dimensions are observed even during low levels of LBNP, suggesting that arterial as well as cardiopulmonary baroreflexes may be deactivated throughout graded hypovolemia. Therefore, the specific baroreflexes that were unloaded under these conditions, and thus contributed to the stimulation of MSNA, cannot be determined with certainty. Because there is no reflex tachycardia suggestive of robust arterial baroreflex involvement during low levels of LBNP, however, it is likely that cardiopulmonary baroreflexes play a major role in the regulation of MSNA during mild hypovolemia in humans. With increasing levels of hypovolemia that produce a progressively greater challenge to arterial blood pressure maintenance, arterial baroreflex deactivation presumably contributes in an increasingly greater manner to the stimulation of MSNA. In the present study, we found that $\Delta$MSNA/$\Delta$PVP was $\approx 150\%$ greater in the older subjects during both the lower and higher levels of hypovolemia. As such, our results are consistent with the concept that both the cardiopulmonary and the integrative (cardiopulmonary and arterial) sympathetic baroreflexes may have been more sensitive in the older men.

Second, we measured SNA in the lower leg and blood flow in the forearm in the present study. However, Rea et al\textsuperscript{29} have established that graded LBNP elicits similar increases in MSNA in the leg and arm in humans.

Third, it is possible that the attenuated limb vasoconstrictor response to sympathetic stimulation in the older subjects was due in part to a smaller rate of release from sympathetic nerve endings and consequent lower synaptic concentration of norepinephrine (ie, rather than reduced vasomotor responsiveness to norepinephrine). Without more direct measures of PNE kinetics from the forearm, we cannot determine the likelihood of this possibility. However, the facts that (1) PNE concentrations obtained from antecubital venous blood samples were similar in the 2 groups and (2) PNE clearance, including the reuptake 1 mechanism, decreases with age in humans\textsuperscript{30} (which would act to elevate rather than reduce synaptic concentrations) are inconsistent with this idea. Moreover, the limited available data suggest that sympathetic innervation of skeletal muscle arterioles is not related to age in healthy adult humans.\textsuperscript{31}

Finally, because our interest in gerontology and geriatric medicine centers on the role of primary aging in cardiovascular health and disease, we studied older subjects who were normotensive and otherwise free of overt cardiovascular disorders. In this context, we should emphasize that less healthy older adults may demonstrate impaired baroreflex control of SNA secondary to the effects of hypertension and/or other forms of cardiovascular disease.

In conclusion, the present findings provide experimental support for the concept that cardiopulmonary and integrative baroreflex control of SNA during acute hypovolemia is enhanced rather than depressed in healthy older humans. This may help minimize the functional impact of a marked age-related reduction in peripheral vasoconstrictor responsiveness to sympathetic neural stimulation and contribute to the effective regulation of arterial blood pressure in older adults during orthostatic challenge.

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