Influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult humans

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Davy, Kevin P., Hirofumi Tanaka, Elizabeth A. Andros, John G. Gerber, and Douglas R. Seals. Influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult humans. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1768–H1772, 1998.—Resting levels of muscle sympathetic nerve activity (MSNA) increase markedly with age in healthy adult humans. An age-related reduction in arterial baroreflex inhibition of MSNA could contribute to these elevations. To test this hypothesis, we measured MSNA using peroneal microneurography in young (age, 25 ± 1 yr; n = 8) and older (69 ± 1 yr; n = 7) healthy normotensive men before (baseline control) and during graded constant infusion of phenylephrine hydrochloride (0.5–2.0 µg·kg⁻¹·min⁻¹) that produced a sustained ~10-mmHg increase in arterial blood pressure. Central venous pressure was controlled at baseline levels (±1 mmHg) using lower body negative pressure. Resting MSNA was ~95% higher in the older compared with the young subjects (43 ± 5 vs. 22 ± 3 bursts/min; P < 0.05). However, arterial baroreflex MSNA inhibitory responsiveness was similar in the older compared with the young subjects (254 ± 112 vs. 259 ± 40 arbitrary integration units/mmHg, respectively), although the percent reduction in MSNA was smaller in the older men (8.9 ± 0.7 vs. 5.2 ± 1.1% mmHg/mmHg), due to their elevated baseline levels. The reflex increase in the R-R interval was not different in the two groups (13 ± 10 vs. 16 ± 7 ms/mmHg). In summary, our findings suggest that arterial baroreflex inhibition of MSNA is preserved with age in healthy normotensive adult humans. As such, this mechanism does not appear to contribute to the age-related rise in tonic MSNA.

autonomic nervous system; heart rate

MUSCLE SYMPATHETIC NERVE activity (MSNA) at rest increases markedly with advancing age in humans (11, 19, 27). Recently, we (12, 13) demonstrated that this age-associated increase in MSNA is related in part to increases in total body and abdominal adiposity. However, other mechanisms that may contribute to this elevation in MSNA remain undetermined. Arterial baroreflexes exert a strong tonic inhibitory influence on MSNA in humans (15). Therefore, a reduction in this inhibitory effect with age could contribute to the age-associated rise in MSNA. Consistent with this idea, arterial baroreflex inhibition of renal SNA is reduced in senescent compared with mature adult beagles (10). However, no age-related differences in arterial baroreflex inhibition of MSNA have been reported to date in humans (4, 17).

There are two potentially important limitations in the previous investigations in humans that could explain these seemingly divergent observations. First, in the studies in beagles (10) the age-related impairment in arterial baroreflex inhibition of sympathetic outflow was due to an inability to sustain the inhibitory effect in response to increases in carotid sinus pressure, i.e., the initial (immediate) reflex inhibition was preserved with age. Previous studies in humans (4, 17) have used bolus administration of a vasoconstrictor drug (phenylephrine) that produces a rapid, dynamic increase in the arterial blood pressure stimulus; as such, their data represent only the initial reflex response. Thus potential age-related changes in the ability to sustain a baroreflex inhibition of MSNA cannot be ascertained from the results of these earlier investigations.

The second limitation is that intravenous administration of phenylephrine evokes increases not only in arterial pressure but also in central venous pressure (24), the latter being a stimulus for cardiopulmonary baroreflex inhibition of MSNA in humans (16). We recently reported (3) that the reflex MSNA adjustments to changes in central venous pressure are augmented with age in humans. Therefore, in these previous studies in humans it is possible that a reduced sympathoinhibitory influence of the arterial baroreflex with age was masked by a greater cardiopulmonary baroreflex-mediated suppression of MSNA.

Accordingly, the purpose of the present investigation was to test the hypothesis that arterial baroreflex inhibition of MSNA is impaired with age in adult humans. To address this, we performed intraneuronal recordings of MSNA in young and older healthy adults during graded constant infusion of phenylephrine intended to produce a sustained increase in arterial blood pressure, while central venous pressure was maintained at baseline levels.

METHODS

Subjects. Experiments were completed on eight young and seven older healthy adult male subjects. All were free of overt cardiopulmonary disease as assessed from medical history, physical examination, and a resting electrocardiogram. Older subjects were further evaluated for clinical evidence of cardiopulmonary disease with a maximal exercise electrocardio-
gram. In addition, all subjects demonstrated normal labora-
tory screening that included a complete blood count, urinalysis,
and levels of serum electrolytes, blood urea nitrogen, creati-
nine, glucose, calcium, aspartate aminotransferase, alkaline
phosphatase, bilirubin, and thyroid-stimulating hormone.
None of the subjects were smokers or were taking medica-
tions that could influence autonomic-circulatory function. All
subjects were sedentary and were not participating in any
program of regular exercise. The nature, purpose, and risks of
the study were explained to each subject before informed
consent was obtained. The experimental protocol was ap-
proved by the Colorado Multiple Institutional Review Board.

Measurements. Heart rate was measured from the lead of
an electrocardiogram that produced the highest R wave
amplitude. Respiratory excursions were measured by a pneu-
nobelt placed around the upper abdomen.

Beat-to-beat arterial blood pressure was measured with
finger photoplethysmography (Finapres model 2300, Ohm-
eda). This device has been validated with direct radial artery
determination of arterial blood pressure during vasoactive
drug infusions (23). Mean arterial blood pressure was calcu-
lated as one-third pulse pressure plus diastolic blood pres-
sure. Brachial arterial blood pressure also was measured
with an oscillographic device (Dynamap, Critikon) on the right
arm immediately before the baseline measurement period.
These pressure readings were used to “calibrate” the baseline
levels of arterial blood pressure obtained from the Finapres.

Recordings of multunit MSNA were obtained from the
right peroneal nerve, using the microneurographic technique
as described previously (19–22). The neural activity was
amplified, filtered (700–2,000 Hz), full-wave rectified, and
integrated (time constant, 0.1 s) (nerve traffic analyzer, model
662c-3, University of Iowa Bioengineering). Neurograms
were considered acceptable as recordings of efferent MSNA
according to previously published criteria (30).

Central venous pressure was measured (Hewlett-Packard
model M1960 A) from a catheter (19-gauge, 24 in., Intra-Cath,
Sandy, UT) inserted into an antecubital vein and advanced to
an intrathoracic position at which right atrial pressure waves
were identified. The position of the catheter tip was confirmed
by chest X-ray.

Data analysis. The electrocardiogram, respiration, beat-to-
beat arterial blood pressure, and MSNA were digitized at 500
samples/s for subsequent off-line analysis with signal-
processing software (CODAS, Dataq Instruments; DADISP,
DSP Development). Average MSNA was calculated from the
neurogram normalized for burst amplitude. The largest sym-
pathetic burst occurring during the baseline measurement
period was assigned a value of 1,000 arbitrary integration
units (AIU) for each subject; all other bursts were calibrated
against that standard (29). Muscle sympathetic nerve activity
was quantified by custom software designed to identify
sympathetic bursts above baseline noise with the appropriate
delay from the R wave of the electrocardiogram (~1.3 s) (6).
Only bursts with a signal-to-noise ratio >2:1 were included
for analysis. The area under each burst was measured, and
the average burst area was derived for each subject for cal-
culation of average MSNA. Average MSNA was calculated
as a function of time both as bursts per minute and as total
activity (average burst area × bursts/min).

Experimental protocol. Subjects were admitted to the Gen-
ergical Clinical Research Center and studied after a 12-h
overnight fast. After steady-state levels of all variables were
established and baseline data were obtained over a subse-
quent 10-min period, graded constant intravenous infusions
of phenylephrine hydrochloride were administered. The ini-
tial dosage was 0.5 µg·kg⁻¹·min⁻¹, and the dose was in-
creased progressively by increments of 0.5 µg·kg⁻¹·min⁻¹
until a sustained elevation of ~10 mmHg in diastolic blood
pressure was observed. The dosage that was required to
produce the desired increase in diastolic blood pressure was
1.5–2.0 µg·kg⁻¹·min⁻¹ for all subjects. Each infusion step
was ~5 min in duration. In general, “new” steady-state levels
were achieved within 2–3 min after the start of a higher dose,
after which data were acquired for an additional 2–3 min.
During all phases of the infusions, central venous pressure
was maintained within ±1 mmHg of baseline control levels in
all subjects with the use of nonhypotensive lower body
negative pressure (~20 mmHg).

Statistical analyses. Differences in dependent variables
between young and older subjects were assessed with un-
paired Student’s t-tests. The regression line relating the
change in arterial pressure (both diastolic and mean) to the
change in MSNA in response to phenylephrine infusions
exhibited a nonzero intercept. Therefore, the use of a ratio
(i.e., Δdiastolic blood pressure/ΔMSNA) as an expression of
baroreflex responsiveness may lead to spurious results (1).
Accordingly, an analysis of covariance (ANCOVA) (diastolic
and mean arterial pressure were used as covariates in
separate analyses) was also used to test for differences in
MSNA in response to phenylephrine infusion in the two
groups. The significance level was set a priori at P < 0.05.

RESULTS

Subjects. Subject characteristics are shown in
Table 1. There was an approximate 45-yr age difference
between the young and older subjects. No significant
differences in body mass, height, or body mass index
were observed between the groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25 ± 1</td>
<td>69 ± 2*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>84.8 ± 5.0</td>
<td>76.6 ± 5.6</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.04</td>
<td>1.74 ± 0.03</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7 ± 1.0</td>
<td>25.3 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 young and 7 older subjects. *P < 0.05 vs. young subjects.
Table 2. Hemodynamics and MSNA at baseline and in response to phenylephrine infusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval, ms</td>
<td>Young (975±30)</td>
<td>Older (1,007±62)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>59±1</td>
<td>60±1</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>130±2</td>
<td>127±3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>68±3</td>
<td>74±1f</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>88±2</td>
<td>92±1</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>6.8±0.6</td>
<td>6.2±0.7</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>22±3</td>
<td>43±5f</td>
</tr>
<tr>
<td>MSNA, AIU/min</td>
<td>2.815±317</td>
<td>6,177±1,300†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 young and 7 older subjects. Arterial blood pressure values are from brachial sphygmomanometry. MSNA, muscle sympathetic nerve activity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; CVP, central venous pressure. *P < 0.05 vs. baseline. †P < 0.05 vs. young controls.

blood pressure also increased during phenylephrine infusion; however, the increases were not significantly different in the two groups. By experimental design, central venous pressure was unchanged (P > 0.05) from baseline, whereas MSNA burst frequency and total activity decreased (P < 0.05) in both the young and older men; there were no significant group differences in the change in MSNA from baseline control levels in response to phenylephrine.

Using diastolic blood pressure as the stimulus, we found that arterial baroreflex responsiveness for inhibition of MSNA burst frequency (Fig. 1, top) and total activity (Fig. 1, bottom) was similar in the young and older subjects. The older subjects did demonstrate greater interindividual variability in their arterial baroreflex responsiveness. However, the percent reduction in MSNA per unit increase in diastolic blood pressure was significantly smaller (8.9 ± 0.7 vs. 5.2 ± 1.1%/mmHg) in the older men. Similar results to those described above were obtained when mean arterial blood pressure was used as the baroreflex stimulus.

The magnitude of the reflex increase in R-R interval (reduction in heart rate) was not significantly different in the older and young subjects. Similarly, arterial baroreflex R-R interval inhibitory responsiveness was not different in the two groups (Fig. 2). When ANCOVA was performed to examine arterial baroreflex MSNA and heart rate inhibitory responsiveness (i.e., compared with the traditional ratio method), the same results were obtained.

DISCUSSION

The primary finding of the present study is that healthy aging does not obviously influence the ability of the arterial baroreflex to produce an inhibition of MSNA in response to a sustained increase in arterial pressure. Taken together with the results of previous studies (4, 17) that examined dynamic responsiveness, the present findings support the view that the ability of the arterial baroreflex to inhibit central sympathetic outflow is preserved with age in healthy normotensive adult humans. Thus this potential mechanism does not appear to contribute to the elevation in MSNA with age in healthy normotensive adults.

The present findings agree with the conclusions of two earlier investigations (4, 17) on age and dynamic baroreflex control of MSNA in humans. However, our results extend these previous findings in at least two important ways. First, we have shown that the ability of the arterial baroreflex to sustain its inhibition of MSNA in response to an elevation in pressure remains intact with age. Second, by experimentally controlling the potentially confounding effects of changes in central venous pressure (a stimulus for cardiopulmonary reflexes), our findings demonstrate that the sympathoinhibitory responsiveness of the arterial baroreflex per se is preserved with age in healthy adult humans.

There are several possibilities for the different results of the present and earlier (4, 17) studies in humans and those reported previously in beagles (10). For example, in the latter study, the senescent animals...
were hypertensive compared with the mature adult controls. However, this does not appear to explain their findings, because reduced sympathoinhibition was observed with age in a subgroup of normotensive older animals. Moreover, the older subjects in previous investigations (4, 17) in humans also had higher mean levels of resting blood pressure than the young adult controls earlier. Rather, in addition to a possible species influence, the differences more likely are due to other factors, such as the use of anesthesia, the type of carotid-sinus preparation employed, or the use of renal SNA (vs. MSNA).

The present findings suggest that mechanisms other than reduced arterial baroreflex inhibition are involved in mediating the marked age-related increases in MSNA in humans. One such mechanism appears to be associated with the increase in adiposity that occurs with adult aging. Recently (12, 13) we reported that ~60% of the variance in the elevation in MSNA with age in healthy normotensive adult males can be accounted for greater abdominal and total body adiposity. The exact central nervous system (CNS) signal associated with adiposity that stimulates MSNA with age has not been determined, although preliminary data from our laboratory (18) suggest that circulating levels of leptin may be involved. However, whereas this mechanism likely contributes on average to the age-related increase in MSNA, elevated total body and abdominal fat may not have played a major role in the present study because body mass index was similar in the young and older men. More precise measures of adiposity would be needed to address this issue more definitively in the present study.

Another possibility is an age-related increase in central (preganglionic) sympathetic drive, as suggested previously by Hajduczok and colleagues (10). This possibility is supported by recent findings (14) that norepinephrine spillover from the subcortical brain regions (measure of central sympathetic drive) is strongly related to MSNA in healthy young and early middle-aged adult humans. In this context, it is possible that 1) CNS concentrations of, or sensitivity to, certain excitatory or inhibitory amino acids (N-methyl-D-aspartate, GABA, etc.) and/or 2) circulating levels of other hormones with access to the CNS change with age and stimulate preganglionic sympathetic neurons.

We should emphasize that our conclusion of a preserved arterial baroreflex inhibitory responsiveness for MSNA with age in healthy adult humans, as well as that in a previous report by Ebert and colleagues (4), is based on the absolute unit reflex reductions in MSNA. Due to their markedly higher baseline (control) levels of MSNA, when inhibitory responsiveness was expressed as percent change, values were significantly smaller in the older men. Certainly, the issue of whether the most appropriate manner in which to view the functional performance of this reflex is using absolute or percent changes in MSNA is complex. As we have argued previously (21), we believe that the absolute expression of baroreflex responsiveness has the most physiological significance (meaningfulness). This idea is based in part on the fact that it is the changes in absolute levels of MSNA that are most closely related to norepinephrine release from the sympathetic nerve endings and the subsequent target organ responses in humans (25, 30). Moreover, we should also point out that healthy older adults, such as those studied in the present investigation, regulate MSNA and arterial blood pressure as well or better than young adults during a variety of acute stressors (2, 3, 21, 22, 28). This observation is consistent with the concept of properly functioning arterial sympathetic baroreflexes with age in this population. However, this clearly represents only one interpretation of our findings and the reader should be aware of the alternative view.

In the present investigation, consistent with our finding of maintained sympathetic-vasoconstrictor neural inhibition with age, we observed similar arterial baroreflex suppression of heart rate in the young and older subjects. These observations conflict with the results of several previous reports (4, 5, 9) that found a smaller reflex-mediated increase in R-R interval with age in adult humans. However, in support of the present findings, Shi et al. (26) recently reported that carotid arterial baroreflex control of heart rate does not decline with age in healthy adults. The differing results may be due in part to factors related to subject selection, particularly in the older groups. For example, in the present study our older subjects were rigorously screened for the presence of overt cardiovascular disease, obesity, and hypertension, all of which may be associated with reduced arterial baroreflex sensitivity (4, 5, 7–9). Thus factors independent of primary aging may have contributed to the differences observed to date among studies.

In summary, the results of the present investigation support the idea that the ability of the arterial baroreflex to sustain inhibition of MSNA is preserved with age in healthy normotensive adult humans. Therefore, a reduction in arterial baroreflex tonic sympathoinhibition does not appear to contribute to the age-related increase in MSNA in this population.

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