ONLINE SUPPLEMENT

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 al[1] 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, respectively[4-7] 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] The 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 tonometory. 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]

$$\beta - \text{Stiffness} = \frac{\ln\left(c\text{SBP}/c\text{DBP}\right)}{\left(\text{Area}_\text{S} - \text{Area}_\text{D}\right)/\text{Area}_\text{D}}$$

where *c*SBP and *c*DBP 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 $\text{cm}\cdot\text{sec}^{-1}$, 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)[1] 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

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Table S1. Association of the arterial stiffness and baroreflex sensitivity

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

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

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

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

Variables	Men	Women	All Subjects
Carotid artery area (diastole), cm^2	0.47±0.02	0.43±0.01	0.45±0.11
Strain of the carotid artery	12±1	10±1	11±1
Aortic area (diastole), mm^2	483±17	432±16 *	457±12
Strain of the aorta	0.25±0.02	0.18±0.02 *	0.22±0.01

Values are means±SEM. *, P<0.05 vs. men.

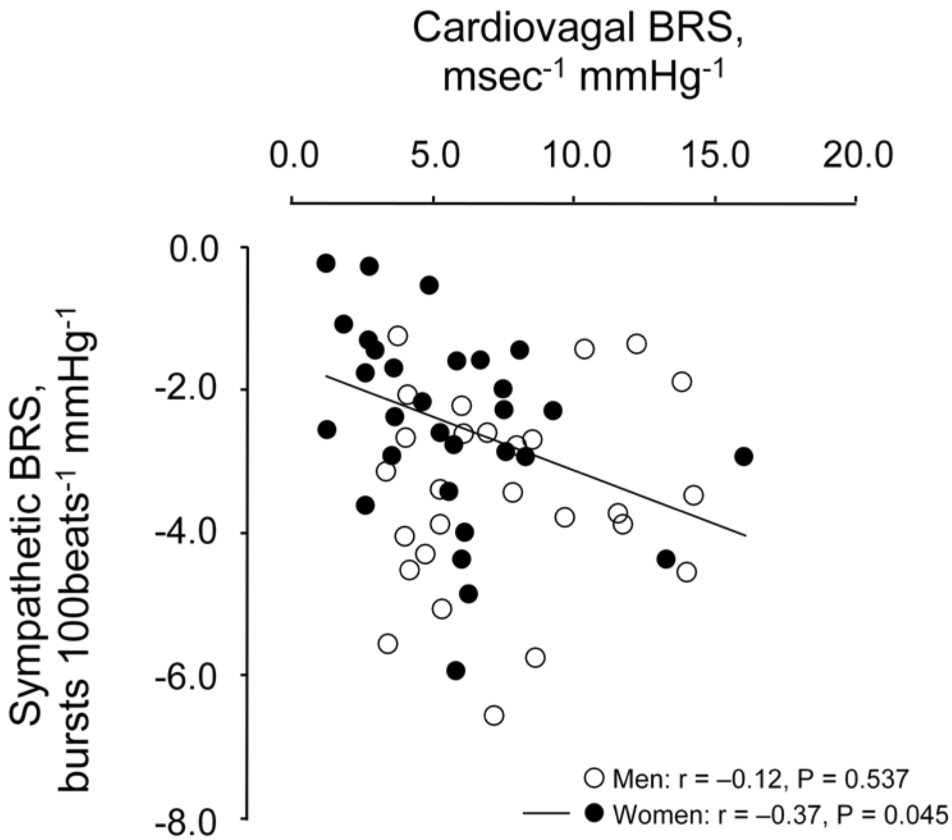

Figure S1: Linear regression analysis of the inter-individual relationship between sympathetic baroreflex sensitivity (BRS) and cardiovascular BRS in elderly men (○) and women (●), separately.

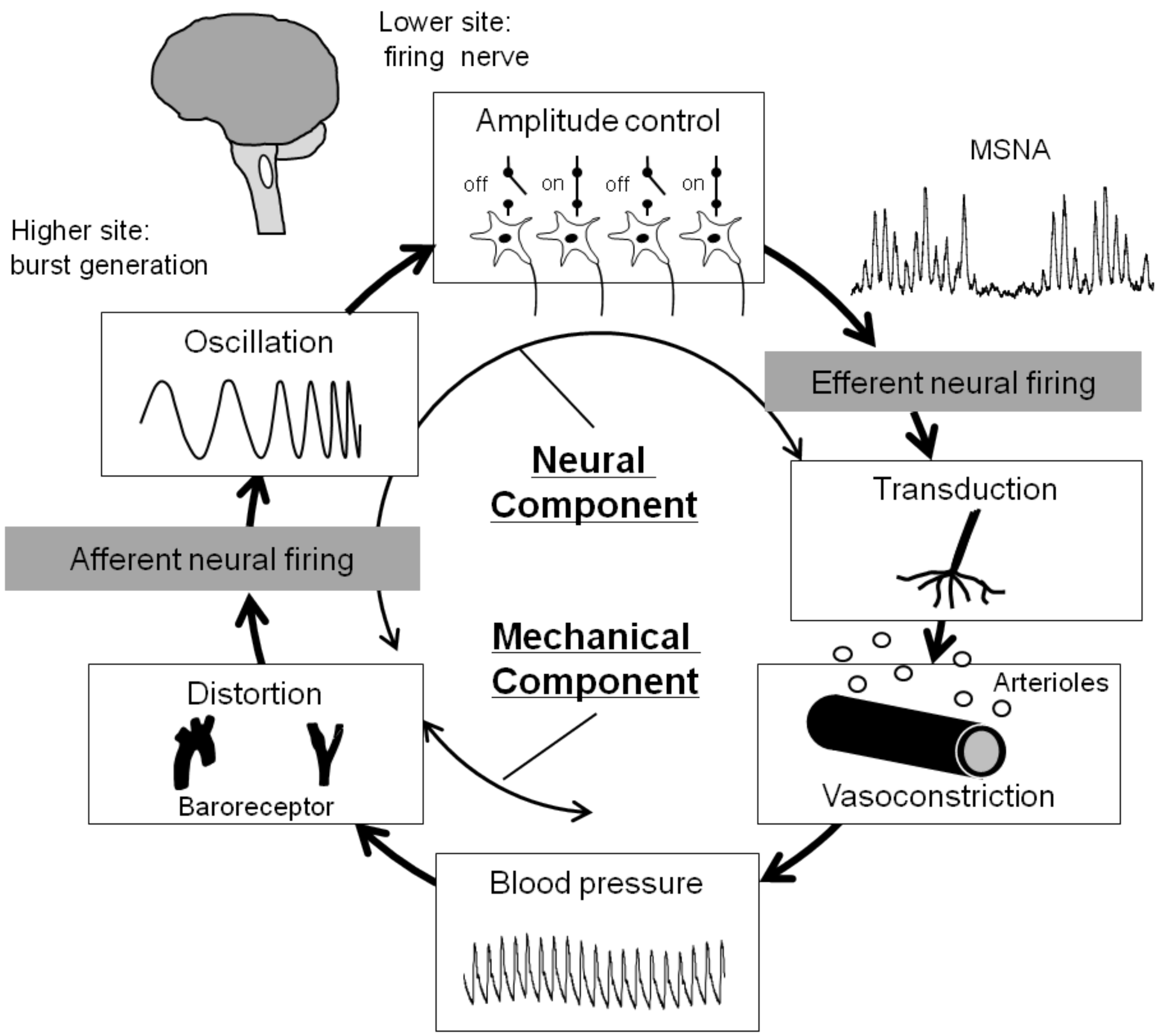

Figure S2. Summary of sympathetic baroreflex components