Vasomotor sympathetic neural control is maintained during sustained upright posture in humans

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Vasomotor sympathetic activity plays an important role in arterial pressure maintenance via the baroreflex during acute orthostasis in humans. If orthostasis is prolonged, blood pressure may be supported additionally by humoral factors with a possible reduction in sympathetic baroreflex sensitivity. We tested the hypothesis that baroreflex control of muscle sympathetic nerve activity (MSNA) decreases during prolonged upright posture. MSNA and haemodynamics were measured supine and during 45 min 60 deg upright tilt in 13 healthy individuals. Sympathetic baroreflex sensitivity was quantified using the slope of the linear correlation between MSNA and diastolic pressure during spontaneous breathing. It was further assessed as the relationship between MSNA and stroke volume, with stroke volume derived from cardiac output (C_2H_2 rebreathing) and heart rate. Total peripheral resistance was calculated from mean arterial pressure and cardiac output. We found that MSNA increased from supine to upright (17 ± 8 (s.d.) *versus* 38 ± 12 bursts min^{-1}; $P < 0.01$), and continued to increase to a smaller degree during sustained tilt (39 ± 11, 41 ± 12, 43 ± 13 and 46 ± 15 bursts min^{-1} after 10, 20, 30 and 45 min of tilt; between treatments $P < 0.01$). Sympathetic baroreflex sensitivity increased from supine to upright (-292 ± 180 *versus* -718 ± 362 units $beat^{-1}$ $mmHg^{-1}$; $P < 0.01$), but remained unchanged as tilting continued (-611 ± 342 and -521 ± 221 units $beat^{-1}$ $mmHg^{-1}$ after 20 and 45 min of tilt; $P = 0.49$). For each subject, changes in MSNA were associated with changes in stroke volume ($r = 0.88 \pm 0.13$, $P < 0.05$), while total peripheral resistance was related to MSNA during 45 min upright tilt ($r = 0.82 \pm 0.15$, $P < 0.05$). These results suggest that the vasoconstriction initiated by sympathetic adrenergic nerves is maintained by ongoing sympathetic activation during sustained (i.e. 45 min) orthostasis without obvious changes in vasomotor sympathetic neural control.

(Resubmitted 27 July 2006; accepted after revision 27 September 2006; first published online 28 September 2006)
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Vasomotor sympathetic activity plays an important role in arterial pressure maintenance mainly through baroreflex-mediated vasoconstriction during short-term orthostasis in humans (Johnson *et al.* 1974; Wallin & Sundlof, 1982; Fu *et al.* 2004). If upright posture is prolonged, blood pressure may be supported additionally by vasoactive humoral factors (Rowell, 1993), with a possible reduction in baroreflex regulation of vasomotor sympathetic activity (Sanderford & Bishop, 2000, 2002).

Conversely, despite the importance of neural control in the rapid stabilization of arterial pressure, it has been proposed that the sympathetic baroreflex is not important in the long term regulation of blood pressure (Cowley, 1992). For example, previous studies in animals have shown that the strength or sensitivity of the baroreceptor feedback control system is insufficient to account for the prolonged constancy of blood pressure (Donald & Edis, 1971; Cowley *et al.* 1973); moreover, the arterial baroreceptors adapt quickly (Cowley *et al.* 1973; Fisher *et al.* 1984). Furthermore, one human study showed that patients with baroreflex failure have little orthostatic hypotension (Robertson *et al.* 1993). It seems likely that the vasoconstriction in the upright posture may be initiated by sympathetic adrenergic nerves and maintained by circulating vasoactive humoral factors in humans. As direct intraneural sympathetic recordings during sustained orthostasis have never been measured in healthy individuals, this speculation has not yet been proven.

We speculated that an increase in vasomotor sympathetic activity, which can be recorded as muscle sympathetic nerve activity in humans (Wallin *et al.* 1974; Vallbo *et al.* 1979), may not be well maintained during prolonged upright posture, and therefore vasomotor sympathetic control may play a smaller role during sustained compared with acute orthostasis. Thus, the present study was performed to test the hypothesis that baroreflex control of muscle sympathetic nerve activity decreases during prolonged upright posture in healthy humans. Specifically, we determined (1) whether the increase in muscle sympathetic nerve activity was attenuated, and (2) whether sympathetic baroreflex sensitivity decreased during sustained upright posture in humans.

Methods

Subjects

Twenty healthy volunteers aged from 19 to 51 years were studied. Thirteen of these completed a 45 min 60 deg upright tilt test (9 men, 4 women), while 7 subjects (2 men, 5 women) developed presyncope during tilting. The data reported in this study are from those 13 subjects who did not have presyncope to avoid the confounding effects of triggered sympathetic withdrawal and hypotension (Kamiya *et al.* 2005*a*) on the calculations of baroreflex sensitivity. The mean age ($\pm$ S.D.) of this group was 32 $\pm$ 10 years, mean body weight 73 $\pm$ 14 kg and mean height 175 $\pm$ 7 cm. No subject smoked, used recreational drugs, or had significant medical problems. None was an endurance-trained athlete (Levine *et al.* 1991). All females were normally menstruating ($\sim$28 days cycle), and had never taken or had not taken oral contraceptives for $\geq$ 6 months. They were not pregnant during the study. The subjects were screened with a careful medical history, physical examination, electrocardiogram and a 15 min 60 deg upright tilt test. Individuals with a history of fainting or neurally mediated syncope were excluded. All subjects were informed of the purpose and procedures used in the study and gave their written informed consent to a protocol approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. The study followed guidelines set forth in the *Declaration of Helsinki*.

Measurements

Muscle sympathetic nerve activity. Muscle sympathetic nerve activity signals were obtained with the microneurographic technique (Wallin *et al.* 1974; Vallbo *et al.* 1979). Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The nerve signals were amplified (gain 70 000–160 000), band-pass filtered (700–2000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 s). Criteria for adequate muscle sympathetic nerve activity recording included: (1) pulse synchrony; (2) facilitation during the hypotensive phase of the Valsalva manoeuvre, and suppression during the hypertensive overshoot after release; (3) increases in response to breath holding; and (4) insensitivity to emotional stimuli (Wallin *et al.* 1974; Vallbo *et al.* 1979).

Heart rate and blood pressure. Heart rate was determined from lead II of the electrocardiogram, and beat-by-beat arterial pressure was measured non-invasively from the middle finger using photoplethysmography (Finapres, Ohmeda, A BOC Health Care Company, Louisville, CO, USA), supported by an arm board at heart level to standardize hydrostatic pressure effects between supine and upright positions. Cuff blood pressure was measured by electrosphygmomanometry (model 4240, SunTech Medical Instruments Inc., Raleigh, NC, USA), with a microphone placed over the brachial artery to detect Korotkoff sounds. Respiratory excursions were detected by a nasal cannula (model 1265, Respironics California Inc., Carlsbad, CA, USA).

Cardiac output. Cardiac output was measured with the acetylene rebreathing technique (Triebwasser *et al.* 1977). Cardiac output is calculated from the disappearance rate of acetylene in expired air, measured with a mass spectrometer (model MGA1100, Marquette, Milwaukee, WI, USA), after adequate mixing in the lung has been confirmed by a stable helium concentration. This method has been validated against standard invasive techniques, including thermodilution and direct Fick at rest and during orthostasis with a typical error (expressed as coefficient of variation) of 4–5% (Fu *et al.* 2005*a*).

Stroke volume was calculated from cardiac output and the heart rate measured during the rebreathing. Total peripheral resistance was calculated as the quotient of mean arterial pressure and cardiac output, multiplied by 80 (expressed as dyn s cm^{-5}). Mean arterial pressure was calculated as [(systolic blood pressure − diastolic blood pressure)/3] + diastolic blood pressure, where blood pressure was measured by arm cuff during the rebreathing.

Protocol

The experiment was performed in the morning or afternoon $\geq$ 2 h after a light breakfast or lunch, and $\geq$ 12 h after the last caffeinated or alcoholic beverage in a quiet, environmentally controlled laboratory with an ambient

temperature of ~25°C. Three females were studied during the luteal phase (from 20 to 22 days after the onset of menstruation, when both oestrogen and progesterone were high), and one was studied during the early follicular phase (2 days after the onset of menstruation, when both sex hormones were low) of their menstrual cycles.

After ≥ 30 min of quiet rest in the supine position, baseline data were collected for 6 min. The subject was then tilted passively to 60 deg upright tilt for 45 min. A belt was placed across the subject's waist to make sure he or she would not fall. A bicycle saddle was used to support approximately two-thirds of the body weight, while the subject stood on a plate at the end of the tilt bed on one leg, allowing the other leg to be relaxed for microneurography. After that, the subject was returned to the supine position for recovery. Heart rate, blood pressure, respiratory waveforms and muscle sympathetic nerve activity were recorded continuously. Cardiac output was measured while supine, and at the 5th, 10th, 20th, 30th and 40th minutes of tilting.

Data analysis

Data were sampled at 500 Hz with a commercial data acquisition system (Biopac System, Santa Barbara, CA, USA) and analysed using LabView software (National Instruments, Austin, TX, USA). Beat-by-beat heart rate was calculated from the R–R interval of the electrocardiogram. Beat-by-beat systolic and diastolic blood pressures were obtained from the arterial pressure waveform.

Sympathetic bursts were identified by a computer program using a 3 : 1 signal-to-noise ratio threshold within a 0.5 s search window and an expected burst reflex latency from the preceding R-wave of 1.3 s (Cui *et al.* 2001), and then were 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 min resting supine baseline. All bursts for that trial were then normalized against that value (Halliwill, 2000). Burst areas of the integrated neurogram, and systolic and diastolic blood pressure were measured simultaneously on a beat-by-beat basis. Total activity of the burst was defined as the burst area of the rectified and integrated neurogram. The number of bursts per minute (burst frequency) and total activity were used as quantitative indices.

Assessment of sympathetic baroreflex control

Baroreflex control of muscle sympathetic nerve activity was assessed by using the slope of the linear correlation between total activity and diastolic blood pressure during spontaneous breathing (Wallin *et al.* 1974; Sundlof & Wallin, 1978; Kienbaum *et al.* 2001), in the supine

position and during the early (from the 2nd to the 5th minute), middle (from the 17th to the 20th minute), and late stages (from the 42nd to the 45th minute) of upright tilt. To perform a linear regression, values for total activity were averaged over a 3 mmHg diastolic pressure bin. This pooling procedure reduces the statistical impact of inherent beat-by-beat variability in nerve activity attributable to non-baroreflex influences (e.g. respiration) (Sundlof & Wallin, 1978). However, minor variations of bin width or bin position may affect the baroreflex sensitivity (Kienbaum *et al.* 2001). To minimize such variations, a statistical weighting procedure was adopted (Kienbaum *et al.* 2001); each data point was entered once for each heart beat in the bin, and total activity was expressed as arbitrary units per heart beat (i.e. units beat^{-1}). The fluctuation of diastolic blood pressure during spontaneous breathing was quantified as the difference between the maximal and the minimal values of the bin.

Additionally, we used the muscle sympathetic nerve activity and stroke volume relationship to further evaluate sympathetic baroreflex control for each subject during changes in posture and 45 min upright tilt, since muscle sympathetic nerve activity has been demonstrated to be related to the change in stroke volume in the supine position (Charkoudian *et al.* 2005) and during orthostasis (Levine *et al.* 2002; Convertino *et al.* 2004; Fu *et al.* 2005*a*,*b*).

Statistical analysis

Data are expressed as mean ± standard deviation (s.d.). Supine baseline muscle sympathetic nerve activity, blood pressure and heart rate were averaged for 6 min. During upright tilt, data were collected and averaged from the 2nd to 5th minutes (Tilt5), 7th to 10th minutes (Tilt10), 17th to 20th minutes (Tilt20), 26th to 29th minutes (Tilt30), 36th to 39th minutes (Tilt40) and 42nd to 45th minutes (Tilt45). Haemodynamic and muscle sympathetic nerve activity responses to upright tilt were analysed using Friedman repeated-measures analysis of variance on rank. The Student-Newman-Keuls method was used *post hoc* for multiple comparisons. The relationship between total activity and diastolic pressure during spontaneous breathing in the supine and upright positions was determined for each subject by least-squares linear regression analysis, and the slopes were compared using Friedman repeated-measures analysis of variance on rank. The relationship between muscle sympathetic nerve activity and stroke volume as well as total peripheral resistance in the supine position and during 45 min upright tilt or in the middle and the late stages of tilt (e.g. Tilt20, Tilt30 and Tilt40) was determined by least-squares linear regression for each subject. All statistical analyses

were performed with a personal computer-based analysis program (SigmaStat, SPSS). A *P* value of < 0.05 was considered statistically significant.

Results

Vasomotor sympathetic and haemodynamic responses during 45 min upright tilt

Figure 1 shows original tracings of blood pressure, heart rate, respiratory waveforms and muscle sympathetic nerve activity from one subject. Muscle sympathetic nerve activity increased on moving from supine to upright; it continued to increase, and reached a peak at 42.5 ± 2.4 min during tilt (Fig. 2*A*, $P < 0.01$). Systolic blood pressure was well maintained (Fig. 2*B*), while diastolic blood pressure increased during 45 min upright tilt (Fig. 2*C*, $P < 0.01$). Heart rate increased from supine to upright, and continued to increase during 45 min tilt (Fig. 2*D*, $P < 0.01$).

Table 1 shows haemodynamic responses to 45 min upright tilt. Both stroke volume and cardiac output decreased from supine to upright, and further decreased during 45 min tilt. Total peripheral resistance increased during changes in posture, and it continued to increase during 45 min upright tilt. Total peripheral resistance was positively related to muscle sympathetic nerve activity during changes in posture and 45 min upright tilt in each subject, and the correlation coefficient (r) was 0.82 ± 0.15 and 0.75 ± 0.18 for Fig. 3*A* and *B*. The positive linear correlation still existed between total peripheral resistance and muscle sympathetic nerve activity in the middle and the late stages of tilt, and the mean of the individual correlation coefficient was 0.70 ± 0.31.

Sympathetic baroreflex control during 45 min upright tilt

The fluctuation of diastolic blood pressure during spontaneous breathing was 15 ± 6 mmHg in the supine position, and 19 ± 5, 21 ± 4 and 22 ± 5 mmHg during the early, middle and late stages of tilting. Sympathetic baroreflex sensitivity increased on moving from supine to upright ($P < 0.01$); however, the sensitivity remained unchanged during the middle and the late stages of sustained upright tilt (between treatments, $P = 0.49$; Fig. 4). The correlation coefficient for the linear relationship between total activity and diastolic pressure was 0.84 ± 0.23 in the supine position, 0.92 ± 0.12, 0.91 ± 0.13 and 0.94 ± 0.04 during the early, middle and late stages of 45 min upright tilt.
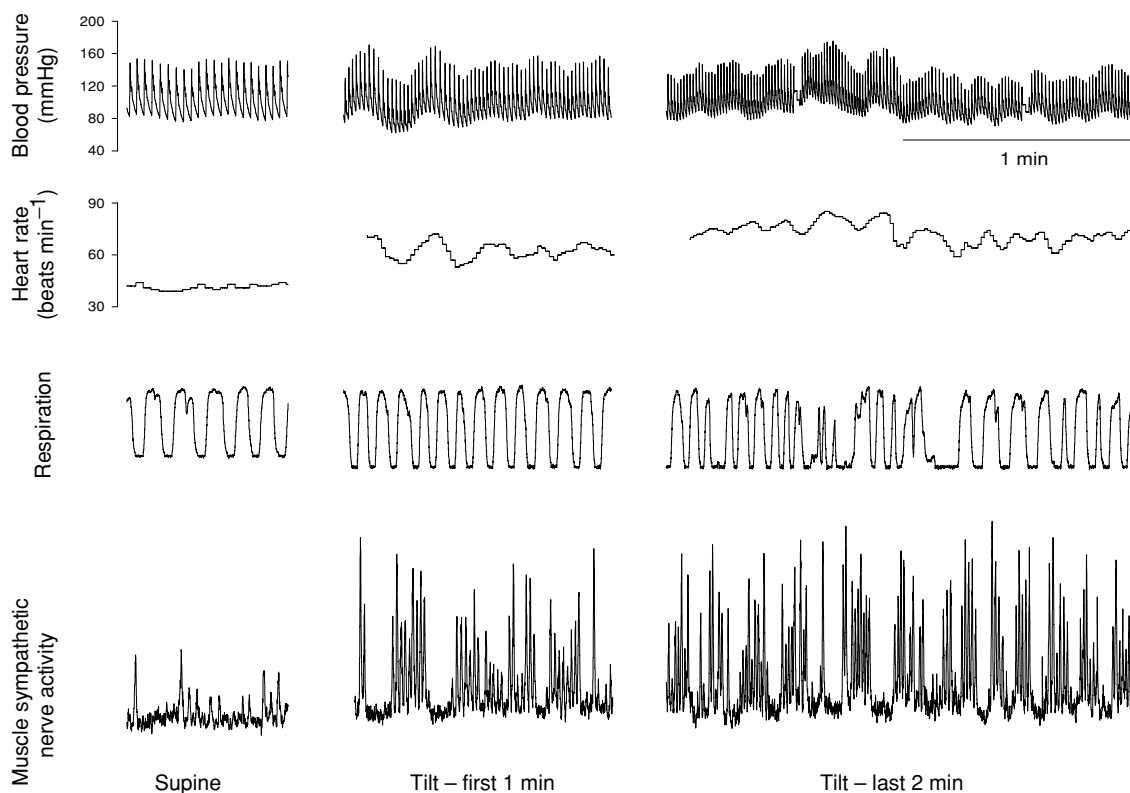

Figure 1

Original tracings of blood pressure, heart rate, respiratory waveforms, and muscle sympathetic nerve activity from one subject in the supine position, and during the initial 1 min and the last 2 min of 60 deg upright tilt.

Table 1. Haemodynamic responses to 45 min 60 deg upright tilt

Variables	Supine	60 deg upright tilt				
		5th minute	10th minute	20th minute	30th minute	40th minute
Stroke volume (ml)	103 ± 23	$75 \pm 23^*$	$61 \pm 15^*$†	$61 \pm 21^*$†	$57 \pm 22^*$†	$55 \pm 16^*$†
Cardiac output (l min^{-1})	7.74 ± 1.23	$6.31 \pm 1.71^*$	$5.24 \pm 0.97^*$†	$5.24 \pm 1.08^*$†	$4.89 \pm 1.18^*$†	$4.91 \pm 1.08^*$†
Total peripheral resistance (dyn s cm^{-5})	873 ± 126	$1247 \pm 349^*$	$1445 \pm 256^*$†	$1453 \pm 306^*$†	$1579 \pm 362^*$†	$1541 \pm 279^*$†

Data are mean $\pm$ s.d. $^*P < 0.05$ compared to the supine position; †$P < 0.05$ compared to the 5th minute of upright tilt.

Muscle sympathetic nerve activity was inversely related to stroke volume during changes in posture and 45 min upright tilt (Fig. 5). A strong linear relationship was observed in each subject, and the mean correlation coefficient was 0.88 ± 0.13. A negative linear correlation still existed between stroke volume and muscle sympathetic nerve activity in the middle and the late stages of tilt, and the mean correlation coefficient was 0.79 ± 0.23.

Discussion

The major findings from this study are that (1) muscle sympathetic nerve activity increased progressively during sustained upright posture; (2) sympathetic baroreflex sensitivity increased immediately after tilting, but did not change further during 45 min upright tilt; and (3) increases in muscle sympathetic nerve activity were associated with decreases in stroke volume during acute and prolonged upright posture in healthy individuals. These results do not support our hypothesis. Rather, they suggest that the vasoconstriction initiated by sympathetic adrenergic nerves is maintained by ongoing sympathetic activation. Therefore vasomotor sympathetic neural control plays an important role not only in acute but also in sustained (i.e. 45 min) arterial pressure maintenance in humans.

Unaltered vasomotor sympathetic control during sustained upright posture

Despite much research on the neural, hormonal and intrinsic mechanisms involved in the regulation of arterial
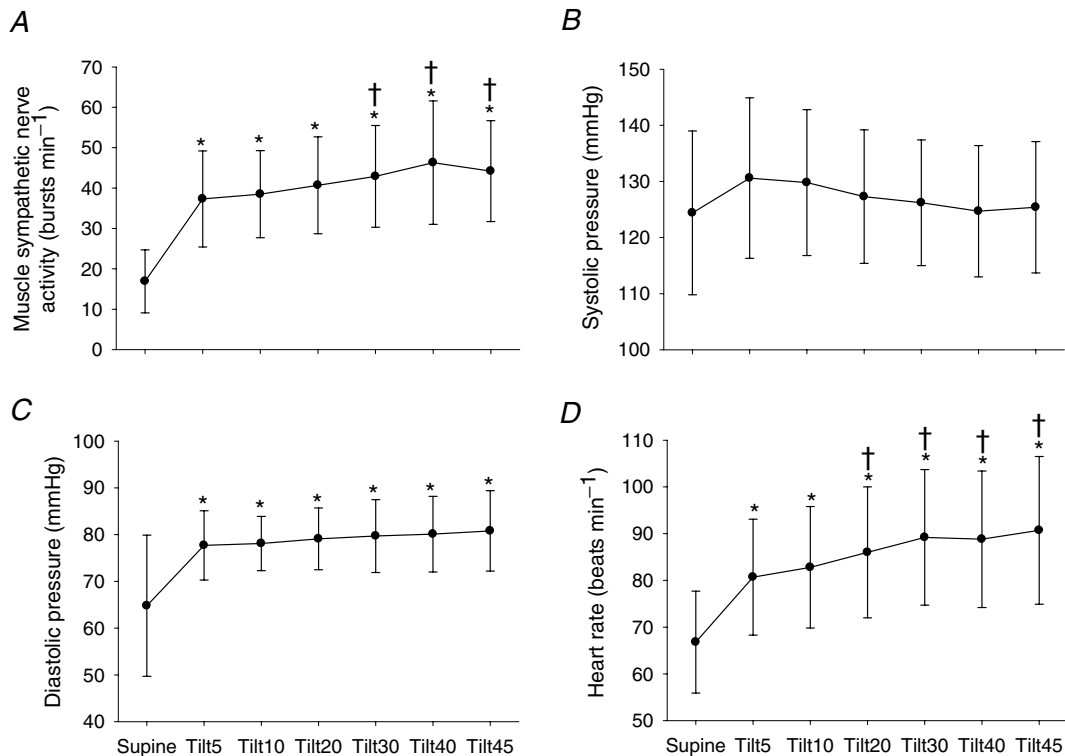
Figure 2
Muscle sympathetic nerve activity (*A*), systolic blood pressure (*B*), diastolic blood pressure (*C*), and heart rate (*D*) responses during 45 min of 60 deg upright tilt. Values are mean $\pm$ s.d. $^*P < 0.05$ compared to the supine position. †$P < 0.05$ compared to the 5th minute of upright tilt.
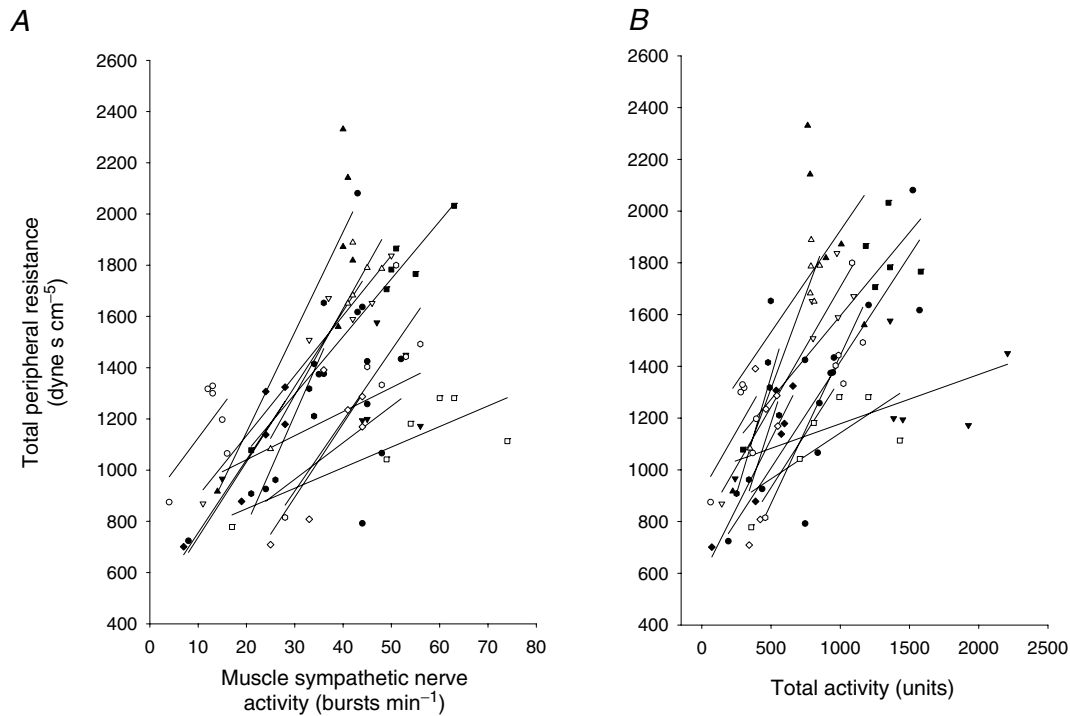
A

B

Figure 3

Correlation between total peripheral resistance and muscle sympathetic nerve activity in the supine position and during 45 min upright tilt in each subject.

pressure in humans, our understanding of how blood pressure is controlled over the long-term is limited (Barrett & Malpas, 2005). We found that vasomotor sympathetic neural control still plays an important role in arterial pressure maintenance during prolonged orthostasis. The underlying mechanism(s) are unknown. One potential explanation may be that physiological levels of increases in humoral factors do not affect vasomotor sympathetic control. Indeed, in contrast to animal studies (Sanderford

Figure 4

Sympathetic baroreflex sensitivity in the supine position, and during the early, middle and late stages of 60 deg upright tilt. Values are mean ± s.d. Tilt5, between the 2nd and 5th minute; Tilt20, between the 17th and 20th minute; Tilt45, between the 42nd and 45th minute of upright tilt. *$P < 0.05$ compared to the supine position.

& Bishop, 2000, 2002), some earlier studies showed that physiological elevations of arginine vasopressin did not alter cardiac or sympathetic baroreflex function, while much higher levels of vasopressin enhanced the vasomotor sympathetic response to unloading of baroreceptors in healthy humans (Ebert & Cowley, 1992; Goldsmith, 1994). It was also found that subpressor doses of angiotensin II did not affect plasma noradrenaline (norepinephrine) concentration (Goldsmith & Hasking, 1990); however, much higher doses of angiotensin II increased muscle sympathetic nerve activity (Matsukawa *et al.* 1991). Although humoral changes were not measured in our subjects, the data suggest that their influences on vasomotor sympathetic neural control were limited.
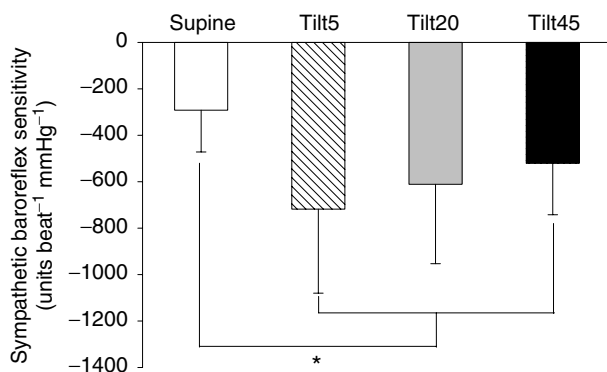
The continuous increase in muscle sympathetic nerve activity during sustained upright posture was related to the progressive decrease in stroke volume. This result expands previous findings from our laboratory (Levine *et al.* 2002; Fu *et al.* 2005*a*,*b*) and others (Convertino *et al.* 2004; Charkoudian *et al.* 2005), and further suggests that changes in muscle sympathetic nerve activity may be associated with changes in stroke volume. During prolonged orthostasis, transudation of fluid out of the capillaries and into the tissue space in the legs gradually decreases central blood volume and subsequently stroke volume in sustained upright humans (Watenpaugh *et al.* 1995). Recently, we found in healthy subjects that an index of carotid artery distortion, assessed by high resolution

ultrasonography, was closely correlated to stroke volume during a graded upright tilt, while the increase in muscle sympathetic nerve activity was related to the decrease in this index of carotid distortion (Hastings *et al.* 2006). These preliminary data support the notion that stroke volume may influence the primary stimulus to the baroreceptors (distortion) during orthostasis. It has been demonstrated that stroke volume changes translate into arterial pulse amplitude and pressure changes, which modulate arterial baroreceptor activity (Angell James, 1971; Chapleau & Abboud, 1989). In addition, stroke volume is one of the determinants of flow in baroreceptive arteries (Hajduczok *et al.* 1988). Moreover, stroke volume is a function of central blood volume and left ventricular end-diastolic volume, and thereby may reflect the stimulus to the myriad receptor populations termed 'cardiopulmonary' (Persson *et al.* 1988).

Since the upright tilt test lasted for only 45 min in the present study, we cannot exclude the possibility that vasomotor sympathetic neural control may decrease during more prolonged (i.e. several hours or days) upright posture in humans. It was difficult for the subjects to stand on one leg, allowing the other leg to be completely relaxed for microneurography for over 45 min in the upright position. Future investigations with improved microneurographic technique, improved tilt beds, and a much longer period of upright tilt are needed to evaluate

sympathetic control of vascular resistance over longer time periods.

Baroreflex sensitivity assessment during spontaneous breathing

Sympathetic baroreflex sensitivity was quantified during spontaneous breathing. We recognize that the blood pressure fluctuations during spontaneous breathing were not as large as those obtained using other methods such as the neck-chamber technique or invasive pharmacological manipulation. Therefore the entire baroreflex stimulus–response curve cannot be evaluated in this study. Consequently, we cannot determine whether the operating point on this stimulus–response curve has simply shifted to a steeper part of the curve during upright posture or whether an entirely new relationship is achieved. This problem is compounded by the differential effect of upright posture on hydrostatic gradients at the carotid and aortic baroreceptor populations. Still, a 20 mmHg change in diastolic pressure is within the physiological range and should be a good reflection of the dynamic baroreflex control of muscle sympathetic nerve activity under physiological conditions regardless of whether the curve has shifted or not. Additionally, previous studies have demonstrated that pharmacological and spontaneous baroreflex sensitivity values are closely
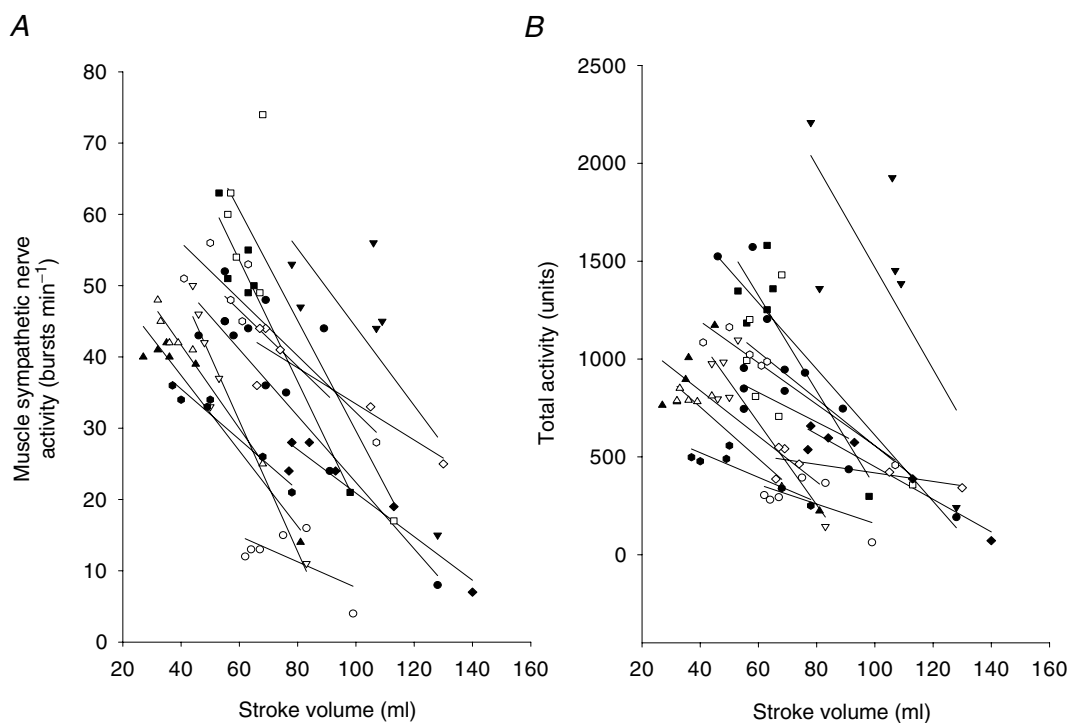

Figure 5
Correlation between muscle sympathetic nerve activity and stroke volume in the supine position and during 45 min upright tilt in each subject.

correlated in most instances (Parlow *et al.* 1995; Pitzalis *et al.* 1998). Thus, our data can be used to reveal the physiological modulation of vasomotor sympathetic control around the prevailing, regulated operating point in upright humans.

The baroreflex system is a feedback control system from baroreceptor distortion to systemic arterial pressure. It has been proposed that the baroreflex system has two subsystems; the central arc from baroreceptor distortion to efferent sympathetic nerve activity via the central nervous system and the peripheral arc from efferent sympathetic nerve activity to systemic arterial pressure (Kamiya *et al.* 2005*b*). Thus spontaneous arterial pressure and muscle sympathetic nerve activity in a closed-loop condition theoretically result from both central and peripheral arcs. However, due to the limitations of the methodology, it is impossible to isolate one unique subsystem (i.e. baroreflex control of muscle sympathetic nerve activity) from the total system output in human research.

Although diastolic pressure has been found to have the best correlation to the occurrence of sympathetic bursts (Sundlof & Wallin, 1978), it cannot be the primary parameter that determines when a burst occurs. For example, it was observed that the start of the burst began approximately 0.1–0.3 s before the end-diastolic point was reached (B. G. Wallin, personal communication). Therefore, other factors which have an intimate relationship to diastolic pressure may be the primary stimulus to the baroreceptors. We assumed but did not verify in this study that stroke volume may be an index of the primary stimulus (distortion of baroreceptor populations) and assessed sympathetic baroreflex control as the relationship between muscle sympathetic nerve activity and stroke volume. Further studies are needed to verify this assumption.

In conclusion, muscle sympathetic nerve activity increased from supine to upright and continued to increase during sustained upright posture. Sympathetic baroreflex sensitivity increased during changes in posture, but it remained unchanged as upright posture continued. The increase in muscle sympathetic nerve activity was associated with the decrease in stroke volume. The positive relationship between total peripheral resistance and muscle sympathetic nerve activity on an individual basis indicates that vasomotor sympathetic neural control is still important for 'prolonged' (i.e. 45 min) arterial pressure control during upright posture in healthy humans.

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