Low LBNP tolerance in men is associated with attenuated activation of the renin-angiotensin system

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Greenleaf, John E., Trine Welløw Petersen, Anders Gabrielsen, Bettina Pump, Peter Bie, Niels Juel Christiansen, Jørgen Warberg, Regitze Videbaek, Shawn R. Simonson, and Peter Norsk. Low LBNP tolerance in men is associated with attenuated activation of the renin-angiotensin system. Am J Physiol Regulatory Integrative Comp Physiol 279: R822–R829, 2000.—Plasma vasoactive hormone concentrations [epinephrine (pEpi), norepinephrine (pNE), ANG II (pANG II), vasopressin (pVP), endothelin-1 (pET-1)] and plasma renin activity (pRA) were measured periodically and compared during lower body negative pressure (LBNP) to test the hypothesis that responsiveness of the renin-angiotensin system, the latter being one of the most powerful vasoconstrictors in the body, is of major importance for LBNP tolerance. Healthy men on a controlled diet (2,822 cal/day, 2 mmol/kg·day NaCl) were exposed to 30 min of LBNP from –15 to –50 mmHg. LBNP was uneventful for seven men [25 ± 2 yr, high-tolerance (HiTol) group], but eight men [26 ± 3 yr, low-tolerance (LoTol) group] reached presyncope after 11 ± 1 min [P < 0.001, low-tolerance (LoTol) group]. Mean arterial pressure (MAP) did not change measurably, but central venous pressure and left atrial diameter decreased similarly in both groups (5–6 mmHg, by ∼30%, P < 0.05). Control (0 mmHg LBNP) hormone concentrations were similar between groups, however, pRA differed between them (LoTol 0.6 ± 0.1, HiTol 1.2 ± 0.1 ng ANG I·ml⁻¹·h⁻¹, P < 0.05). LBNP increased (P < 0.05) pRA and pANG II, respectively, more in the HiTol group (9.9 ± 2.2 ng ANG I·ml⁻¹·h⁻¹ and 58 ± 12 pg/ml) than in LoTol subjects (4.3 ± 0.9 ng ANG I·ml⁻¹·h⁻¹ and 28 ± 6 pg/ml). In contrast, the increase in pVP was higher (P < 0.05) in the LoTol than in the HiTol group. The increases (P < 0.05) for pNE were nonsignificant between groups, and pET-1 remained unchanged. Thus there may be a causal relationship between attenuated activation of pRA and pANG II and presyncope, with pVP being a possible cofactor. Measurement of resting pRA may be of predictive value for those with lower hypotensive tolerance.

The mechanism for reduction of cerebral blood pressure resulting in syncope (fainting) in humans is not clear.

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for differentiating men with lower hypotensive tolerances.

METHODS

Fifteen healthy, nonsmoking, male volunteers were selected as test subjects after passing a medical examination and giving informed consent. After LBNP testing was completed, they were allocated into two groups: those who tolerated 20 min of 50 mmHg LBNP (HiTol, n = 7, 25 ± 2 yr (SD), 184 ± 11 cm height, 78.2 ± 15.1 kg weight, and 2.01 ± 0.24 m² surface area) and those who did not (LoTol, n = 8, 26 ± 3 yr, 185 ± 6 cm height, 81.8 ± 8.7 kg weight, and 2.05 ± 0.12 m² surface area). This study was approved by the Regional Scientific Ethics Committee of Copenhagen and Frederiksberg (KF 01–299/96) and performed according to the Declaration of Helsinki.

All subjects consumed a controlled daily diet for 3 days before testing that consisted of normal food (bread, meat, fruit, vegetables, milk, etc.) containing 2,822 kcal/day (16% protein, 55% carbohydrate, 29% fat). Dietary NaCl content was 2 mmol·kg⁻¹·day⁻¹, i.e., 156 ± 29 (SD) mmol Na⁺/day (HiTol) and 161 ± 15 mmol Na⁺/day (LoTol). Mean (±SE) respective urinary excretion rates in the HiTol and LoTol groups were 1.8 ± 0.4 and 1.7 ± 0.4 ml min⁻¹·day⁻¹ [not significant (NS)], and urinary Na⁺ excretions were 135 ± 24 and 135 ± 14 mmol/day (8.6 ± 1.2 and 8.0 ± 0.8 g Na⁺/day, NS).

The men slept in the laboratory the night before testing and consumed no food or fluid after 2100 from the previous day to experiment termination. They were awakened at 0745, urinated to close their 24-h sample, were weighed, and consumed no food or fluid after 2100 from the previous day to experiment termination. They were awakened at 0800 when the central venous pressure (CVP) catheter was inserted and electrocardiogram electrodes applied. The protocol for the 165-min ambient control period was 60 min rest supine with the last 15 min for plasma volume (PV) measurement, then 60 min sitting (last 15 min for PV) and a final 45 min supine to determine that PV increases from sitting to supine (Fig. 1). Changing body position required ~2 min. This 165-min ambient control period was followed by the LBNP period (supine): 10 min at ambient pressure, 10 min at −15 mmHg, 20 min at −50 mmHg or until onset of presyncopal signs or symptoms (point of intolerance), and finally 10 min of recovery at ambient pressure. Presyncopal signs and symptoms included stomach awareness, nausea, sweating, narrowing of vision, or dizziness or, if those did not occur, a rapid drop in mean arterial pressure and/or bradycardia. Time (min) on the figures indicated the start of the 3-min interval when blood sampling commenced. The final −50-mmHg blood sample was taken as close as possible to the onset of presyncopal signs or symptoms. Mean (±SE) ambient dry-bulb temperature was 23.6 ± 1.3°C, and relative humidity was 38 ± 4%.

The integrated heart rate was displayed on a Diacor monitor (model DS 521, Simonsen and Weel, Copenhagen, Denmark) and was counted manually from a three-lead electrocardiogram. Arm cuff blood pressures were displayed on a Protocol Systems monitor (model Proapq 102, Beaverton, OR), but PP and MAP [1/3 {systolic blood pressure (SBP) – diastolic blood pressure (DBP)} + DBP] pressures were calculated manually from a Finapres Monitor trace (model Ohmeda 2300, Englewood, CO) with the sensor positioned on the mid- and distal phalanx of the right middle or third finger. A CVP catheter assembly (16 gauge, 1.7 mm ID with a 14-gauge injection catheter, model Cavafix Certo, B. Braun, Melsungen, Germany) was inserted via a right antecubital vein. Another catheter (18 gauge, model Venflon 2, BOC Ohmeda AB, Helsingborg, Sweden) was inserted into a left forearm vein for injection of Evans blue dye (Pharmacor Hopital E. Herriot, Lyon, France). Heart rate, systemic (Propac) blood pressures, and CVP data were displayed and recorded continuously on a Gould (model V1000, Ballaïnvilliers, France) oscilloscope and model ES1000 strip-chart recorder, respectively. Heart images were displayed on an echocardiograph (model SSD-500, Aloka, Tokyo, Japan), recorded on a Sony (model SVO 9500 MPD) videocassette recorder, and printed for analysis later.

The CVP catheter was advanced to an intrathoracic vein near the superior vena cava, where its position was verified by the characteristic waveform and responses to respiratory maneuvers. The subject’s arms were supported horizontally at heart level, and the electronically integrated pressure trace was calibrated frequently. Pressure calibration levels were determined manually with a water column.

Mean end-expiratory left atrial diameter (LAD) measurements, obtained at 3-min intervals during the LBNP period from 3 M-mode prints, were taken by echocardiography from the parasternal long-axis view. The LAD were measured blind (3).

The two PV determinations required 10 3-ml blood samples (30 ml) and four 2-ml samples (8 ml) for hematocrit (Hct) plus a maximum of 14 20-ml samples (280 ml) for pRA and for plasma vasoactive hormone concentrations [epinephrine (pEpi), norepinephrine (pNE), ANG II (pANG II), and vasopressin (pVP)] analyses (at 3-min intervals) and five 5-ml samples for plasma vasoactive hormone concentration of endothelin-1 (pET-1, 25 ml at 10-min intervals) during the LBNP period.

Fig. 1. Experimental protocol. PV, plasma volume; Hct, hematocrit; B, blood sample; D, diameter of left atrium; LBNP, lower body negative pressure.
depending on the onset of presyncopal signs or symptoms (when the experiment was terminated; Fig. 1). Maximal blood volume withdrawn was 3-4 ml/experiment; of that, the discarded presample dead-space volume was 42 ml, whereas that from the Evans blue test was reinjected.

The blood was transferred to chilled polyethylene tubes containing appropriate anticoagulants and buffers. For p<sub>PEP</sub> and p<sub>NE</sub>, the tubes contained 20 μl/ml of blood with 0.195 M reduced glutathione and 0.250 M EGTA adjusted with NaOH to pH range 6–7. For p<sub>VP</sub>, p<sub>ANG II</sub>, and p<sub>ET-1</sub>, the tubes contained 25 μl EDTA and 2,700 KIU of Trasylol R, i.e., aprotinin (Novo Nordisk, Bagsvaerd, Denmark). The tubes were oscillated gently, centrifuged at 1,500 g for 10 min at 4°C, and stored at −18°C for batch analysis.

Albumin space was determined after 45 min rest supine and sitting with intravenous injection of Evans blue dye via the forearm catheter in eight subjects (5 HiTol and 3 LoTol group). The central venous catheter allowed for a 3-ml preinjection blood sample taken at −1 min and postinjection samples at 5, 7, 10, and 15 min into tubes containing 12.5 IU heparin/ml. Plasma dye concentration was measured on each sample at 620 and 740 nm (Hitachi, model U-1000 spectrophotometer) before each injection of dye and also in the postinjection samples, so extraction was unnecessary. Quadruplicate microhematocrit tubes were centrifuged at 12,000 g for 5 min in a Struers Kebo model 3K10 centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany) and corrected with a plasma centrifugation trapping factor of 0.96 and body-venous F-cell factor of 0.91. Plasma and blood volumes were measured and calculated (4).

p<sub>ET-1</sub> was measured by radioimmunoassay (2) with plasma extracted by Sep-Pak C<sub>18</sub> columns (Waters, Milford, MA) preconditioned sequentially with 5 ml each of 4% acetic acid in 96% ethanol, 100% methanol, water, and 4% acetic acid. Two milliliters of plasma acidified with 6 ml of 4% acetic acid were run through the columns that were then washed with 5 ml of water. The peptide was eluted with 3 ml of 4% acetic acid in 96% ethanol into minisorp R tubes (Nunc, Roskilde, Denmark) containing 10 μl (0.1%) of Triton X-100. The air-dried eluate was adjusted to pH 7.4 with 550 μl of assay buffer (0.01 M phosphate buffer with 0.01 M K<sub>2</sub>EDTA and 0.001 M Na<sub>2</sub>EDTA and 1.0 mg/ml of human serum albumin) (Behringwerke, Marburg, Germany). Then 200 μl each of test sample and standard were incubated for 24 h with 100 μl of antiserum RAS6901 (Peninsula Laboratories Europe, St. Helens, UK). Then, 50 μl (6,000 disintegrations/min) of 12<sup>5</sup>I labeled ET-1 (New England Nuclear Life Sciences Products, Boston, MA) were added, and incubation was continued for an additional 24 h. Bound was separated from free antigen with 1,050 μl of a charcoal-plasma suspension (10.8 g charcoal and 60 μl of plasma in 300 μl of buffer). After centrifugation, the supernatant radioactivity was measured. The detection limit of the assay was <0.6 pg/tube, and the extraction recovery of unlabeled ET-1 was 100% from plasma. Interassay assay was 10% at an ET-1 concentration of 6 pg/ml.

p<sub>E</sub> and p<sub>NE</sub> were measured with a radioenzymatic assay (14). Plasma was precipitated with an equal volume of perchloric acid, and 100 μl of the supernatant were incubated with the enzyme carboxy-O-methyl-transferase. After incubation, unlabeled metanephrine and normetanephrine were added to the supernatant, and samples were extracted with an isooamylalcohol-toluene mixture. These extracted samples were acidified with HCl, and 120 μl were injected into an HPLC (Waters model 600) where a 120×3 mm Nucleosil C<sub>18</sub> reverse-phase column separated the metanephrine and normetanephrine. The mobile phase consisted of dilute phosphoric acid (30 mM, pH 1.8) with 2% methanol added before use. The cold metanephrines were measured at 276 nm (ultraviolet) to ensure that the labeled samples were collected at appropriate elution times (for example, at 2.6 min for normetanephrine and 4.0 min for metanephrine). The eluate was collected in 1.0-min fractions centered on those intervals. There was no crossover between the two peaks. Then, 3H-labeled metanephrine and normetanephrine were oxidized to vanillin and counted with liquid scintillation spectrometry. Intra-assay CV for normal, basal levels of epinephrine and norepinephrine were 8% and 6%, respectively; corresponding interassay CV were 11% and 7%, respectively. Intra-assay sensitivity (3 x standard deviation of the blank) was 0.3 pg/assay for epinephrine and 0.5 pg/assay for norepinephrine; corresponding inter-assay sensitivity was 0.5 pg/assay for both variables.

Statistical analyses. Anthropometric, environmental, dietary control, and LBNP tolerance data were analyzed with the t-statistic for independent groups (HP-65, Stat-Pac 1 no. 1–30A, Hewlett-Packard, Cupertino, CA). The difference between PV in the supine and sitting positions was determined with the paired t-test (HP-65, no. 1–29A). Remaining data from the two groups over time were analyzed first with one-way ANOVA (SPSS 7.5 for Windows, SPSS, 1996, Chicago, IL). Because of the inconsistent experiment termina-
PRESYNCOPEAL VASOACTIVE HORMONAL RESPONSES

Table 1. Mean systolic and diastolic blood pressures during the control, LBNP, and recovery periods for the higher and lower tolerance groups

<table>
<thead>
<tr>
<th></th>
<th>Control (0 mmHg)</th>
<th>15 mmHg</th>
<th>50 mmHg</th>
<th>Recovery (0 mmHg)</th>
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<td></td>
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<td><strong>Higher Tolerance</strong></td>
<td></td>
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<td>Systolic pressure</td>
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<tr>
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LBNP, lower body negative pressure. *P < 0.05 from mean control value. n, No. of subjects.

tion times in the LoTol group, subsequent differences between groups were also determined with the Kruskal-Wallis H-test (15), a nonparametric analog of one-way ANOVA. All presyncopal data through 12 min were included in the analysis; data for the one subject at 15 min were omitted. Significant data indicated in the figures were significant by both ANOVA and the Kruskal-Wallis tests. An intercorrelation matrix (Pearson-product moment, SPSS 7.5 for Windows) was calculated with cardiovascular and hormonal variables. The null hypothesis was rejected when P < 0.05, and nonsignificant differences were denoted NS.

RESULTS

LBNP tolerance. All HiTol subjects completed 20 min of −50-mmHg pressure with no adverse signs, symptoms, or discontinuities in their cardiovascular or hormonal data (Figs. 2 and 3). The LoTol group’s intolerance ranged from 6 to 15 min (mean = 11 ± 1 min, t = 9.01, P < 0.001). Numbers of subjects at each intolerance time at −50 mmHg were as follows: 6 min for all 8 subjects, 9 min for 7 subjects, 12 min for 6 subjects, and 15 min for 1 subject. The mean values at 18 min in the figures are their final intolerance levels irrespective of time.

Plasma and blood volumes. PV data from the two groups (n = 8) were not significantly different, so they were combined for analysis. Mean (±SE) PV in the sitting position (3,557 ± 126 ml, 42 ml/kg) was increased in the supine position to 3,911 ± 153 ml (10.0%, t = 5.01, P < 0.001); corresponding calculated blood volumes increased from 5,975 ± 229 ml (71 ml/kg) sitting to 6,403 ± 259 ml (7.2%, t = 4.18, P < 0.002) supine.

Cardiovascular responses. After 60 min supine, there were no significant differences in heart rate (range 52 ± 3 to 60 ± 4 beats/min) or in SBP (range 116 ± 3 to 127 ± 3 mmHg), DBP (range 72 ± 2 to 77 ± 2 mmHg), or PP (range 40 ± 3 to 50 ± 2 mmHg) over time or between groups during the supine 10-min control period and the ensuing 10 min of 15 mmHg LBNP (Table 1, Fig. 2). Onset of −50 mmHg by minute 3 resulted in increases (P < 0.05) in heart rate in both groups of 76 ± 5 to 97 ± 6 beats/min, no significant changes in SBP (121 to 116 mmHg) or DBP (76 to 79 mmHg), but PP decreased (P < 0.05) in both groups. From 9 to 12 min at −50 mmHg, there were no significant changes in heart rate or in SBP, DBP, or PP in either group. Essentially all SBP at −50 mmHg were decreased from their respective mean control levels, whereas DBPs were not (Table 1).

MAP was not different between groups or over time during the control, LBNP, or recovery periods, but it tended to decrease (NS) in the LoTol group after 6 min at −50 mmHg (Fig. 2). CVP was sensitive to progressive negative pressure, but there was no significant difference in CVP between groups during the entire experiment (Fig. 2). CVP, unchanged (range 5.4–6.9 mmHg) during the control period, decreased significantly to 3.3–3.9 mmHg at −15 mmHg and further to 0.4–1.2 mmHg at −50 mmHg and had not increased to control levels by 5 min of recovery. Like CVP, the LAD was also sensitive to the progressive negative pressure (Fig. 2), but the LoTol group’s LAD were significantly greater than those for the HiTol group in the control, −15 mmHg, and recovery periods, but not during −50 mmHg. From the 9 min control level to 12 min at −50 mmHg, the LoTol group’s LAD decreased from 37 to 25 mm (by 32%, P < 0.05); that in the HiTol group de-
creased similarly from 30 to 21 mm (by 30%, $P < 0.05$), and both groups’ LAD had recovered by 5 min.

**Plasma enzyme-hormonal responses.** There were no statistically significant differences in plasma endothelin-1, a potent vasoconstrictor, between groups or over time during control through recovery: mean ($\pm SE$) values ranged from 3.2 $\pm$ 0.4 to 4.1 $\pm$ 0.3 pg/ml.

With the exception of similar control levels of $p_{\text{ANG II}}$ in both groups and its significant increase in the HiTol group during $-15$ mmHg, the responses of $p_{\text{RA}}$ and $p_{\text{ANG II}}$ were remarkably similar during $-50$ mmHg and recovery (Fig. 3). The HiTol group’s increases in $p_{\text{RA}}$ and $p_{\text{ANG II}}$ from control 9 min to $-50$ mmHg at 18 min (intolerance level) were $-8.0$- and $7.7$-fold, respectively, whereas those for LoTol were $-6.9$- and $6.1$-fold, respectively. Mean LoTol group tolerance ($11 \pm 1$ min) was reached at lower ($P < 0.05$) levels of $p_{\text{RA}}$ and $p_{\text{ANG II}}$ in the control and all subsequent periods. Higher tolerance individuals could be characterized by their higher ($P < 0.05$) resting levels of $p_{\text{RA}}$.

$p_{\text{VP}}$ was not significantly different between groups or over time in the control periods (range 0.4 $\pm$ 0.1 to 0.7 $\pm$ 0.3 pg/ml) or between groups during $-15$ mmHg (Fig. 2). But the important differences between groups and over time occurred during $-50$ mmHg, where $p_{\text{VP}}$ increased more slowly but significantly in the HiTol group from $1.0 \pm 0.3$ (at 9 min $-15$ mmHg) to reach $13.0 \pm 4.8$ pg/ml [change ($\Delta$) = $12.0$ pg/ml, $P < 0.05$] at 18 min, but it increased more quickly and significantly in the LoTol group from $0.9 \pm 0.2$ to $54.7 \pm 18.3$ pg/ml ($\Delta = 53.8$ pg/ml, $P < 0.05$) at 18 min (mean intolerance). Vasopressin in the LoTol group at 12 min and $-50$ mmHg was probably higher than that in the HiTol group because of impending onset of presyncopal signs and symptoms. One LoTol group subject’s $p_{\text{VP}}$ even reached 168.8 pg/ml at intolerance at 9 min and $-50$ mmHg. Thus compared with HiTol, accentuated $p_{\text{VP}}$ responses were found in the LoTol group in contrast to the attenuated responses of $p_{\text{RA}}$ and $p_{\text{ANG II}}$.

$p_{\text{Epi}}$ was not significantly different between groups in the control, $-15$ and $-50$ mmHg, or recovery periods (Table 2). However, from control, it was significantly increased over time only at $-50$ mmHg in HiTol and at $-15$ and $-50$ mmHg in the LoTol group. On the other hand, the $p_{\text{NE}}$ concentration was more sensitive, whereas the mean values of the two groups were similar in all periods, it increased significantly above control levels in both groups during $-15$ and $-50$ mmHg and did not recover until 10 min in the LoTol group (Fig. 3). The $p_{\text{NE}}$ response pattern during $-50$ mmHg and recovery was similar to that of $p_{\text{VP}}$; although the $p_{\text{NE}}$ maximum response in the LoTol group occurred at 9 min and $-50$ mmHg, its maximal $p_{\text{VP}}$ response occurred at intolerance.

Because of the different scales for $p_{\text{RA}}, p_{\text{ANG II}}, p_{\text{VP}}$, and $p_{\text{NE}}$ on the y-axis in Fig. 3, it is difficult to determine possible onset sequences for a presyncopal enzyme-hormonal cascade, especially $p_{\text{ANG II}}$ and $p_{\text{NE}}$ at $-15$ mmHg, where there was significantly increased activity even when heart rate and various blood pressures were unchanged. When these combined hormonal responses from all subjects at 3, 6, and 9 min during $-15$ mmHg were expressed as respective percent changes in those intervals, there does not seem to be a general sequence of onset beginning with catecholamines and progressing to the RAA system and on
to p vp (18). In fact, the p vp concentrations had the greater ranges and variability (±SD) of percent changes when compared with the other hormonal responses. When these data were ranked according to the upper end of their respective ranges (±SD), we observed a different order beginning with p vp and ending with p RA: p vp (−10.4 ± 19.0 to 102.9 ± 105.8), p Epi (−12.3 ± 27.2 to 42.7 ± 66.3), p NE (−2.4 ± 16.6 to 37.2 ± 32.6), p ANG II (0.0 ± 17.4 to 24.2 ± 47.2), and p RA (0.2 ± 19.3 to 13.4 ± 25.7%).

**DISCUSSION**

Our protocol was designed to prolong the presyncopal period to better delineate vasoactive enzyme-hormonal activation by using an initial negative pressure of only 15 mmHg for 10 min and progressing to 50 mmHg for 20 min or until onset of presyncopal responses. This protocol was sufficient to allocate otherwise unresponsive men with “normal” tolerance into approximately equal higher and lower tolerance groups where there appears to be a wide range of normal LBNP tolerance from 16 min to more than 30 min. None of the subjects was aware of any abnormal syncopal responses in their daily lives.

It is clear that significant hormonal activation occurred in both groups at −15 mmHg when heart rate, PP, and MAP were unresponsive. Our data indicated the onset sequence was p vp → p Epi → p NE → (p RA → p ANG II) in the LoTol group and essentially the same in the HiTol group [p vp → p NE → p Epi (→ p RA → p ANG II)], with the RAA system at the terminal end in both. The activation patterns of p RA and p ANG II were also similar in both tolerance groups at −50 mmHg (with due regard for the large difference in their y-axis scales in Fig. 3), i.e., they increased progressively during both lower and especially the higher levels of LBNP.

This RAA system response was probably not due to large variations in plasma cations, because the subjects were consuming the same sodium intake per kilogram of body weight coupled with similar urinary Na⁺ excretions before the experiment. Also, plasma cation and osmotic concentrations remain within normal control limits during the hypotensive hypovolemia that occurs during 70° head-up tilt (6). Controlled sodium intake is important because it controls the extracellular fluid volume and renal sodium excretion (including distal tubular delivery) and because of its close interaction with control of blood pressure; e.g., exaggerated symptoms of hypotension are attenuated in...
orthostatically intolerant patients on a high-sodium diet (20).

$\text{pRA}$ in the LoTol group was significantly lower than that in the HiTol group in the control period and throughout LBNP. Jacob et al. (11) suggested that reduced $\text{pRA}$, possibly from defective sympathetic activation of the kidney, could have facilitated fainting in chronically orthostatically intolerant patients via hypovolemia. They found a significant correlation ($r = 0.84$) between $\text{pRA}$ and blood volume. It is well known that total body dehydration and hypovolemia reduce tilt tolerance (8) and accentuate $\text{pVA}$, $\text{pRA}$, and plasma aldosterone responses during tilt (7). However, the differences in LAD between the two groups and different LADs imply low compliance in the low-pressure system. Even though the LoTol group had a large pVP response was induced by a sinoaortic baroreceptor response or vice versa. The similar significant rise in $\text{pNE}$ in both groups might be an early stimulus that signals the possible impending vasopressor reaction. Thus increased sympathetic activity rather than the attenuated response to secrete more ANG II may have been a major initiating stimulus that resulted in the hypotensive intolerance. If the LoTol group was more predisposed to the vasopressor syncope by the increased sympathetic activity that resulted in a strong vagal stimulus, this stimulus could have inhibited RAA system activity. This explanation implies that the mechanism of the intolerance was a graded response in subjects of varying intolerance thresholds and should function similarly in all subjects at their varying presyncopal points. The question arises if the depressed $\text{pRA}$ and/or $\text{pANG II}$ in the LoTol group was increased to equal that in the HiTol group, would the LoTol group’s tolerance be increased significantly? If so, it would confirm the pivotal role of the RAA system in this hypotensive mechanism.

Our results confirm those of Mark et al. (17) who first reported that $-40 \text{mmHg LBNP}$ decreased CVP and arterial PP which attenuated, respectively, both low- and high-pressure baroreceptor inhibition, i.e., reacti-

### Table 2. Mean plasma epinephrine (ng/ml) values during the control, LBNP, and recovery periods for the higher and lower tolerance groups

<table>
<thead>
<tr>
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<th>Control (0 mmHg)</th>
<th>LBNP 15 mmHg</th>
<th>LBNP 50 mmHg</th>
<th>Recovery (0 mmHg)</th>
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<td>3 6 9 12</td>
<td>5 10</td>
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$P < 0.05$ from mean control value. $n$, No. of subjects.
vated baroreceptor function. But their $p_{\text{RA}}$ increased significantly (to $7.4 \pm 1.4 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$) only when both low- and high-pressure inhibition occurred and not with low pressure ($-10$ to $-20 \text{ mmHg LBNP}$) inhibition alone. This pressure was comparable with the $-15 \text{ mmHg LBNP}$ used in the present study, in which $p_{\text{RA}}$ was also unchanged in both groups, whereas $p_{\text{ANG II}}$ was increased significantly in the HiTol group but was unchanged (inhibited?) in the LoTol group due to presumably reactivated low-pressure baroreceptors from the LBNP.

Norsk et al. (18) indicated that $p_{\text{VP}}$ during LBNP responds more to narrowing of the PP than to reduction of CVP. Results from the present study support this conclusion from the significant correlation between PP and $p_{\text{VP}}$ ($r = -0.56$, $P < 0.01$) in the HiTol group, but not in the LoTol group ($r = -0.12$, NS). We found similar results in the RAA system only in our HiTol group between PP vs. $p_{\text{RA}}$ and PP vs. $p_{\text{ANG II}}$: the $r$ was $-0.55$ and $-0.56$ (both $P < 0.01$), respectively. However, the respective $r$ in our LoTol group were $-0.36$ and $-0.24$ (both NS). These findings strengthen the high-pressure hypothesis regarding the hormonal release action on PP, but it may not apply to lower-tolerance subjects.

It is concluded that the response of the renin-angiotensin system appears to be linked inversely to the occurrence of presyncopal symptoms, and measurements of resting $p_{\text{RA}}$ may have predictive value for those with lower hypotensive tolerance.

**Perspectives**

If sufficiently confirmed, one major practical application of these findings concerning attenuation of the increase in $p_{\text{RA}}$ and $p_{\text{ANG II}}$ during hypotensive stress might be to provide a method for prognosticating lower orthostatic tolerance in normal, healthy people. An increase of hypotensive tolerance by infusing ANG II during LBNP would help to confirm this hypothesis.

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