Vasoconstrictor Reserve and Sympathetic Neural Control of Orthostasis

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Background—We tested the hypothesis that individual variability in orthostatic tolerance is dependent on the degree of neural and vasomotor reserve available for vasoconstriction.

Methods and Results—Muscle sympathetic nerve activity (MSNA) and hemodynamics were measured in 12 healthy young volunteers during 60° head-up tilt (HUT), followed by a cold pressor test (CPT) in HUT. Orthostatic tolerance was determined by progressive lower-body negative pressure (LBNP) to presyncope. The same protocols were performed randomly in normovolemic and hypovolemic conditions. We found that mean arterial pressure increased and stroke volume decreased, whereas heart rate (HR), MSNA, and total peripheral resistance (TPR) increased during HUT (all $P<0.01$). Application of the CPT in HUT did not increase HR or decrease stroke volume further but elevated mean arterial pressure ($P<0.01$) and increased MSNA and TPR in some subjects. There was a positive correlation between the time to presyncope from -50 mm Hg LBNP (equivalent to 60° HUT alone) and the changes in MSNA produced by the CPT under both conditions ($r=0.442$, $P=0.039$). Those who had greater increases in MSNA had greater increases in TPR during the CPT and longer time to presyncope (both $P<0.05$). One subject had dramatic increases in MSNA but small increases in TPR during the CPT, which indicates a disassociation between sympathetic activity and the increase in peripheral vascular resistance.

Conclusions—These results support our hypothesis and suggest that vasoconstrictor capability is a contributor to orthostatic tolerance in humans. Vasoconstrictor reserve therefore may be one mechanism underlying individual variability in orthostatic intolerance. (***Circulation***. 2004;110:2931-2937.)

Key Words: vasoconstriction ■ nervous system, sympathetic ■ blood pressure

Orthostatic intolerance is a common clinical problem that occurs in patients with autonomic dysfunction. It may also occur in healthy individuals, such as endurance athletes,[1,2] astronauts returning from space,[3] or after a period of bed rest.[4,5] However, the individual variability in the development of orthostatic intolerance is large, and the underlying mechanisms remain unclear.

Normally, orthostatic stress evokes compensatory vasoconstriction in skeletal muscle via an increase in sympathetic nerve traffic, which can be recorded as muscle sympathetic nerve activity (MSNA) in humans.[6–8] When this compensatory mechanism fails, arterial pressure will drop, and syncope may occur.[6,8,9] A progressive withdrawal of MSNA before vasovagal syncope was observed in previous studies[6,10,11]; it was thereby assumed that a reduced sympathetic activity might play a causal role in orthostatic intolerance.[10] On the other hand, one study by Levine et al[12] showed that MSNA increased appropriately during upright tilt, but without a commensurate increase in peripheral vascular resistance in most astronauts returning from a 16-day space shuttle mission. A dissociation between the sympathetic activity and the increase in vascular resistance could also be a mechanism that explains the orthostatic intolerance after microgravity exposure or, more broadly, after cardiovascular deconditioning in general.

We speculate that each human individual may have a finite range of maximal vascular resistance that can be mediated by adrenergic activity. A limited vasoconstrictor reserve may result in reduced orthostatic tolerance, and orthostatic intolerance during hypovolemia may be a direct function of the capacity for vasoconstrictor reserve. It is likely that the vasoconstrictor reserve significantly affects the maintenance of orthostatic tolerance.[13,14] However, these speculations have not yet been proven.

The purpose of this study was to test the hypothesis that individual variability in orthostatic tolerance is dependent on the degree of neural and vasomotor reserve available for vasoconstriction. To accomplish this objective, we measured

the amount of additional adrenergic activity and vasoconstriction that could be made available during orthostatic stress and determined the relationship between the vasoconstrictor reserve and orthostatic tolerance in healthy individuals under both normovolemic and hypovolemic conditions.

Methods

Subjects

Twelve healthy volunteers (7 men and 5 women; 28.3 ± 1.7 [mean $\pm$ SE] years old, 67.5 ± 2.6 kg body weight, and 175.7 ± 2.0 cm in height) participated. No subject smoked, used recreational drugs, or had significant medical problems. None was an endurance-trained athlete.[1] No woman was pregnant during the experiment. All subjects gave their written informed consent, approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas.

Measurements

Heart Rate and Blood Pressure

Heart rate (HR) was monitored from the ECG (Hewlett-Packard), and beat-to-beat arterial pressure was derived by finger photoplethysmography (Finapres, Ohmeda). Cuff blood pressure (BP) was measured by electrosphygmomanometry (model 4240, Suntech), with a microphone placed over the brachial artery to detect Korotkoff sounds. Respiratory excursions were detected by a nasal cannula.

Cardiac Output

Cardiac output was measured with the acetylene rebreathing technique.[15] Cardiac output is calculated from the disappearance rate of acetylene in expired air, measured with a mass spectrometer (model MGA1100, Marquette), 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, during exercise and changes in orthostatic stress, with a typical error (expressed as coefficient of variation) of 4% to 5%.[16] This method has been used extensively in our laboratory and others,[1,2,5,12,15,17] and it has been reviewed recently in detail.[18]

Stroke volume (SV) was calculated from cardiac output and the HR measured during rebreathing. Total peripheral resistance (TPR) was calculated as the quotient of mean arterial pressure and cardiac output, multiplied by 80 (expressed as dyne $\cdot$ s $\cdot$ cm^{-5}). Mean arterial pressure was calculated as $[(SBP-DBP)/3]+DBP$, where SBP and DBP are cuff systolic and diastolic BP measured during rebreathing, respectively.

Muscle Sympathetic Nerve Activity

MSNA signals were obtained with the microneurographic technique.[19] Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2 to 3 cm from the recording electrode. The nerve signals were amplified (gain 70 000 to 160 000), band-pass filtered (700 to 2000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 second). Criteria for adequate MSNA recording included the following: (1) pulse synchrony; (2) facilitation during the hypotensive phase of the Valsalva maneuver, and suppression during the hypertensive overshoot after release; (3) increases in response to breath holding; and (4) insensitivity to emotional stimuli.[19]

Blood Samples

Blood samples were drawn from an intravenous catheter placed in the antecubital vein. Plasma catecholamine was measured with high-precision liquid chromatography.[20] Hematocrit was determined with a microcentrifuge. The percentage change in plasma volume ($\Delta PV\%$) with administration of a diuretic (furosemide) in the hypovolemic condition was estimated from hematocrit according to the method described by Van Beaumont,[21] namely, $\Delta PV\%=[100/$ $(100-Hct1)]\times[100(Hct1-Hct2)/Hct2]\%$, where Hct1 and Hct2 are original and final hematocrit, respectively.

Acute Hypovolemia

Plasma volume was reduced with the administration of 20 mg of furosemide. This dosage was chosen because it induced a reduction in plasma volume of 7% to 14% after administration for $\approx$2 hours, equivalent to the loss of plasma volume observed after 2 weeks of head-down bed rest.[17,22] An oral potassium supplement of 20 mEq was given before the injection of furosemide. After injection, urine was collected and cuff BP was measured every 15 minutes. Approximately 2 hours later, the following protocols were performed.

Protocols

The experiment was performed in the morning $\geq$2 hours after a light breakfast and $\geq$12 hours after the last caffeinated or alcoholic beverage in a quiet, environmentally controlled laboratory with an ambient temperature of $\approx$25°C. The same protocols were performed randomly in the normovolemic and hypovolemic conditions with an $\approx$4-week interval, and therefore, females were in the same phases of their menstrual cycles each time.

Protocol to Measure Vasoconstrictor Reserve

After $\geq$30 minutes of quiet rest in the supine position, baseline data were collected for 6 minutes. The subject was then tilted passively to a 60° head-up tilt (HUT) for 6 minutes, followed by a cold pressor test (CPT) during tilting for another 3 minutes. A belt was placed across the subject's waist to make sure he or she would not fall. The subject supported the body weight by standing on a plate at the end of the tilt bed on one leg, allowing the other leg to be relaxed for microneurography. The CPT was performed by immersing the subject's hand into an ice-water bath ($\approx$4°C). Subjects were instructed to avoid breath holding during the CPT. After that, the subject was returned to the supine position for recovery.

HR, BP, respiratory waves, and MSNA were recorded continuously. Cardiac output was measured and a blood sample was taken when supine, at the sixth minute of tilting, and at the third minute of the CPT. After completion of this protocol, the microneurography electrodes and intravenous catheter were removed.

Protocol for Maximal Orthostatic Tolerance Test

After a sufficient recovery period ($\geq$20 minutes), the subject was placed in the supine position in a Plexiglas lower-body negative pressure (LBNP) tank sealed at the iliac crest level. Suction was provided by a vacuum pump and controlled with a variable autotransformer calibrated against a mercury manometer. After $\geq$30 minutes of quiet rest, baseline measurements were repeated to confirm a return to the hemodynamic steady state. Maximal orthostatic tolerance was determined by the use of progressive LBNP to presyncope. LBNP was begun at -15 mm Hg for 5 minutes, then increased to -30 and -40 mm Hg for 5 minutes each, followed by an increase in LBNP by -10 mm Hg every 3 minutes until presyncope was achieved. Presyncope was defined as a decrease in systolic BP to <80 mm Hg; a decrease in systolic BP to <90 mm Hg associated with symptoms of lightheadedness, nausea, sweating, or diaphoresis; or progressive symptoms of presyncope accompanied by a request from the subject to discontinue the test.[2] A true hypotensive end point was reached in all subjects in this study. The recovery lasted for 5 minutes. A cumulative stress index was calculated by adding the product of negative pressure and duration at each level of LBNP and was used as a continuous measure of orthostatic tolerance.

Data Analysis

MSNA signals were identified by a computer program[23] and confirmed by an experienced microneurographer. The number of bursts per minute (burst frequency), the number of bursts per 100 heartbeats (burst incidence), and the sum of the integrated burst area per minute (total activity) were used as quantitative indexes. Because the amplitude of bursts of sympathetic activity depends critically on electrode position, whereas determinations of burst frequency are

Hemodynamics and Orthostatic Tolerance in Normovolemic and Hypovolemic Conditions

Variables	Normovolemia	Hypovolemia Before Furosemide	Hypovolemia After Furosemide
Body weight, kg	67.5±2.6	68.3±2.7	66.7±2.5*
Systolic BP, mm Hg	113±3	111±3	112±2
Diastolic BP, mm Hg	61±2	61±1	63±2
Cardiac output, L/min	7.54±0.56	7.32±0.64	5.53±0.35*‡
Norepinephrine, pg/mL	194.8±19.2	180.5±14.0	246.8±20.7*†
Epinephrine, pg/mL	18.8±3.7	14.4±1.9	15.5±2.9
Hematocrit, %	40.3±1.2	39.7±1.2	43.1±1.2*‡
Orthostatic tolerance			
CSI, mm Hg×min	835±71		722±95
Time to presyncope, s	413±61		274±108

CSI indicates cumulative stress index. Values are mean±SE.
*$P<0.01$ vs before administration of furosemide.
†$P<0.05$ and ‡$P<0.01$ vs normovolemic condition.

stable between recording sessions,[24] total activity was normalized to the resting supine value to allow comparisons between normovolemic and hypovolemic conditions. Therefore, the supine baseline recording was assigned a value of 100%, and subsequent changes of total activity were expressed as percentages of this baseline value.

HR, BP, and MSNA were averaged for 6 minutes during supine baseline. Data were collected from the third to the fifth minute during HUT and were averaged for 3 minutes. During the CPT, data were collected during the initial 2 minutes and averaged for every 0.5 minute, and the highest value was used.

Statistical Analysis

Data are presented as mean±SE. Comparisons at baseline and LBNP tolerance between normovolemic and hypovolemic conditions were made with paired t tests. Changes in MSNA and hemodynamics due to HUT and the CPT in HUT under both conditions were analyzed with 2-way repeated-measures ANOVA, with Bonferroni method post hoc for multiple comparisons. The relationship between the time to presyncope from −50 mm Hg LBNP (equivalent to 60° HUT alone) during the orthostatic tolerance test and the changes in MSNA produced by the CPT under both conditions were determined by linear regression analysis. All statistical analyses were performed with a personal computer–based analysis program (SigmaStat, SPSS). A probability value of <0.05 was considered statistically significant.

Results

Supine Resting Values

Furosemide induced a diuresis of 1.6±0.1 L of urine volume and increased hematocrit (Table; $P<0.01$), which resulted in a 10.9±1.3% reduction in plasma volume. Mean arterial pressure and HR did not change, but SV decreased ($P<0.01$) in the hypovolemic condition (Figure 1). MSNA burst frequency (Figures 2 and 3) and burst incidence (19.6±2.7 bursts/100 heartbeats in normovolemia versus 26.4±3.2 in hypovolemia), plasma norepinephrine (Table), and TPR (Figure 4) increased in the hypovolemic condition (all $P<0.05$). Maximal LBNP tolerance tended to decrease in the hypovolemic compared with the normovolemic condition (Table; $P=0.129$ and 0.135 for the cumulative stress index and the time to presyncope).
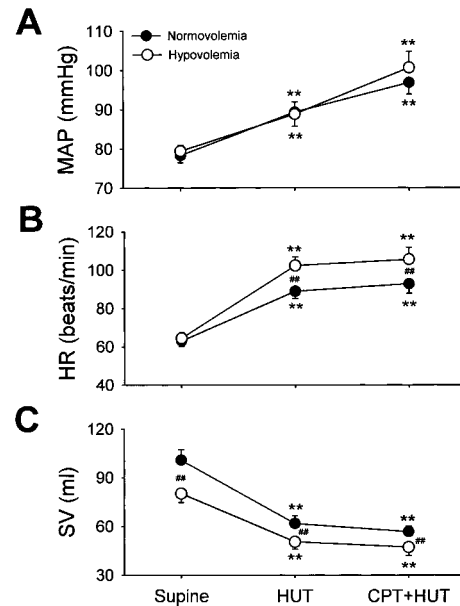

Figure 1. Mean arterial pressure (MAP; A), HR (B), and stroke volume (C) in response to 60° HUT and CPT during tilting (CPT+HUT) in normovolemic and hypovolemic conditions. Values are mean±SE. **$P<0.01$ compared with supine; ##$P<0.01$ compared with normovolemia.

Hemodynamic and MSNA Responses to HUT

Mean arterial pressure and HR increased, whereas SV decreased during HUT (Figure 1; all $P<0.01$). HR was higher and SV lower in the hypovolemic than in the normovolemic condition (both $P<0.01$). MSNA increased during HUT, and burst frequency was greater (Figure 3; $P<0.01$), whereas burst incidence tended to be greater in the hypovolemic than in the normovolemic condition (42.6±4.4 versus 36.7±3.1 bursts/100 heartbeats, $P=0.108$). However, normalized total activity was not different between conditions during HUT (435±68% in normovolemia versus 387±62% in hypovolemia, $P=0.297$). TPR increased during HUT under both conditions (Figure 4; $P<0.05$).

Hemodynamic and MSNA Responses to CPT During HUT

Application of the CPT in upright tilt elevated mean arterial pressure (Figure 1A; $P<0.01$) but did not increase HR or decrease SV further (Figure 1). HR was higher and SV lower during the CPT in the hypovolemic condition than in the normovolemic condition (Figures 1B and 1C; both $P<0.01$). MSNA and TPR increased in some subjects during the CPT under both conditions (Figures 3 and 4). There was a significant positive correlation between the time to presyncope from −50 mm Hg LBNP (equivalent to 60° HUT alone) during the orthostatic tolerance test and the changes in MSNA produced by the CPT under both conditions (Figure 5; $r=0.442$, $P=0.039$). Those who had greater increases in MSNA also had greater increases in TPR during the CPT and longer time to presyncope during progressive LBNP (Figure 6; both $P<0.05$).

For some subjects, MSNA and/or TPR during the CPT in upright tilt under the normovolemic condition appeared to be
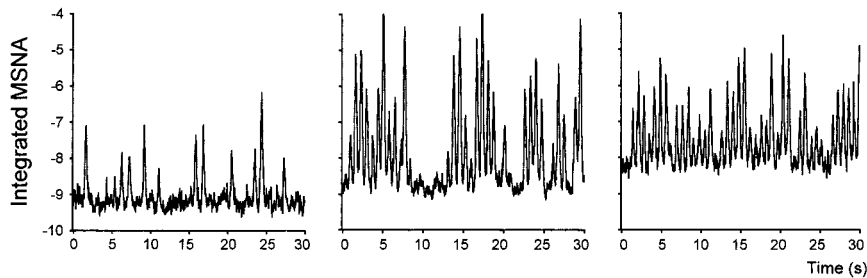
Normovolemia
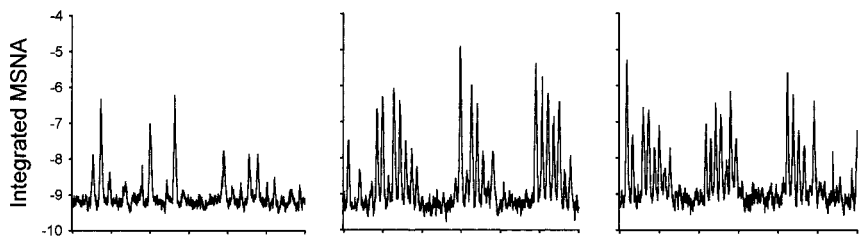
Hypovolemia

Figure 2. Original tracings of MSNA of 1 typical subject in supine position, at 60° HUT, and during CPT+HUT under both normovolemic and hypovolemic conditions.

maximal; in these subjects, MSNA/TPR during hypovolemic tilt was equivalent to this maximal value and did not increase further during the CPT, suggestive of limited further vasoconstrictor reserve (Figures 3 and 4 solid lines a and b). These subjects had the largest reductions in orthostatic tolerance in the hypovolemic condition (Figure 3, cumulative stress index for a and b). One female subject had dramatic increases in MSNA but only small increases in TPR during the CPT under both conditions (Figures 3 and 4 solid line c), which indicates a disassociation between sympathetic activity and the increase in peripheral vascular resistance and suggests a limited maximal vascular resistance that was mediated by adrenergic

activity. The time to presyncope during the orthostatic tolerance test was decreased prominently in the hypovolemic condition in this subject. Her data were excluded from the linear regression analysis shown in Figure 5 because they were clearly outliers.

Discussion

The major findings from this study are that (1) application of the CPT in upright tilt increased MSNA and TPR in some subjects; (2) the time to presyncope from −50 mm Hg LBNP (equivalent to 60° HUT alone) during the orthostatic tolerance test was positively correlated with an index of neural sympathetic reserve, that is, the changes in MSNA from HUT to the CPT during tilting; (3) those who had greater increases in MSNA also had greater increases in TPR during the CPT and longer time to presyncope during progressive LBNP; and
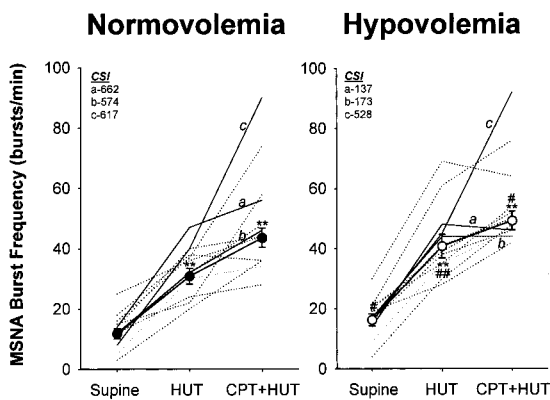

Figure 3. Individual and mean MSNA burst frequency responses to HUT and CPT+HUT. Solid lines (a, b, and c) are from 3 individual subjects. Subjects a and b had limited sympathetic neural reserve, and their orthostatic tolerance decreased significantly in hypovolemic condition. Subject c had dissociation between sympathetic activity and increase in peripheral vascular resistance during CPT in upright position and had limited vasoconstrictor reserve, and orthostatic tolerance was also decreased in hypovolemic condition. CSI indicates cumulative stress index. Grouped values are mean±SE. **P<0.01 compared with supine; #P<0.05 and ##P<0.01 compared with normovolemia.
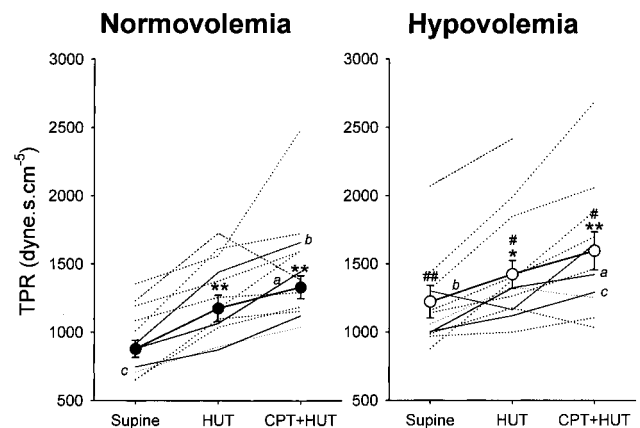

Figure 4. Individual and mean TPR responses to HUT and CPT+HUT. Solid lines (a, b, and c) are from 3 individual subjects. Grouped values are mean±SE. **P<0.01 compared with supine; #P<0.05 and ##P<0.01 compared with normovolemia.
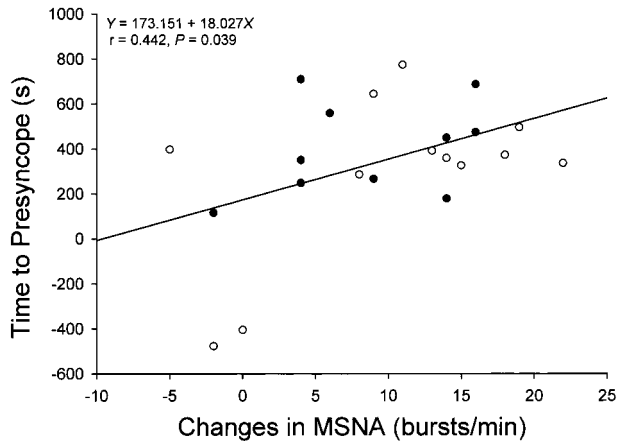
Figure 5. Correlation between time to presyncope from −50 mm Hg LBNP (equivalent to 60° HUT alone) during orthostatic tolerance test and changes in MSNA from 60° HUT to CPT in HUT under both normovolemic (●) and hypovolemic (○) conditions.

(4) 1 subject had a disassociation between the increase in MSNA and the increase in TPR during the CPT, which suggests a limited maximal vascular resistance that was mediated by adrenergic activity. Thus, our results support the hypothesis that individual variability in orthostatic tolerance is dependent on the degree of neural and vasomotor reserve
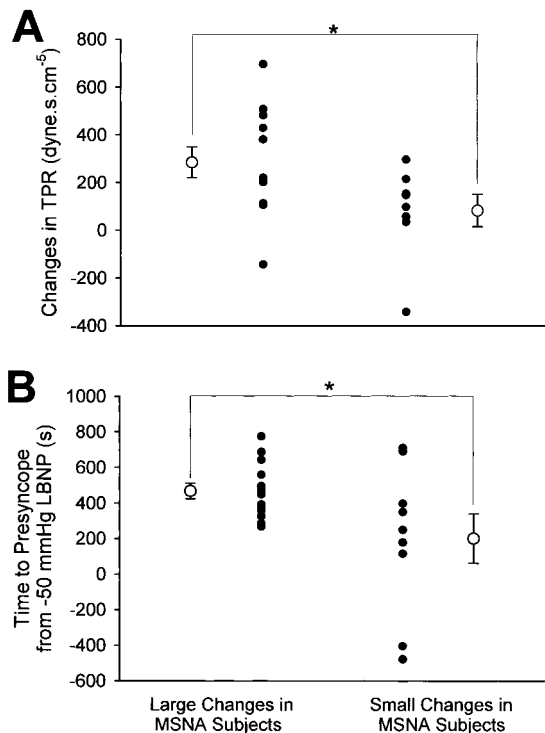

Figure 6. Comparisons of changes in TPR produced by CPT in upright tilt (A) and time to presyncope during orthostatic tolerance test (B) in subjects with large sympathetic reserve (ie, increases in MSNA >5 bursts/min produced by CPT) and small sympathetic reserve (ie, increases in MSNA ≤5 bursts/min produced by CPT). Grouped values are mean±SE. Comparisons were made with unpaired *t* tests. *$P<0.05$, subjects with large vs small changes in MSNA.

available for vasoconstriction and may determine which individuals will develop orthostatic intolerance during hypovolemic conditions, such as bed rest or space flight.

Individual Variability in Orthostatic Intolerance

The individual variability in the development of orthostatic intolerance after conditions such as space flight and bed rest is large, but the underlying mechanisms are unclear. Although certain individual physical and physiological factors (ie, height and resting arterial pressure), and physiological changes induced by simulated or real microgravity (ie, hypovolemia, changes in cardiac distensibility, and cardiovascular regulation by the autonomic nervous system) have been proposed to contribute to the occurrence of orthostatic intolerance,[25–27] no predicting factor has been identified.

The susceptibility to orthostatic intolerance differs among astronauts returning to Earth, and some differences have been found between those who did and did not complete a 10-minute stand test after spaceflight.[28,29] For instance, it was observed that the postural vasoconstrictor response was significantly smaller in astronauts who could not complete the stand test than in those who could.[29] Similarly, the occurrence of orthostatic intolerance after bed rest varies from individual to individual and has been reported to be associated with a lack of augmentation of the increase of TPR during orthostatic stress.[30] Additionally, diminished vascular resistance responses during orthostatic challenges have been shown in many patients with neurally mediated syncope well before the onset of syncope.[13,31–33] It has also been reported that administration of midodrine (α_1-agonist drug) at the end of bed rest significantly ameliorated the excessive decreases in blood pressure and presyncope during a provocative tilt test[34] and that enhancement of sympathetic tone by yohimbine (an α_2-antagonist) markedly improved orthostatic tolerance in patients with neurally mediated syncope.[35] Taken together, these results indicate that vasoconstrictor capacity may be a contributor to the individual variability in orthostatic intolerance.

Vasoconstrictor Reserve and Orthostatic Tolerance

The present study is the first to demonstrate directly a clear link between the vasoconstrictor reserve and orthostatic tolerance in healthy individuals. Our data showing a significant positive correlation between changes in MSNA and TPR produced by CPT in the upright position and time to presyncope during the orthostatic tolerance test support the assumptions that each human individual may have an intrinsic, limited reserve for sympathetically mediated vasoconstriction and that vasoconstrictor reserve could affect the maintenance of orthostatic tolerance.

The mechanisms for the individual differences in vasoconstrictor reserve are unknown; however, factors such as genetic influence or physical fitness may have to be considered. Wallin et al[36] demonstrated that the strength of sympathetic outflow to muscle is controlled genetically in humans, which may contribute to the heritability of blood pressure both in normotensive and hypertensive individuals. Interindividual differences in resting MSNA are highly reproducible over a long time[37,38]; such differences have been proposed to be

associated with the individual variability in the number of active vasoconstrictor neurons.[39] It was reported that initial MSNA levels could influence the magnitude of sympathetic responses to orthostatic challenges,[40,41] which suggests that the potential for maximal sympathoexcitation diminishes with higher resting activity. A "ceiling effect" may be an explanation, namely, sympathetic activity simply cannot increase further. Moreover, high levels of sympathetic activity may completely saturate postsynaptic adrenergic receptors, leading to maximal levels of smooth muscle constriction. Further increases in sympathetic activity under such circumstances might not result in more vasoconstriction.

Physical fitness may be another factor underlying the individual differences in vasoconstrictor reserve. It was found that exercise training in initially sedentary healthy individuals decreased resting arterial pressure and TPR.[42] However, the decreases in BP and TPR were proposed to be due to a decrease in resting renal but not cardiac sympathetic activity and MSNA.[43,44] On the other hand, muscular vasodilatory capacity was found to increase after exercise training,[45] which might result in an increase in vasoconstrictor reserve. Human vasoconstrictor reserve, particularly during exercise, is complex, because it is dependent not only on the amount of available sympathetic vasoconstrictor activity but also on the amount of competing vasodilator activity that is directed to vascular beds.[14]

Assessment of Vasoconstrictor Reserve by CPT

The CPT has been used as a nonspecific and strong stimulus to sympathetic neural outflow in humans. It evokes remarkable increases in BP and MSNA with no significant changes in HR.[46,47] The reflex pathway to activate MSNA may originate from cold nociceptors in the skin that conduct afferent signals by unmyelinated C-fibers, and the pathway may involve a central vasomotor center that serves to regulate MSNA.[47,48] It is independent of the baroreflex and is used to test the efferent limb of the sympathetic arc.[49]

Whether activation of the skin's cold nociceptors by the CPT can interact with the baroreflexes activated by postural changes in humans has not been determined with certainty; however, additive rather than potentiating effects on sympathetic activity were found during LBNP in combination with the CPT.[50] Moreover, cardiovascular responses to combined CPT and static exercise summed additively,[51] which indicates independence of sympathetic excitation by the baroreflex and somatic pressor reflex mechanisms. Nevertheless, we cannot exclude completely the possibility that activation of the skin's cold nociceptors attenuates sympathetic excitation by the baroreflexes, because we noticed that MSNA was slightly suppressed by the CPT during HUT in some subjects, especially in the hypovolemic condition. Additionally, we recognize that application of the CPT in upright tilt may not have elicited maximal sympathetic excitation and vasoconstriction in all subjects in the present study; however, by assessing the changes in MSNA and TPR produced by the CPT in upright tilt, it is highly likely that information on vasoconstrictor reserve could be gained.

In summary, the present study demonstrates that each human may have an intrinsic, limited reserve for sympathet-

ically mediated vasoconstriction, and the individual variability in orthostatic tolerance is dependent on the degree of neural and vasomotor reserve available for vasoconstriction. Our results suggest that vasoconstrictor capacity may be a contributor to orthostatic intolerance in humans. It is likely that vasoconstrictor reserve is one of the mechanisms underlying individual variability in orthostatic tolerance.

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