The influence of barosensory vessel mechanics on the vascular sympathetic baroreflex: insights into ageing and blood pressure homeostasis

Running title: Barosensory vessel mechanics and the baroreflex

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ABSTRACT

Changes in the arterial baroreflex arc contribute to elevated sympathetic outflow and altered reflex control of blood pressure with human ageing. Utilizing ultrasound and sympathetic microneurography (muscle sympathetic nerve activity; MSNA) we investigated the relationships between aortic and carotid artery wall tension (indices of baroreceptor activation) and the vascular sympathetic baroreflex operating point (OP; MSNA burst incidence) in healthy, normotensive young (n = 27, 23 ± 3 years) and middle-aged men (n = 22, 55 ± 4 years). In young men, the OP was positively related to the magnitude and rate of unloading and time spent unloaded in the aortic artery (r = 0.56, 0.65 and 0.51, *P* = 0.02, 0.003 and 0.03), but not related to the magnitude or rate of unloading or time spent unloaded in the carotid artery (r = -0.32, -0.07 and 0.06, *P* = 0.25, 0.81 and 0.85). In contrast, in middle-aged men, the OP was not related to either the magnitude or rate of unloading or time spent unloaded in the aortic (r = 0.22, 0.21 and 0.27, *P* = 0.41, 0.43 and 031) or carotid artery (r = 0.48, 0.28 and -0.01, *P* = 0.06, 0.25 and 0.98). In conclusion, in young men, aortic unloading mechanics may play a role in determining the vascular sympathetic baroreflex OP. In contrast, in middle-aged men, barosensory vessel unloading mechanics do not appear to determine the vascular sympathetic baroreflex OP, and therefore do not contribute to age-related arterial baroreflex resetting and increased resting MSNA.

KEYWORDS: muscle sympathetic nerve activity; barosensory vessel unloading mechanics; healthy ageing; sympathetic nervous system; baroreflex

NEW AND NOTEWORTHY

- We assessed the influence of barosensory vessel mechanics (magnitude and rate of unloading and time spent unloaded) as a surrogate for baroreceptor unloading.
- In young men, aortic unloading mechanics are important in regulating the operating point of the vascular sympathetic baroreflex, whereas in middle-aged men, these arterial mechanics do not influence this operating point.
- The age-related increase in resting muscle sympathetic nerve activity does not appear to be driven by altered baroreceptor input from stiffer barosensory vessels.

FIGURE LEGENDS

Figure 1 – Example of aortic wall thickness measurement (blue line) and aortic diameter measurement (red line) in systole and diastole for one cardiac cycle. “A” indicates the time caliper measurement between systolic and diastolic diameters (time taken to unload) and “B” indicates the time caliper measurement between diastolic diameter measurement and end of the cardiac cycle (time spent unloaded).

Figure 2 – Relationships between the magnitude of aortic unloading, rate of unloading and time spent unloaded and the MSNA OP of the vascular sympathetic baroreflex in young (filled circles) and middle-aged men (open diamonds)

Figure 3 - Relationships between aortic unloading and vascular sympathetic baroreflex gain in young (filled circles) and middle-aged men (open diamonds)

INTRODUCTION

An age-associated increase in arterial blood pressure is evident for apparently healthy humans, and is frequently attributed to structural and functional changes in central arteries (19). Another feature of cardiovascular ageing is the progressive elevation in sympathetic outflow, however this is not necessarily accompanied by increased arterial blood pressure. Over the age of 50 years, ageing results in an upward resetting of the operating point (OP) of the vascular sympathetic baroreflex (20); this occurs with no change in baroreceptor reflex responsiveness, referred to as baroreflex gain (7, 17). In contrast, ageing impairs cardiovagal baroreflex gain (7, 21), indicating a divergent impact of age on the cardiac and vascular components of the arterial baroreceptor reflex. The arterial baroreceptor reflex regulates blood pressure primarily via changes in sympathetic vasomotor activity. Indeed, it is suggested that elevated sympathetic outflow may mitigate age-associated adaptation of the cardiovascular system, and thus preserve homeostatic control of blood pressure (30).

The vascular sympathetic baroreflex arc consists of mechanoelectrical transduction by arterial baroreceptors, a central neural component, and efferent neurotransmission. Baroreceptors are activated by deformation or strain of the arterial wall where they are located. Therefore, age-related changes within the arterial wall could be an important influence on vascular sympathetic baroreflex resetting with human ageing. Indeed, a study of integrated vascular sympathetic baroreflex control observed that age-related barosensory artery stiffening may alter the mechanical component of baroreflex control of sympathetic outflow. However, overall vascular sympathetic reflex responsiveness appeared to be well maintained (27), which was attributed to more sensitive central neural control. This change may be necessary to offset the age-associated reduction in cardiovagal baroreflex gain; that is, dependence on vascular sympathetic baroreflex control, and outflow, for blood pressure homeostasis increases with advancing age (11). However, increased sympathetic outflow is also associated with an increased risk of developing hypertension and susceptibility to CVD (16). Importantly, mechanism(s) responsible for the age-related rise in sympathetic

outflow remain incompletely understood, and it is not clear how these overlap with mechanisms of abnormal sympathoexciation.

Previous assessment of the mechanical component of the arterial baroreflex has only quantified the systolic diameter of the common carotid artery in response to the corresponding pressure (10). However, this approach does not give an indication of the magnitude or time course of baroreceptor loading and unloading. Nor does it address mechanical pressure transduction in aortic baroreceptors, which also play an important role in reflex control of sympathetic vasomotor activity. Furthermore, as a burst of MSNA occurs in the diastolic period of the cardiac cycle, a lack of detail about unloading events is, in our view, a notable gap in existing knowledge. Indeed, dynamic events at aortic and carotid baroreceptors may be characterised relatively easily using the magnitude and rate of change in wall tension within these arteries. Therefore, this approach could provide a better index of the baroreceptor stimulus than previous attempts to characterize the mechanical component. That is, acquisition of important information about recoil could be important for developing understanding of reflex control of sympathetic outflow. Notably, it is suggested that the occurrence of a burst of outflow is determined via a central gating mechanism, whereby a burst of sympathetic activity only occurs if there is sufficient time between the removal of afferent inhibition and generation of efferent vasomotor activity within the cardiac cycle (12); this “gate” could be influenced by barosensory vessel wall mechanics. Furthermore, identifying whether stiffer and less compliant arteries (29) and altered vessel wall mechanics contribute to age-related changes in reflex control of MSNA is worthy of consideration.

With healthy ageing, barosensory vessels are stiffer and less compliant (9, 29) across a wide range of static and dynamic pressures; this may reduce the magnitude and rate of baroreceptor unloading, in barosensory vessels. Hypothetically, this could result in a longer period of reflex inhibition within each cardiac cycle, and subsequently a reduction in MSNA burst probability. In fact, with healthy ageing there is an increase, rather than a decrease, in the proportion

of cycles with a burst of activity, that is MSNA burst incidence, or baroreflex OP, increases (18, 23, 28). Consequently, it appears the OP of the vascular sympathetic baroreflex may be regulated independently of barosensory vessel unloading mechanics above a certain age.

The aim of this study was therefore: 1) to utilise a new method of analysis to effectively quantify dynamic changes in barosensory vessel wall tension as a surrogate for baroreceptor unloading across the cardiac cycle and 2) to establish the impact of the magnitude and rate of unloading and time spent unloaded during the cardiac cycle in the aortic and carotid arteries on the OP of the vascular sympathetic baroreflex in young compared to middle-aged men. We hypothesized that: 1) the magnitude and rate of baroreceptor unloading in the aortic and carotid arteries would be lower, and therefore time spent unloaded, relative to the cardiac cycle, would be shorter, in middle-aged compared to young men and 2) the magnitude and rate of unloading in the aortic and carotid arteries and time spent unloaded, relative to the cardiac cycle, would be related to the OP of the vascular sympathetic baroreflex in young but not middle-aged men.

METHODS

Ethical approval

The study was approved by the Cardiff School of Sport and Health Sciences Research Ethics Committee (Approval code: 16/7/02R) and conformed to the most recent Declaration of Helsinki, except for registration in a database. Prior to testing, participants all provided written informed consent.

Participants

Seventy men (age 21-63 years old) were screened to take part in this study. Following exclusion, data were obtained from 27 young men and 22 middle-aged men (see Table 1). All subjects were normotensive and free from diagnosed cardiovascular or metabolic disease and had a BMI of <30. Participants were recruited across a range of fitness status, and all were recreationally active. Participants abstained from caffeine, alcohol and strenuous exercise for twenty-

four hours before testing. Participants attended the laboratory on two separate occasions, with visits 1 and 2 separated by a minimum of one week. This study design also facilitated the completion of a previous study (33).

Visit 1 - Screening visit and $\dot{V}O_2$ peak testing

Participants attended the laboratory at Cardiff Metropolitan University having eaten >2 hours prior to the testing visit. The first testing session included measures of height (Stadiometer, Holtain ltd, Crosswell, UK) and body mass (Scales, SECA 770, Vogel & Halke, Hamburg, Germeny) to ensure BMI <30 $kg \cdot m^2$ and resting blood pressure (manual sphygmomanometer, Welch Allyn, UK) to confirm participants were normotensive (<140/90 mmHg supine). Participants also reported both prescription and over the counter medication use and were excluded if regularly taking any form of medication.

Participants completed an incremental exercise test to exhaustion on a cycle ergometer (Lode Corival, Gronigen, The Netherlands) to assess $\dot{V}O_2$ peak with increments of 20 watts per minute as previously described (33).

Visit 2 - Physiological Assessments

On a separate occasion, participants arrived at the laboratory having fasted for six hours. Following 20 minutes of supine rest, participants underwent a period of assessment to quantify sympathetic vasomotor outflow and spontaneous sympathetic baroreflex function. During this time, ultrasound images of the aortic and carotid arteries were obtained for the assessment of the magnitude and rate of unloading, and the time spent unloaded (relative to the cardiac cycle).

Hemodynamics and resting sympathetic neural activity

Heart rate (HR) and systolic (SBP) and diastolic blood pressure (DBP) were monitored using a 3-lead ECG and finger photoplethysmography, respectively (FinometerPro, FMS, Groningen, Netherlands). SBP and DBP values were calibrated against manual brachial blood pressure measurements. Mean arterial pressure (MAP) was calculated using the equation ((2xDBP) + SBP)/3. Multiunit

MSNA was obtained from the peroneal nerve by an experienced microneurographer (JPM) in line with current guidelines (28). The acceptability of the MSNA neurogram was based upon established criteria (34) and was amplified, band-pass filtered, rectified and integrated (Nerve Traffic Analyser, Model 663 C, University of Iowa, Iowa City, IA). Once an appropriate site was identified for recording, following 10-minutes of supine rest, baseline MSNA and hemodynamic data were acquired for 10 minutes using a commercial data acquisition system (LabChart 8, LabChart Pro, AD Instruments, UK). Immediately following the 10-minute recording period described above, six minutes of hemodynamic and neural data were recorded during spontaneous breathing to characterize the vascular sympathetic baroreflex regulation of MSNA. Resting MSNA was quantified as burst frequency (bursts per minute [bursts·min^{-1}]) and the OP of the sympathetic baroreflex was quantified as MSNA burst incidence (bursts per 100 heart beats [bursts·100hb^{-1}]). Vascular sympathetic baroreflex gain was assessed using the slope of the stimulus-response relationship between DBP and MSNA burst probability. The MSNA OP was determined from burst incidence (i.e. probability) over the six-minute period.

Ultrasound procedures

During 10 minutes of basal MSNA data collection, ultrasound assessment of left ventricular stroke volume and aortic and carotid arteries was undertaken. Echocardiograms were acquired using a commercially available ultrasound system (Vivid E9, GE Medical, Norway) with a 4 MHz array probe. Images were obtained from apical 4 and 2 chamber views of the left ventricle by a single experienced sonographer (RNL) and saved for offline analysis of stroke volume (SV) using Simpson's biplane method, allowing for the calculation of cardiac output (Q_C: HR x SV) and total peripheral resistance (TPR: Q_C/mean arterial pressure). Longitudinal B-mode images (12-MHz linear array transducer, Vivid Q, GW Medical, Norway) of the right common carotid artery 2cm proximal to the carotid bulb were recorded for 1 minute at an insonation angle of 60° for later offline analysis of carotid artery blood flow. Analysis of carotid artery blood flow

was performed using semi-automated custom-designed edge-detection and wall-tracking software as outlined previously (35).

Aortic (4-MHz phased array transducer, Vivid E9, GE Medical, Norway) and carotid artery (12-MHz linear array transducer, Vivid Q, GW Medical, Norway) images were recorded over 15 cardiac cycles by trained sonographers (RNL and CJP). The suprasternal view was used to obtain images of the aortic arch. The right common carotid artery proximal to the carotid bifurcation was used to obtain common carotid artery images. Anatomical M-mode was applied to aortic images between the brachiocephalic and right common carotid artery branches (13), and to carotid artery images 2cm from the carotid bulb (EchoPac BT13, GE Medical, Norway). Systolic diameters of the aorta and carotid arteries were measured as the peak diameter within the systolic period (maximum barosensory vessel stretch) and diastolic diameter was identified as the diameter at the end of barosensory vessel stretch (see Figure 1). Systolic and diastolic wall thickness was measured in both vessel walls at the corresponsing M-mode point for systolic and diastolic diameters and an average wall thickness from both walls calculated for 5 cardiac cycles. Systolic and diastolic blood pressure for the corresponding cardiac cycles was identified in the reconstructed arterial pressure signal acquired using Lab Chart. Systolic and diastolic wall tension were then calculated in accordance with previous literature (2, 3) as:

$$\text{Wall tension} = \frac{\text{Pressure} \times \left(\frac{\text{Diameter}}{2}\right)}{\text{Wall thickness}}$$

The difference between systolic wall tension and diastolic wall tension was used as our measure of the magnitude of baroreceptor unloading. Time calipers were used to determine the time interval between systolic diameter measurement and diastolic diameter measurement to allow calculation of the rate of unloading (time measurement A, Figure 1) as magnitude of unloading/time taken to unload. The time interval between the diastolic diameter measurement and end of the cardiac cycle (time measurement B, Figure 1) was also assessed using time calipers to allow calculation of % of time spent unloaded, relative to the cardiac cycle, as the

time taken from the diastolic diameter measurement to the end of the cardiac cycle/cardiac cycle length x 100. See Supplementary Data for cardiac timing methods (https://doi.org/10.25401/cardiffmet.12145164.v1).

(FIGURE 1)

Data Analysis

Descriptive statistics were calculated for demographic, anthropometric, and hemodynamic variables. Data are reported as mean ± SD. Shapiro-Wilk tests of normality were used to assess variable distribution. As all data were normally distributed, independent group T-tests were used to assess between-group differences in participant characteristics, haemodynamics, resting vascular sympathetic baroreflex function and barosensory vessel unloading mechanics. Pearson's correlation coefficients, using $\dot{V}O_2$ peak as a covariate to control for the impact of fitness status, were used to assess the relationship between the magnitude of baroreceptor unloading, the rate of unloading, and the time spent unloaded in the aortic and carotid arteries and the OP and reflex gain of the vascular sympathetic baroreflex. Alpha was set a-priori as $P < 0.05$. All statistical analyses were completed using Statistics Package for Social Sciences for Windows, (Version 23, Chicago, IL).

RESULTS

Participant characteristics and resting haemodynamics

By design, middle-aged men were significantly older than young men ($P < 0.001$), whereas anthropometrics (height, body mass and BMI), and aerobic fitness ($\dot{V}O_2$ peak) were similar between groups ($P > 0.05$, see Table 1). Resting diastolic blood pressure and HR were similar between young and middle-aged men. SV and Q_c were all significantly lower in middle-aged men ($P = 0.02$ and <0.001 respectively), whereas carotid artery blood flow was not different between young and middle-aged men. In contrast, TPR and MAP were significantly elevated with age ($P < 0.003$, see Table 1) and systolic pressure was elevated, but not

373 significantly, in middle-age. Cardiac timing data are presented in Supplementary
374 Table 1.
375
376 Table 1 – Participant characteristics and haemodynamics

	Young men (n = 27)	**Middle-aged men (n = 22)**
Age (years)	23 ± 3	55 ± 4 *
Height (cm)	179.1 ± 5.4	175.1 ± 6.5
Body mass (kg)	72.8 ± 12.9	72.6 ± 11.4
BMI ($kg.m^2$)	23.11 ± 3.82	23.67 ± 3.36
SBP (mmHg)	117 ± 8	126 ± 9
DBP (mmHg)	72 ± 9	78 ± 7
MAP (mmHg)	87 ± 9	94 ± 7 *
HR ($beats.min^{-1}$)	51 ± 13	48 ± 11
SV (ml)	77 ± 17	67 ± 10 *
Q_c ($L.min^{-1}$)	3.90 ± 0.59	3.11 ± 0.62 *
TPR ($mmHg.L.min^{-1}$)	22.54 ± 4.30	33.12 ± 5.80 *
Carotid artery blood flow ($ml.min^{-1}$)	660 ± 147	648 ± 130
$\dot{V}O_2$ peak ($ml.kg.min^{-1}$)	50.4 ± 14.2	42.8 ± 11.8

377 Data are presented as mean ± SD. * significantly different between young and
378 middle-aged men, $P < 0.05$
379
380 *Sympathetic activity and barosensory vessel diameters, wall thickness, wall*
381 *tension and unloading mechanics*

The MSNA OP of the vascular sympathetic baroreflex (i.e. MSNA incidence) and MSNA burst frequency were both significantly higher in middle-aged men compared to young men ($P < 0.001$, see Table 2), whereas reflex gain of the vascular sympathetic baroreflex was similar between groups ($P = 0.51$, see Table 2).

Systolic and diastolic wall tension in both the aorta and carotid artery were similar between young and middle-aged men ($P > 0.05$, see Table 2), therefore, the magnitude of aortic and carotid artery unloading were not different between groups ($P = 0.72$ and 0.49 respectively, see Table 2). Despite similar HRs, the rate of unloading in both the aorta and carotid artery was significantly faster in young men compared to middle-aged men (both $P < 0.001$, see Table 2). Consequently, the time spent unloaded relative to the total cardiac cycle length in both the aorta and carotid artery was significantly greater in young men compared to middle-aged men ($P = 0.006$ and 0.004 respectively, see Table 2). Aortic and carotid artery wall thickness and diameter data are presented in Supplementary Table 2.

Table 2 – Resting vascular sympathetic baroreflex function and barosensory vessel unloading mechanics

	Young men (n = 27)	**Middle-aged men (n = 22)**
Vascular sympathetic baroreflex function		
MSNA operating point (burst.100hb^{-1})	32 ± 20	64 ± 23 *
MSNA frequency (burst.min^{-1})	16 ± 9	30 ± 10 *
Vascular sympathetic baroreflex gain (%.mmHg^{-1})	-6.13 ± 3.02	-6.03 ± 3.05
Aortic wall tension		
Aortic systolic wall tension (dynes.mm^{-2})	854.5 ± 220.3	821.4 ± 225.9

Aortic diastolic wall tension (dynes.mm^{-2})	358.0 ± 103.5	348.1 ± 104.6
Aortic unloading mechanics		
Magnitude of aortic unloading (dynes.mm^{-2})	490.0 ± 188.6	471.5 ± 143.5
Rate of aortic unloading (dynes.mm^{-2}.ms.$^{-1}$)	1899.7 ± 689.6	1146.1 ± 529.9 *
Aortic time spent unloaded (%)	51 ± 9	43 ± 10 *
Carotid artery wall tension		
Carotid systolic wall tension (dynes.mm^{-2})	485.5 ± 153.2	470.8 ± 113.4
Carotid diastolic wall tension (dynes.mm^{-2})	268.5 ± 98.4	232.6 ± 58.6
Carotid artery unloading mechanics		
Magnitude of carotid unloading (dynes.mm^{-2})	222.3 ± 79.4	238.2 ± 69.5
Rate of carotid unloading (dynes.mm^{-2}.ms.$^{-1}$)	844.0 ± 380.4	421.8 ± 191.6 *
Carotid time spent unloaded (%)	50 ± 8	41 ± 11 *

Data are presented as mean ± SD. * significantly different between young and middle-aged men, $P < 0.05$

Relationships between operating point of vascular sympathetic baroreflex and barosensory vessel unloading mechanics

In young men, the MSNA OP of the vascular sympathetic baroreflex was positively related to the magnitude of aortic unloading, the rate of aortic unloading and time spent unloaded in the aorta ($P < 0.03$, see Figure 2), but was not related to the magnitude of carotid unloading, the rate of carotid unloading or time spent unloaded in the carotid artery ($P > 0.05$, see Table 3). In contrast, in middle-aged men, the MSNA OP of the vascular sympathetic baroreflex was not related to the magnitude of either aortic or carotid artery unloading, the rate of unloading or the time spent unloaded in either aorta or carotid artery ($P > 0.05$, see Figure 2 and

Table 3). In young men, the magnitude of aortic but not carotid artery unloading was related to vascular sympathetic baroreflex gain (P = 0.03, see Figure 3), whereas in middle-aged men the magnitude of neither aortic nor carotid artery unloading was related to vascular sympathetic baroreflex gain. In both young and middle-aged men, neither the rate of unloading or time spent unloaded was related to vascular sympathetic baroreflex gain.

(FIGURE 2)

(FIGURE 3)

Table 3 - Relationships between the magnitude of carotid unloading, rate of unloading and time spent unloaded and the MSNA OP of the vascular sympathetic baroreflex

	Young men (n = 27)		**Middle-aged men (n = 22)**	
	***r* value**	***P* value**	***r* value**	***P* value**
Relationship with MSNA operating point				
Magnitude of aortic unloading (dynes.mm^{-2})	0.56	0.02*	0.22	0.41
Rate of aortic unloading (dynes.mm^{-2}.ms.$^{-1}$)	0.65	0.003*	0.21	0.43
Aortic time unloaded (%)	0.51	0.03*	0.27	0.31
Relationship with MSNA operating point				
Magnitude of carotid unloading (dynes.mm^{-2})	-0.32	0.25	0.06	0.48
Rate of carotid unloading (dynes.mm^{-2}.ms.$^{-1}$)	-0.07	0.81	0.28	0.25
Carotid time unloaded (%)	0.06	0.85	-0.01	0.98

* significant relationship, $P < 0.05$

DISCUSSION

Our primary aim was to use a new methodological approach to investigate relationships between indices of barosensory vessel unloading mechanics and indices of vascular sympathetic baroreflex function. Therefore, we determined the following: the magnitude of aortic and carotic artery unloading, the rate of unloading, and the time spent unloaded (%); and, vascular sympathetic baroreflex MSNA OP and gain. The key findings are threefold: 1) in young men, aortic unloading mechanics, but not carotid artery unloading mechanics, are related to the OP of the vascular sympathetic baroreflex, implying that the aortic unloading mechanics may be important in regulating this OP, whereas carotid artery unloading mechanics are not, 2) in middle-aged men, neither aortic nor carotid artery unloading mechanics are related to the OP of the vascular sympathetic baroreflex, suggesting that barosensory vessel unloading mechanics likely do not have a role in determining this OP with advancing age, and 3) in young men, but not middle-aged men, aortic unloading is related to the reflex gain of the vascular sympathetic baroreflex, indicating that reflex responsiveness is not dependent upon unloading mechanics in healthy older men. These key findings provide new insight into reflex control of sympathetic outflow and the mechanisms underlying age-related vascular sympathetic baroreflex resetting.

Impact of barosensory vessel unloading mechanics on baroreflex function in young men

The key novel aspect of the study was measurement of the magnitude and rate of change in wall tension within the arterial walls of barosensory vessels during the recoil phase of mechanical deformation. For the younger men studied here, a striking finding is that aortic unloading, the rate of unloading, and the time spent unloaded in the aorta all correlated positively with the MSNA OP. The potential role that aortic vessel unloading mechanics play in determining the OP of the vascular sympathetic baroreflex in young men may be explained by the interaction between these mechanics and the potential for a burst to occur via the

proposed “gate” system (12). Presumably, a shorter proportion of the cardiac cycle with baroreceptor afferent input to the nucleus tractus solitarius (NTS), would increase the likelihood of a burst, dependent upon a sufficient period between the removal of inhibition and initiation of a burst of efferent activity within the cardiac cycle (12). Over time, in the resting state, the rate of unloading and time spent unloaded may therefore represent an important control input to the brainstem in determining the OP of the sympathetic baroreflex in young men. Indeed, animal data supports the notion that baroreceptors have a long-term control input on resting SNA (32).

Furthermore, only the magnitude of aortic unloading was related to reflex gain of the vascular sympathetic baroreflex in the young men studied here. This is intriguing given that arterial baroreceptors are located at both sites. Previous studies have suggested that the aortic baroreflex is more important than the carotid baroreflex in blood pressure regulation in young men (26) and that aortic baroreceptors have a higher pressure mechanosensitivity than carotid baroreceptors (15). In addition, the aortic baroreflex in isolation can produce a sustained inhibition of MSNA, whereas the carotid baroreflex cannot achieve this (26). Aortic distensibility has also been shown to be more important than carotid distensibility with respect to cardiovagal baroreflex gain (13); however, until now, no studies have determined the relative contribution of the aortic and carotid artery to vascular sympathetic baroreflex gain.

Impact of barosensory vessel unloading mechanics on baroreflex function in middle-age

In this study, we observe proportionately less time *per* cardiac cycle with the baroreceptors unloaded in middle-aged men compared with younger individuals. Despite this, older men operate with predictably higher MSNA burst probability. There is, however, no relationship between unloading mechanics in barosensory vessels and sympathetic baroreflex OP or reflex gain. It seems reasonable, therefore, to speculate that age-related resetting of the vascular sympathetic baroreflex is not determined by altered barosensory vessel unloading mechanics.

We cannot, however, discount changes at the baroreceptors themselves, and/or changes in central mechanisms that generate and modulate MSNA (see below).

Neither aortic nor carotid artery unloading mechanics were related to reflex gain of the vascular sympathetic baroreflex in middle-aged men, implying that unloading does not have an important role in determining baroreflex responsiveness in this age group. However, our data contrasts with a previous study reporting a significant negative correlation between barosensory vessel distensibility and reflex gain of the vascular sympathetic baroreflex (24); this difference could be explained by an older sample population, of approximately 15 years, for that study compared to the middle-aged cohort examined in the present study; or, the different methodology used to quantify the mechanical component of reflex gain.

Potential modulators of the operating point of the vascular sympathetic baroreflex

A potential mechanism to explain why unloading mechanics of barosensory vessels do not determine the MSNA OP of the vascular sympathetic baroreflex in middle-aged men could be related to adaptation of the baroreceptor with age. Animal studies show that baroreceptor activity declines following a period of sustained pressure elevation with increased vessel stiffness (6). Moreover, increased vessel stiffness with age is related to reduced stretch sensitivity (1), and therefore a higher threshold to initiate baroreceptor afferent activity is evident (5). Either the combination of these adaptations, or one in isolation, would cause reduced baroreceptor afferent firing at a given pulsatile pressure (31) and therefore elevate the OP of the vascular sympathetic baroreflex, independent of barosensory vessel mechanics; however this cannot be assessed easily *in vivo* in humans.

The possibility that central neural remodeling may also alter sympathetic outflow at rest is conceivable (4). Cerebral noradrenaline turnover increases with age (8) which may be a contributor to increased central sympathetic outflow. Indeed, animal studies have suggested brain structures that influence baroreceptor control of sympathetic bursts (22) and brain imaging studies in humans also

support these structures as regions of baroreflex control (14). In addition, Osborn and colleagues (2005) propose that there is a baroreceptor-independent central nervous system set point for sympathetic outflow to the kidney (25). Taken together, these data suggest that neural plasticity may result in a central resetting of sympathetic outflow, distinct to barosensory vessel mechanical input.

Limitations

The association between spontaneous fluctuations in DBP and occurrence of bursts of MSNA were used to calculate vascular sympathetic baroreflex gain, therefore we did not take burst amplitude into account in our analysis. Baroreceptor signals modulate burst occurrence via a gating system (12), whereas little is known about the mechanisms that control burst amplitude. In addition, we did not assess vascular sympathetic reflex gain to rising and falling pressure independently and therefore do not have a measure of baroreflex hysteresis in our data (27). Given the *a priori* aim of this study, we do not present data relating these vessel unloading mechanics to MSNA burst frequency. However, post hoc analysis of our data revealed the same significant relationships as we report with the MSNA OP in young but not middle-aged men.

We have not reported the relationships between barosensory vessel unloading mechanics and the OP or gain of the cardiovagal baroreflex, as it is not known whether the cardiovagal baroreflex also operates via a similar gating system. That said, our data demonstrates the same significant relationships as we report with the MSNA OP in young but not middle-aged men. In this exploratory investigation, we only examined men and therefore further studies with female participants across the age range are required to establish potential sex differences.

The data we obtained for carotid unloading was acquired from the common carotid artery which does not harbour baroreceptors, these are located in the carotid sinus. However, previous studies imaging both aortic and carotid artery sinus have only reported a significant contribution from the aorta, not carotid

sinus, to cardiovagal baroreflex gain (13) and previous studies assessing the mechanical component of both the cardiovagal (10) and sympathetic baroreflex (27) have imaged the common carotid artery, not the carotid sinus.

Future research directions

This study is the first to apply this new methodology and suggest a control input for unloading mechanics on the MSNA OP. Further investigation designed to influence unloading mechanics using manipulations of volume/pressure in barosensory vessels, and beat-by-beat analsysis of this response, are required to establish the importance of unloading mechanics in determining the OP of the vascular sympathetic baroreflex. In addition, the impact of unloading mechanics on burst amplitude, onset and latency should be explored.

PERSPECTIVES

In our view, the increase in MSNA with healthy ageing may indicate an increased dependence on the vascular sympathetic baroreflex to maintain blood pressure. Notably, we find that the MSNA OP of the vascular sympathehic baroreflex of healthy middle-aged men is not related to aortic or carotid artery unloading mechanics. In contrast, indices of aortic unloading mechanics are associated with the MSNA OP, and reflex gain, for younger men. Thus, we infer that age-related elevation of MSNA OP and basal sympathetic outflow is not driven by vascular ageing and stiffening of barosensitive vessel walls. Although not tested here, central mechanisms that subserve baroreflex resetting likely underpin elevated basal vasomotor outflow with human ageing. Nevertheless, abnormally elevated sympathetic nervous system activity is a feature of hypertension and other diseases of the circulation. Therefore, further exploration of mechanism(s) responsible for increased central sympathetic outflow, and delineating physiological from pathological processes, is fundamental to our understanding of the progression of hypertension and other CVD.

CONCLUSIONS

This study demonstrates that in young, healthy, normotensive men, aortic unloading mechanics contribute to the resting OP of the vascular sympathetic baroreflex, whereas carotid artery unloading mechanics do not influence this OP. With advancing age, neither aortic nor carotid artery unloading mechanics have a role in determining the resting OP of the vascular sympathetic baroreflex. Taken together, these data suggest that although advancing age alters barosensory vessel unloading mechanics, these mechanics do not control the OP of the vascular sympathetic baroreflex in middle-aged men, and are therefore not driving the well documented age-related increase in resting MSNA.

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