Relationship Between Sympathetic Baroreflex Sensitivity and Arterial Stiffness in Elderly Men and Women

Yoshiyuki Okada, M. Melyn Galbreath, Shigeki Shibata, Sara S. Jarvis, Tiffany B. VanGundy, Rhonda L. Meier, Wanpen Vongpatanasin, Benjamin D. Levine, Qi Fu

See Editorial Commentary, pp XX–XX

Abstract—Previous human studies have shown that large-artery stiffness contributes to an age-related decrease in cardiovagal baroreflex sensitivity. Whether this is also true with sympathetic baroreflex sensitivity is unknown. We tested the hypothesis that sympathetic baroreflex sensitivity is associated with the stiffness of baroreceptor segments (the carotid artery and the aorta) in elderly individuals and that sex affects this relationship. Sympathetic baroreflex sensitivity was assessed from the spontaneous changes in beat-by-beat diastolic pressure and corresponding muscle sympathetic nerve activity (microneurography) during supine rest in 30 men (mean±SEM: 69±1 years) and 31 women (68±1 years). Carotid artery stiffness (B-mode ultrasonography) and aortic stiffness (MRI) were also determined. We found that elderly women had lower sympathetic baroreflex sensitivity than elderly men (−2.33±0.25 versus −3.32±0.25 bursts·100 beats⁻¹·mm Hg⁻¹; P=0.007). β-Stiffness indices of the carotid artery and the aorta were greater in elderly women than in men (6.68±0.48 versus 5.10±0.50 and 4.03±0.47 versus 2.68±0.42; both P<0.050). Sympathetic baroreflex sensitivity was inversely correlated with carotid artery stiffness in both men and women (r=0.49 and 0.50; both P<0.05), whereas this relation was shifted in parallel upward (toward a reduced sensitivity) in women with no changes in the slope (0.26 versus 0.24 arbitrary units). Sympathetic baroreflex sensitivity and aortic stiffness showed similar trends. Thus, barosensory artery stiffness seems to be one independent determinant of sympathetic baroreflex sensitivity in elderly men and women. The lower sympathetic baroreflex sensitivity in elderly women may predispose them to an increased prevalence of hypertension. (Hypertension. 2012;59:00-00.) ● Online Data Supplement

Key Words: baroreceptors • β-stiffness • muscle sympathetic nerve activity • aging • sex differences

The arterial baroreflex is a primary mechanism through which the autonomic nervous system regulates blood pressure (BP). Previous human studies have shown that cardiovagal baroreflex sensitivity (BRS) is blunted in normal aging,1–3 as well as in patients with hypertension.4,5 The blunted cardiovagal BRS has been found to be a risk factor for life-threatening arrhythmias and a predictor of sudden cardiac death.6,7 Thus, understanding the mechanisms underlying age-related changes in BRS has significant clinical relevance.

Because the deformation of the baroreceptors rather than direct intravessel pressure during acute changes in arterial pressure is required to initiate neural firing,8 the stiffness of large elastic arteries where the baroreceptors exist (the carotid artery and the aortic arch) could be associated with a decrease in BRS. Indeed, a number of studies have demonstrated that an age-related increase in the stiffness of these arteries is responsible for the decline in cardiovagal BRS.2,3,9–11 Whether this is also the case with sympathetic BRS is unknown.

It was reported recently that sympathetic BRS was correlated with cardiovagal BRS in healthy young women.12 On the other hand, O’Leary et al13 showed that the difference in mechanical change of the carotid artery between nitroprusside and phenylephrine injection was related to hysteresis of the cardiovascularg baroreflex but not the sympathetic baroreflex. A similar observation was made during a cold pressor test,14 head-up tilt,15 and volume expansion,16 suggesting that the relation between arterial stiffness and sympathetic BRS is complex. Surprisingly, there is no information available regarding the relationship between the stiffness of the aorta and sympathetic BRS in humans. Recent evidence of sex differences in age-related increases of arterial stiffness17,18 and in autonomic control of BP19–22 suggests that sex may
affect the relationship between arterial stiffness and sympathetic BRS.

In the present study, we hypothesized that sympathetic BRS would be associated with both carotid and arterial stiffness in elderly people and that sex would affect these relationships. To test this hypothesis, we assessed sympathetic BRS from the linear correlation between muscle sympathetic nerve activity (MSNA) and beat-by-beat diastolic BP during spontaneous breathing and the stiffness of the carotid artery and the aorta with ultrasound imaging and MRI, respectively. We then determined the relationships between these variables in all of the subjects, as well as in men and women separately.

Methods

Subjects
A total of 175 elderly individuals were contacted between August 2008 and September 2010. 92 of them were interested in participating in research and were screened, and 61 (30 men and 31 women) were eventually qualified and enrolled in this study. They were nonsmokers and had no overt history of cardiovascular, neuromuscular, or renal diseases. Women taking hormone replacement therapy were excluded. All of the subjects gave their written informed consent to a protocol approved by the institutional review boards of the University of Texas Southwestern Medical Center at Dallas and Texas Health Presbyterian Hospital Dallas. Subjects’ physical characteristics and fasting glucose and insulin are presented in Table 1.

Measurements

Muscle Sympathetic Nerve Activity
MSNA signals were obtained with microneurography. In brief, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2 to 3 cm apart from the recording electrode. The nerve signals were amplified (70,000- to 160,000-fold), band-pass filtered (700–2000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant: 0.1 second). Criteria for adequate MSNA recording include the following: pulse synchrony; facilitation during the hypotension phase of the Valsalva maneuver and suppression during the hypertensive overshoot phase after release; increase in response to breath holding; and insensitivity to emotional stimuli.

Hemodynamics
Heart rate (HR) was determined from lead II of the ECG (Hewlett-Packard), and beat-by-beat arterial BP was derived by finger plethysmography (Nexfin). Arm-cuff BP was measured by electrophysgmomanimetry (SunTech) with a microphone placed over the brachial artery to detect Korotkoff sounds. Beat-by-beat carotid and radial pressures were obtained with a pencil-sized tonometer containing a high-fidelity strain gauge transducer (SphygmoCor). Because the absolute levels of these arterial pressures are subjected to hold-down force, the pressure signals obtained by tonometry were calibrated by equating their DBP and mean BP to those obtained at the brachial artery, as described previously. Cardiac output (CO) was measured with the modified acetylene rebreathing technique. Stroke volume was calculated from CO divided by HR, and total peripheral resistance (TPR) was calculated as the quotient of mean BP (DBP + systolic BP (SBP) – DBP)/3 from arm cuff BP and CO, multiplied by 80, where all of the variables were measured during rebreathing, CO, stroke volume, and TPR were normalized to body surface area as cardiac index, stroke index, and TPR index.

Arterial Images
Cross-sectional images of the right common carotid artery were captured using a phase-locked echo tracking system coupled to a B-mode ultrasonic imager (iE33, Philips), which allows sequential measurements of the arterial wall movement noninvasively through a high-resolution transducer (7.5 MHz) positioned at 2 cm proximal to the carotid bifurcation with simultaneous ECG recordings. MRI of the aortic arch was obtained in the transverse plane at the level of the right pulmonary artery using 1.5-T clinical magnetic resonance scanner (Gyroscan Intera, Philips) to assess aortic pulsative dimension.

Protocol
The experiment was performed in the morning ≥2 hours after breakfast, ≥72 hours after the last caffeinated or alcoholic beverage, and ≤24 hours after strenuous physical activity in a quiet, environmentally controlled laboratory with an ambient temperature of ~25°C. The subject was placed in the supine position. At least 10 minutes after an acceptable nerve-recording site had been found, baseline data were recorded for 6 minutes during spontaneous breathing to assess sympathetic BRS. Subsequently, 2 Valsalva maneuvers (40 mm Hg for 20 seconds) were performed to assess cardiac BRS, separated by ≥2 minutes of recovery. Because the day-to-day variability of MSNA is small and the reproducibility of the measurement is high, arterial stiffness was assessed on the next day in the morning. Baseline hemodynamics were measured in all of the subjects. Both carotid and brachial arterial pressures were obtained using tonometry and arm cuff BP at the brachial artery, followed by ultrasonography on the common carotid artery for the assessment of carotid artery stiffness. Throughout the entire experimental procedures, beat-by-beat BP, HR, and respiratory waveforms were recorded continuously. The aortic MRI (for the assessment of aortic stiffness) was performed at the University of Texas Southwestern Medical Center at Dallas.

Data Analysis
Data were sampled at 625 Hz and stored on personal computer with a commercial data acquisition system (Biopac). Offline data analyses were performed using signal-processing software (LabView). Beat-by-beat HR was calculated from the R-R interval measured by ECG. Beat-by-beat SBP and DBP were obtained from the arterial-pressure waveform. Sympathetic bursts were identified by a computer program and then confirmed by an experienced microneurographer. The integrated neurogram was normalized by assigning a value of 100 to the largest amplitude of a sympathetic burst during the 6-minute baseline. Burst area was measured as the area under the curve of each sympathetic burst of the normalized integrated neurogram on a beat-by-beat basis. The number of bursts per minute (burst frequency), the number of bursts per 100 heartbeats (burst incidence), and total burst area per minute and per 100 beats (total MSNA) were used as quantitative indices.

Table 1. Physical Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men (n=30)</th>
<th>Women (n=31)</th>
<th>All Subjects (n=61)</th>
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</thead>
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<tr>
<td>Age, y</td>
<td>69±1</td>
<td>68±1</td>
<td>68±1</td>
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<tr>
<td>Height, cm</td>
<td>176±1</td>
<td>162±1*</td>
<td>169±1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83±2</td>
<td>71±2*</td>
<td>77±1</td>
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<td>Body surface area, m²</td>
<td>2.01±0.02</td>
<td>1.78±0.03*</td>
<td>1.89±0.02</td>
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<td>Body mass index, kg · m²</td>
<td>26.7±0.5</td>
<td>26.7±0.6</td>
<td>26.7±0.4</td>
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<td>Fasting glucose, mg · dL⁻¹</td>
<td>81±2</td>
<td>80±2</td>
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<tr>
<td>Insulin, µU · mL⁻¹</td>
<td>11±2</td>
<td>11±1</td>
<td>11±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05 vs men.
Table 2. Supine Resting Hemodynamics and MSNA Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men (n=30)</th>
<th>Women (n=31)</th>
<th>All Subjects (n=61)</th>
</tr>
</thead>
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<tr>
<td>Arm-cuff SBP, mm Hg</td>
<td>124±3</td>
<td>122±3</td>
<td>123±2</td>
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<tr>
<td>Arm-cuff DBP, mm Hg</td>
<td>73±2</td>
<td>68±2*</td>
<td>70±1</td>
</tr>
<tr>
<td>Arm-cuff MBP, mm Hg</td>
<td>90±2</td>
<td>86±2</td>
<td>88±1</td>
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<tr>
<td>HR, beats·min⁻¹</td>
<td>60±2</td>
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<td>63±1</td>
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<tr>
<td>CO, L·min⁻¹·m⁻²</td>
<td>2.11±0.06</td>
<td>2.04±0.06</td>
<td>2.08±0.04</td>
</tr>
<tr>
<td>SV, mL</td>
<td>68.1±2.3</td>
<td>51.7±1.5*</td>
<td>58.9±1.7</td>
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<tr>
<td>Stroke index, mL·m⁻²</td>
<td>33.8±1.1</td>
<td>29.1±0.8*</td>
<td>31.4±0.7</td>
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<tr>
<td>TPR, dyn·s·cm⁻⁵</td>
<td>1618±54</td>
<td>1753±60</td>
<td>1687±41</td>
</tr>
<tr>
<td>TPR index, dyn·s·cm⁻⁵·m²</td>
<td>3269±126</td>
<td>3096±95</td>
<td>3181±79</td>
</tr>
<tr>
<td>MSNA burst frequency, bursts·min⁻¹</td>
<td>39±2</td>
<td>42±1</td>
<td>40±1</td>
</tr>
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<td>Burst incidence, bursts·100 beats⁻¹</td>
<td>64±2</td>
<td>66±2</td>
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<td>Total MSNA, units·min⁻¹</td>
<td>565±28</td>
<td>586±31</td>
<td>576±21</td>
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<tr>
<td>Total MSNA, units·100 bursts·min⁻¹</td>
<td>935±49</td>
<td>907±42</td>
<td>930±32</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; MSNA, muscle sympathetic nerve activity. Values are mean±SEM.

*P<0.05 vs men.

Sympathetic BRS was assessed using the slope of the linear correlation between MSNA and DBP during spontaneous breathing, and cardiovagal BRS was assessed by R-R interval and SBP during the Valsalva maneuvers after adjusting all signal and physiological delays (see the online Data Supplement at http://hyper.ahajournals.org). Detailed assessments of carotid artery and aortic stiffness were also reported in the online Data Supplement.

Statistical Analysis

Values are expressed as mean±SEM. Linear regression analysis was used to evaluate the correlation between arterial stiffness and sympathetic BRS. Data between men and women were compared using unpaired t tests. If normality tests and/or equal variance tests failed, we compared the differences between sexes using Mann-Whitney rank-sum tests. β-Stiffness of the aorta calculated with transferred aortic pressure and direct carotid pressure for men and women was evaluated using 2-way repeated ANOVA. A P value of <0.05 was considered statistically significant.

Results

Hemodynamics and Neural Variables

Table 2 depicts supine resting hemodynamics and MSNA. There was no difference between sexes in arm-cuff SBP and mean BP. Women had lower arm DBP, CO, and stroke volume but higher HR compared with men (all P<0.05). Cardiac index showed no sex difference (P=0.392), whereas stroke index was significantly smaller in elderly women than men (P<0.001). TPR trended higher in elderly women than men (P=0.099), but TPR index did not differ between sexes (P=0.458). Although MSNA burst frequency tended to be higher in elderly women (P=0.078), there were no significant differences in burst incidence and total MSNA between sexes.

BRS and Arterial Stiffness

Sympathetic and cardiovagal BRS data are shown in Figure 1. Elderly men had higher sympathetic BRS than elderly women. There were no significant sex differences in cardiovagal BRS assessed during phase II of the Valsalva maneuver; however, cardiovagal BRS was greater in elderly men than women during phase IV. Elderly men had lower β-stiffness indices than women in both the carotid artery and aorta (Figure 2).

Relationship Between Sympathetic BRS and Arterial Stiffness

Sympathetic BRS was significantly and inversely correlated with β-stiffness index of the carotid artery in all, as well as in men and women separately (Figure 3A). The slope of this relationship was 0.26 in women and 0.24 arbitrary units in men, whereas the y intercept was −4.05 in women and −4.55 bursts·100 beats⁻¹·mmHg⁻¹ in men. Sympathetic BRS was not correlated with β-stiffness index of the aorta calculated with aortic pressure derived from transfer function analysis in any groups (Figure 3B). Conversely, sympathetic BRS was correlated with β-stiffness index of the aorta calculated with carotid artery pressure in all of the subjects together, although with less statistical certainty in men and women examined separately (Figure 3C). This relation was
shifted in parallel upward (toward a reduced sensitivity) in women with no changes in the slope.

Discussion

Our major findings are as follows: (1) sympathetic BRS was lower and β-stiffness indices of both the carotid artery and aorta were higher in elderly women than men; (2) sympathetic BRS was correlated with carotid artery stiffness and aortic stiffness in all of the subjects, as well as in elderly men and women separately; and (3) the slope of the correlation between sympathetic BRS and carotid artery and aortic stiffness was similar between sexes; however, the line relating these 2 variables was shifted in parallel upward in elderly women. Thus, barosensory artery stiffness seems to be one independent determinant of sympathetic BRS in elderly men and women. The lower sympathetic BRS in elderly women may predispose them to an increased prevalence of hypertension.

Sex Differences in BRS

Recent studies suggested that there was no sex difference in sympathetic BRS in young individuals. However, our data in elderly subjects showed that women had lower sympathetic BRS than men, suggesting that age-related changes in sympathetic baroreflex function may be different between sexes. Similar sympathetic BRS across all ages was reported in men during steady-state changes in BP with a neck chamber method and continuous phenylephrine infusion. Conversely, lower sensitivity in elderly men than in young men was observed during dynamic changes in BP by the Valsalva maneuver. Although a limited number of studies have included women, Studinger et al found that age did not affect integrated sympathetic BRS in men and women during bolus injection of nitroprusside and phenylephrine. The discrepancy between our results and those of Studinger et al may be attributable to different methods used for the assessment of sympathetic BRS. However, it has been demonstrated that pharmacological and spontaneous BRS values are closely correlated in most instances. Another possible explanation for the discrepancy may be age differences. Our subjects were older than those in the study of Studinger et al (ie, mean age: 68 versus 62 years). It has been shown that sympathetic activity increases with age, whereas the increment is greater in women, especially after menopause, compared with men. Resting MSNA was much lower in the study by Studinger et al (22 bursts·min⁻¹ both in men and women) than ours (39 in men and 42 in women). In addition, we found that resting MSNA trended greater in elderly women than men, whereas Studinger et al did not. It is likely that BP is regulated near the threshold of the baroreflex curve, particularly in elderly women in our study, where the sensitivity is lower than the maximal point, and, therefore, we found sex differences in sympathetic BRS.

We recently found in healthy young individuals that cardiovagal BRS during BP falls (phase II of the Valsalva maneuver) was similar between sexes; however, women had lower cardiovagal BRS during BP rises (phase IV) than men. Similar results were found in elderly subjects in the current study. Advancing age decreases cardiovagal BRS even in the healthy population; men and women seem to have a similar decline in cardiovagal BRS, whereas the rate of increase in arterial stiffness is greater in
women. One animal study showed that cardiovagal BRS was lower in female than in male rats only during BP rises with phenylephrine, and the difference disappeared by infusion of atropine but not propranolol, suggesting that the sex difference in cardiovagal BRS was caused by a lower vagal response to baroreceptor activation. Thus, sex differences in cardiovagal BRS, which impaired the correction of hypertension in women, may originate in the neural component of the baroreflex, altering the relationship between sympathetic and cardiovascular BRS in the elderly compared with the young (see Figure S1 in the online Data Supplement).

### Relation Between Sympathetic BRS and Arterial Stiffness

We found that sympathetic BRS was inversely correlated with the barosensory artery stiffness. To our knowledge, there have been only 2 studies that simultaneously assessed sympathetic BRS and arterial stiffness; however, neither study investigated the correlation between these 2 variables. O’Leary et al reported that the hysteresis during drug-induced changes in BP occurred in the mechanical change of the carotid artery but not sympathetic BRS in middle-aged subjects; therefore, they concluded that there was no association between sympathetic BRS and carotid artery stiffness. Conversely, Studinger et al described the reduction in integrated sympathetic BRS and a greater sensitivity of the neural component in elderly compared with young subjects during decreasing BP, suggesting that there was an association between integrated sympathetic BRS and carotid artery stiffness. Because all of the subjects in our study were seniors, we cannot determine the effects of aging, per se, on the relationship of sympathetic BRS and arterial stiffness.

Within the elderly, sympathetic BRS was lower as carotid and aortic arterial stiffness were higher, which supports the idea proposed by Studinger et al.

This significant correlation was also shown separately in elderly men and women; however, some important sex differences existed. The line relating sympathetic BRS and artery stiffness in elderly women was shifted in parallel upward (less sensitive) compared with elderly men, but the slope of the linear regression line was similar between sexes. These data suggested that the effect of artery stiffness, serving as the mechanical component of sympathetic BRS, is similar between elderly men and women (if the effect were different between sexes, this slope would have been different). However, the higher artery stiffness did not account entirely for the lower sympathetic BRS in elderly women (if the higher arterial stiffness were the only cause of the lower sensitivity, the relation in elderly women would have been shifted right-upward on the same regression line of elderly men). We used DBP and MSNA as the input and output variables, respectively, which contained both the mechanical and neural components of the baroreflex (Figure S2). Therefore, the offset of this relation may indicate the difference of the neural component. Because Studinger et al demonstrated that the neural component of sympathetic BRS increased in the elderly, the lower sympathetic BRS in elderly women found in this study seems to be attributable to the lower increase in the neural component, as well as the higher stiffness of the barosensory artery during BP falls.

We found no correlation between sympathetic BRS and aortic stiffness calculated with aortic pressure derived from transfer function analysis. However, when directly measured carotid artery pressure (calibrated by brachial BP) was used for calculation of aortic stiffness, we found that, although the value was similar to that calculated using transferred aortic pressure, the relationship between sympathetic BRS and aortic stiffness became more clearly discernible. This discrepancy may be caused by an artifact of the general transfer function, especially in the elderly population. It seems to be more reasonable to use carotid artery pressure rather than transferred central pressure for calculation of aortic stiffness in our study (Table S1). Certainly, we recognize that carotid artery pressure is different from real aortic pressure. Sanders et al found that the reduction in sympathetic activity during phenylephrine injection with (isolated aortic baroreceptor loading) and without (both carotid and aortic baroreceptor loading) neck pressure was similar, indicating that aortic baroreceptors play a more important role in BP control. One previous animal study showed that the aortic baroreceptors were mainly involved in the control of high BP, whereas at lower pressures the major control occurred through the carotid baroreflexes. To determine the relative contribution of carotid and aortic baroreceptors in BP regulation, future studies are needed, perhaps with direct invasive measurements of vascular pressures.

### Limitations

First, sympathetic BRS was evaluated during spontaneous breathing with peripheral (finger) DBP, and, therefore, the entire baroreflex stimulus-response curve to central BP cannot be determined. We used the binning method to reduce the impact of nonbaroreflex influence, which allowed us to reveal the physiological modulation of sympathetic control around the prevailing and operating point. Conversely, we could not completely solve the discrepancy between peripheral and central BP, although we used finger DBP, which showed the least error compared with finger SBP (Table S2). Second, aortic stiffness was evaluated with MRI, which could be limited by low spatial resolution. The aortic size may affect the calculation of aortic stiffness (Table S3). However, the reproducibility for the analysis of aortic MRI in our laboratory was high, whereas there seemed to be small sex differences (the typical error of the intraoperator variability of the manual calculation: 2.1% for men and 1.8% for women). Third, the range of the neural component is so wide, containing afferent sites including barosensory segments, the central nervous system, and efferent sites, that we cannot determine which site(s) have been affected by sex (Figure S2).

### Perspectives

Large epidemiological studies have demonstrated the higher prevalence of hypertension and the lower BP control rate in elderly women than men. Because baroreflex function is...
blunted in hypertensive patients,4,5 the lower sympathetic BRS in elderly women than men, combined with the lower cardio vagal BRS during BP rises shown in this study, may be one major mechanism underlying sex differences in hypertension and responses to antihypertensive medications. The lower BP control function in elderly women caused by the higher stiffness of the carotid artery and less sympathetic baroreflex buffering has clinical implications, because the relationship between BP and cardiovascular events is more pronounced in people aged ≥65 years.47 However, which component of the sympathetic baroreflex, arterial stiffness, MSNA response, or sympathetic vascular transduction, is responsible for the risks of hypertension in elderly women remains to be determined in future studies.

Acknowledgments

We are grateful to the study volunteers for their participation. We also thank Jeffrey L. Hastings, M. Dean Palmer, Peggy Fowler, Murugappan Ramanathan, Cyrus Oufi, and Wade Wang for their valuable laboratory assistance.

Sources of Funding

This study was supported by the National Institutes of Health grant R01 HL091078, and UT Southwestern O’Brien Kidney Research Core Center.

Disclosures

None.

References


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Hypertension. published online November 21, 2011;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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RELATIONSHIP BETWEEN SYMPATHETIC BAROREFLEX SENSITIVITY AND ARTERIAL STIFFNESS IN ELDERLY MEN AND WOMEN

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Running head: Sex Differences in Baroreflex and Arterial Stiffness

Supplementary Introduction

Since Dutoit et al¹ reported that sympathetic BRS was positively correlated with cardiovagal BRS in healthy young women, we further tested whether there is a significant relationship between sympathetic and cardiovagal BRS in elderly women.

Carotid-femoral pulse wave velocity (CFPWV) was used as an index of the stiffness of central arteries.² We compared the relationships between CFPWV and sympathetic/cardiovagal BRS with the relationships between arterial stiffness of each baroreflex segment and sympathetic/cardiovagal BRS.

Supplementary Data Analysis

Sympathetic baroreflex sensitivity

Baroreflex control of MSNA was assessed using the slope of the linear correlation between MSNA and DBP during spontaneous breathing in the supine position.³⁻⁵ To perform a linear regression, values for burst incidence and total MSNA were calculated over a 2-mmHg DBP bin increment covering the lowest to highest DBP, respectively⁴⁻⁷ after appropriately accounting for baroreflex delay; 1.3 sec. This pooling procedure reduces the statistical impact of inherent beat-by-beat variability in nerve activity attributable to non-baroreflex influence (e.g., respiration).³ Moreover, a statistical weighting was adopted to minimize the effect of minor variation of bin width and bin position on the slope with respect to the number of cardiac cycle in the bins.⁸ The sensitivity was determined from the slope in each subject after confirming that r value was >0.5 as described previously.⁷

Cardiovagal baroreflex sensitivity

We assessed cardiovagal BRS using the slope of the linear correlation between RRI and SBP during the Valsalva maneuver. To perform a linear regression, values for SBP were linearly regressed against corresponding RRI (lag 1)⁹ on the beat-by-beat basis during early phase II (i.e., a hypotensive stimulus; from the point of the highest SBP value to the lowest value within continuous reduction) and phase IV (i.e., a hypertensive stimulus; from the point where RRI began to lengthen to maximal SBP value within continuous increase), respectively.⁴
sensitivity was determined from the slope in each subject after confirming r value was > 0.8 as described previously.9

**Carotid artery stiffness**
The stiffness of the carotid artery was determined using a combination of ultrasound image and carotid artery pressure measured with tonometry. The operator identified and traced the vessel wall boundary, corresponding to the interface between the lumen and intima to detect luminal area at maximal systolic expansion and at minimal diastolic relaxation with image-analysis software (QLAB, Philips, Andover, MA). In the cross-sectional view of the image for this analysis, we did not identify any plaque in all subjects while longitudinal view of the image showed non-calcified plaque in 2 subjects, which was found not to affect arterial stiffness in the previous study.10 Diastolic and systolic areas were averaged over 4 continuous beats. Then β-stiffness index was calculated to provide an index of arterial stiffness adjusted for distending pressure using the following equation:11

\[
\beta - \text{Stiffness} = \frac{\ln (cSBP/cDBP)}{(\text{Area}_S - \text{Area}_D)/\text{Area}_D}
\]

where cSBP and cDBP are systolic and diastolic carotid artery pressure, Area\(_S\) and Area\(_D\) are cross-sectional areas at maximal systolic and minimal diastolic points of the carotid artery. The denominator of the equation expresses the strain of the carotid artery.

**Aortic arch stiffness**
The stiffness of the aortic arch was determined using a combination of MRI; repetition time 3.70 ms, echo time 1.77 ms, flip angle 15°, slice thickness 8 mm, field of view 36.0×30.5 cm, matrix size 288×288 and velocity encoding 300 cm·sec\(^{-1}\), and aortic pressure from the waveform generated by validated transfer function using radial pressure wave.12 The operator manually traced wall boundary of the descending part of the aorta arch to draw contours on the modulus images of all cardiac phases. The luminal areas at maximal systolic expansion and minimal diastolic relaxation were identified, and then, β-stiffness index and strain of the aorta were calculated as described previously.11 In addition to the aortic pressure derived from transfer function analysis, we also used directly measured carotid artery pressure (calibrated by the brachial blood pressure) to assess aortic arch stiffness in all subjects.

**Pulse wave velocity**
Arterial pressure waveforms were obtained at the carotid artery and femoral artery to assess the stiffness of central arteries by using tonometry and were synchronized with ECG. A foot-to-foot methodology was employed to determine pressure wave transit time in relation to the R-wave. Pulse transit length was estimated by subtracting the distance between sterna notch and the measuring point at the carotid artery from the distance between sterna notch and the measuring point at the femoral artery. CFPWV was calculated from the transit length divided by the transit time (SphygmoCor).13

**Statistical analysis**
Values are expressed as means±SEM. Linear regression analysis was used to evaluate the correlation between cardiovagal and sympathetic BRS, and between CFPWV and
sympathetic/cardiovagal BRS. The effects of sex on other variables were evaluated with unpaired t-tests.

Supplementary Results and Discussion

**Relationship between sympathetic and cardiovagal baroreflex sensitivity**

Sympathetic BRS was weakly correlated with cardiovagal BRS in elderly women ($r = -0.37$, $P = 0.045$) but not in men ($r = -0.12$, $P = 0.573$) (Figure S1). This correlation was reported to be stronger in young women ($r = -0.54; P < 0.01$) than elderly women. The weakened relationship in elderly women may be because that aging differently affected sympathetic and cardiovagal BRS. On the other hand, we evaluated sympathetic BRS with changes in DBP and cardiovagal BRS with changes in SBP. Increases in the stiffness of the arteries may accentuate SBP and pulse pressure rise but not DBP rise. Therefore, it is also possible that the difference of age-related changes in SBP and DBP due to the arterial stiffening would have caused the weakened correlation in elderly women. We compared sympathetic BRS assessed by DBP and that assessed by SBP, and found a significant correlation between them (i.e., sympathetic BRS expressed with total MSNA: $r = 0.63$, $P < 0.001$ in men; $r = 0.53$, $P = 0.002$ in women; and $r = 0.57$, $P < 0.001$ in all; sympathetic BRS expressed with burst incidence: $r = 0.54$, $P = 0.002$ in men; $r = 0.45$, $P = 0.011$ in women; and $r = 0.49$, $P < 0.001$ in all). These results suggest that the weakened relationship between sympathetic and cardiovagal BRS in elderly women was not attributed to the different changes in SBP and DBP with advancing age.

References


**Table S1. Association of the arterial stiffness and baroreflex sensitivity**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sympathetic BRS, bursts 100beats⁻¹ mmHg⁻¹</td>
<td></td>
</tr>
<tr>
<td>CFPWV, m sec⁻¹</td>
<td>0.54 (0.003)</td>
<td>0.43 (0.018)</td>
</tr>
<tr>
<td>β-stiffness of the carotid artery</td>
<td>0.49 (0.006)</td>
<td>0.50 (0.005)</td>
</tr>
<tr>
<td>β-stiffness of the aorta (GTF AP)</td>
<td>0.19 (0.339)</td>
<td>0.03 (0.875)</td>
</tr>
<tr>
<td>β-stiffness of the aorta (CAP)</td>
<td>0.33 (0.088)</td>
<td>0.32 (0.089)</td>
</tr>
<tr>
<td></td>
<td>Cardiovagal BRS (phase IV), msec mmHg⁻¹</td>
<td></td>
</tr>
<tr>
<td>CFPWV, m sec⁻¹</td>
<td>0.27 (0.185)</td>
<td>0.35 (0.058)</td>
</tr>
<tr>
<td>β-stiffness of the carotid artery</td>
<td>0.15 (0.465)</td>
<td>0.40 (0.033)</td>
</tr>
<tr>
<td>β-stiffness of the aorta (GTFAP)</td>
<td>0.36 (0.067)</td>
<td>0.15 (0.459)</td>
</tr>
<tr>
<td>β-stiffness of the aorta (CAP)</td>
<td>0.40 (0.041)</td>
<td>0.19 (0.348)</td>
</tr>
</tbody>
</table>

CFPWV indicates carotid-femoral pulse wave velocity; GTFAP, general transferred functional aortic pressure; CAP, carotid artery pressure; BRS, baroreflex sensitivity. Values are correlation coefficients (P-values).
Table S2. Supine resting blood pressure at different measurement sites

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men (n=30)</th>
<th>Women (n=31)</th>
<th>All Subjects (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger SBP, mmHg</td>
<td>125±4</td>
<td>117±4</td>
<td>121±3</td>
</tr>
<tr>
<td>Finger DBP, mmHg</td>
<td>67±2</td>
<td>63±2</td>
<td>65±2</td>
</tr>
<tr>
<td>Finger MBP, mmHg</td>
<td>86±3</td>
<td>81±2</td>
<td>83±2</td>
</tr>
<tr>
<td>Finger PP, mmHg</td>
<td>59±3</td>
<td>54±4</td>
<td>56±2</td>
</tr>
<tr>
<td>Arm Cuff SBP, mmHg</td>
<td>121±3</td>
<td>115±3</td>
<td>118±2</td>
</tr>
<tr>
<td>Arm Cuff DBP, mmHg</td>
<td>70±2</td>
<td>64±2*</td>
<td>67±1</td>
</tr>
<tr>
<td>Arm Cuff MBP, mmHg</td>
<td>87±2</td>
<td>81±2*</td>
<td>84±1</td>
</tr>
<tr>
<td>Arm Cuff PP, mmHg</td>
<td>51±2</td>
<td>51±2</td>
<td>51±1</td>
</tr>
<tr>
<td>Carotid artery SBP, mmHg</td>
<td>118±3</td>
<td>114±3</td>
<td>116±2</td>
</tr>
<tr>
<td>Carotid artery DBP, mmHg</td>
<td>70±2</td>
<td>64±2*</td>
<td>67±1</td>
</tr>
<tr>
<td>Carotid artery MBP, mmHg</td>
<td>86±2</td>
<td>81±2</td>
<td>83±1</td>
</tr>
<tr>
<td>Carotid artery PP, mmHg</td>
<td>49±2</td>
<td>51±2</td>
<td>50±2</td>
</tr>
<tr>
<td>Aortic SBP, mmHg</td>
<td>118±3</td>
<td>116±3</td>
<td>117±2</td>
</tr>
<tr>
<td>Aortic DBP, mmHg</td>
<td>74±2</td>
<td>67±2*</td>
<td>70±1</td>
</tr>
<tr>
<td>Aortic MBP, mmHg</td>
<td>89±2</td>
<td>83±2</td>
<td>86±1</td>
</tr>
<tr>
<td>Aortic PP, mmHg</td>
<td>45±2</td>
<td>49±2</td>
<td>47±1</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PP, pulse pressure. Values are means±SEM. *, P<0.05 vs. men.
Table S3. Cross-sectional area and strain of carotid artery and aorta

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Women</th>
<th>All Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery area (diastole), cm$^2$</td>
<td>0.47±0.02</td>
<td>0.43±0.01</td>
<td>0.45±0.11</td>
</tr>
<tr>
<td>Strain of the carotid artery</td>
<td>12±1</td>
<td>10±1</td>
<td>11±1</td>
</tr>
<tr>
<td>Aortic area (diastole), mm$^2$</td>
<td>483±17</td>
<td>432±16</td>
<td>457±12</td>
</tr>
<tr>
<td>Strain of the aorta</td>
<td>0.25±0.02</td>
<td>0.18±0.02</td>
<td>0.22±0.01</td>
</tr>
</tbody>
</table>

Values are means±SEM. *, $P<0.05$ vs. men.
Figure S1: Linear regression analysis of the inter-individual relationship between sympathetic baroreflex sensitivity (BRS) and cardiovascular BRS in elderly men (○) and women (●), separately.
Figure S2. Summary of sympathetic baroreflex components