Orthostatic Stimuli Rapidly Change Plasma Adrenomedullin in Humans

Andreas Rössler, Zoltán László, Bernd Haditsch, Helmut G. Hinghofer-Szalkay

Abstract—The aim of this study was to evaluate the effect of orthostasis on the time course of plasma adrenomedullin concentration. On 5 different days, normotensive subjects were randomized to undergo for 30 minutes either 12°, 30°, 53°, or 70° passive head-up tilt or to remain supine. Venous blood was collected from each subject in the supine position before tilting, at 3 and 27 minutes during tilting, and at 2 and 50 minutes after orthostasis. Plasma adrenomedullin increased significantly with tilt of ≥30° in a stimulus-dependent manner. Approximately half of the increase seen at 27 minutes occurred during the first 2 minutes of upright positioning; the maximum effect with 70° tilt was +70%. Elevations in norepinephrine, epinephrine, aldosterone, plasma renin activity, vasopressin, heart rate, and mean arterial pressure were also significant. Hematocrit, blood density, plasma density, and plasma volume loss rose (P<0.05) at 53° and 70° tilt. Our results indicate that adrenomedullin may play an important role in stabilization of hemodynamics during passive orthostasis. In conclusion, plasma adrenomedullin rapidly increases with orthostatic challenge in a stimulus-dependent manner and also swiftly returns to baseline levels after the subject resumes the supine position. (Hypertension. 1999;34:1147-1151.)

Key Words: tilt, head-up □ adrenomedullin □ barorereflex □ volume, plasma □ catecholamines

The 52–amino acid peptide adrenomedullin (ADM) is a potent vasorelaxant1 and natriuretic2 peptide. The strong relationship between plasma ADM and vascular tone functions in water homeostasis by increasing the glomerular filtration rate and natriuresis while lessening renal vascular resistance.2,3 Additionally, ADM might function as an endocrine factor for vascular smooth muscle.4

Until now, no data were available on changes in plasma ADM levels induced by orthostasis, which redistributes blood from central- to lower-body vascular beds and unloads cardiopulmonary receptors5; this causes constriction of resistance and capacitance vessels,6 in part through the action of vasopressin, angiotensin, and endothelin-1.7,8

The present investigation was designed to test the hypothesis that plasma ADM is influenced by head-up tilting (HUT) in normotensive, euhydrated humans; to study the time course of ADM during and after HUT; and to quantify effects in relation to the intensity of orthostatic challenge. As ancillary information, hemodynamic and other endocrine as well as blood volume indicators were determined. The present study shows that ADM responds quickly and in a dose-dependent manner to HUT, and its concentration rapidly declines afterward.

Subjects
Investigations were performed on 8 healthy, nonsmoking male volunteers (age, 24 to 38 years; weight, 62 to 75 kg; height, 170 to 180 cm; body surface area, 1.72 to 1.93 m²; body fat mass as estimated by bioelectrical impedance analysis single-frequency bio-impedance measurement, 13.6% to 17.6%; values are ranges) who had no history of syncope or presyncope episodes of vasovagal origin and were normotensive. Medical clearance to participate was required of all subjects and was based on medical history review, physical examination, and resting 12-lead ECG. Subjects abstained from medication, were fully informed about the purpose and nature of the experiments, and gave informed consent. The experimental protocol was approved by the local Institutional Ethics Committee.

Experimental Protocol
To compare dose-response traits in identical subjects, we used 12°, 30°, 53°, and 70° HUT (sin. 0.21, 0.50, 0.80, and 0.94, respectively). The tilt table had a footboard and chest harness, upright position was assumed within 20 s, and tilting lasted 30 minutes. All subjects underwent 5 experiments (4 HUT, 1 continuously supine [rest control]), in randomized order on different test days.

After a standardized breakfast (100 g bread with butter and jam and 200 g orange juice), each experimental session began at 8:30 AM with a 40-minute supine rest period, during which the blood-pressure cuff was positioned and the left antecubital vein was cannulated with a 17-gauge 1.4×40-mm 3-way-stopcock Teflon catheter (TriCath In, Codan Steritex). Venous blood was poured into prechilled tubes.
containing EDTA and Trasylol (aprotinin; 500 kallikrein inhibition units [KIU]/mL for the samples of plasma ADM and arginine vasopressin [AVP]) and immediately placed on ice.

The arm was positioned such that the lower arm remained near the hydrostatic indifference point at any body posture. Blood samples were taken every minute to measure blood density, plasma density, and hematocrit at the beginning and end of orthostasis and for hormone determinations 10 minutes before orthostasis (10 minutes before tilt in the supine position [baseline]), at 3 and 27 minutes of HUT, and at 2 and 50 minutes after HUT in the supine position (minutes 32 and 50).

Data from actual HUT sessions were compared with rest control (HUT0) data at identical protocol times. Plasma samples prepared by instantaneous centrifugation were frozen at -20°C for hormone determinations.

**Measurements**

Blood pressure (systolic blood pressure, mean arterial pressure, and diastolic blood pressure; in mm Hg) was determined oscillometrically (Dinamap 1846 SX, Critikon) every 20 s. Hematocrit (Hct) was determined in quadruplicate by microcentrifugation (10 minutes at 20,000 rpm) without correction for trapped plasma. Blood density (BD) and plasma density (PD) were measured at 37.00 ± 0.02°C with a high-precision mass densitometry device (model DMA 602 M, Paar KG) on 0.2-mL samples using the mechanical oscillator technique,9 in which the resonant frequency of a U-shaped glass tube is determined and converted to corresponding density values. Mass density (FD) of the fluid shifted into or from the circulating blood was calculated from corresponding PD and Hct values:

\[
FD = PD_d - \left(\frac{[Htc_d(1-Htc_d) - Htc_c(1-Htc_c)]}{Htc_d - Htc_c}\right) \times (PD_d - PD_h),
\]

where c indicates hemocoentrated; d, hemodiluted state. PD was measured in grams per liter. The volume of fluid lost to the extravascular compartment (FV) was computed from plasma density changes and expressed as percentage changes of plasma volume (PV):

\[
FV = 100 \times \left(\frac{(PD_h - PD_d)(PD_d - PD_f)}{PD_f - PD_h}\right) \times PV_d (\%PV_d).
\]

**Hormone Measurements**

Catecholamines were determined using high-pressure liquid chromatography; all other hormones were measured by radioimmunoassay. After blood samples were centrifuged at 2500 rpm at 4°C for 15 minutes, plasma was decanted and stored at -80°C to await analysis. For ADM measurement, 2 mL of plasma was extracted onto C-18 bond elute cartridges (Millipore-Waters) that had been prewashed (1% trifluoroacetic acid). Extraction efficiency was measured by addition of labeled ADM; the calculated recovery rate was 99.3%.

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For graphic presentation, data from 10 minutes before HUT of the experiment were taken as 100%, and all following data were expressed in normalized fashion. These relative values were also used to test differences between HUT and rest control. To calculate correlations between ADM and other variables from corresponding times, linear regression analysis was applied on the normalized (percentage of preHUT) data.

**Data Analysis**

Data are presented as mean±SEM unless otherwise stated. The Shapiro-Wilk W test indicated normal distribution of all data. A 1-way ANOVA for repeated measurements was used to determine the effect of time on variables and differences between test conditions. Post-hoc Student’s paired t test compared HUT and rest control data from identical protocol times. Differences were considered significant if P < 0.05 for the null hypothesis. Data analysis was performed using the Statistica software suite (version 5.0, StatSoft, Inc.).

For graphic presentation, data from 10 minutes before HUT of the experiment were taken as 100%, and all following data were expressed in normalized fashion. These relative values were also used to test differences between HUT and rest control. To calculate correlations between ADM and other variables from corresponding times, linear regression analysis was applied on the normalized (percentage of preHUT) data.

**TABLE 1. Absolute Mean Values ±SEM Before, During, and 5 Minutes After Different HUT Levels**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MAP, mm Hg</th>
<th>SP, mm Hg</th>
<th>DP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUT 0°</td>
<td>Before</td>
<td>71.9±3.8</td>
<td>106.1±3.0</td>
<td>54.8±4.3</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>70.6±3.6</td>
<td>105.0±3.2</td>
<td>53.4±4.1</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>71.0±3.8</td>
<td>105.9±3.2</td>
<td>53.5±4.3</td>
</tr>
<tr>
<td>HUT 12.5°</td>
<td>Before</td>
<td>67.0±2.9</td>
<td>100.4±2.1</td>
<td>50.3±3.4</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>67.0±3.1</td>
<td>100.3±3.0</td>
<td>50.3±3.2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>66.5±3.0</td>
<td>100.7±2.7</td>
<td>49.3±3.2</td>
</tr>
<tr>
<td>HUT 30°</td>
<td>Before</td>
<td>67.4±3.0</td>
<td>102.2±2.5</td>
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</tr>
<tr>
<td></td>
<td>During</td>
<td>69.3±3.1</td>
<td>104.0±2.9</td>
<td>52.0±3.2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>68.0±3.0</td>
<td>102.8±2.4</td>
<td>50.6±3.5</td>
</tr>
<tr>
<td>HUT 53°</td>
<td>Before</td>
<td>69.5±3.6</td>
<td>104.7±3.1</td>
<td>50.8±3.9</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>73.4±4.5</td>
<td>107.5±4.8</td>
<td>55.8±4.4</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>70.5±3.7</td>
<td>106.9±3.6</td>
<td>52.0±3.9</td>
</tr>
<tr>
<td>HUT 70°</td>
<td>Before</td>
<td>67.6±2.9</td>
<td>102.6±2.4</td>
<td>50.0±3.5</td>
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<tr>
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<td>During</td>
<td>72.6±3.9</td>
<td>104.4±4.3</td>
<td>56.7±4.0</td>
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<td></td>
<td>After</td>
<td>71.1±3.3</td>
<td>106.1±2.6</td>
<td>53.6±3.7</td>
</tr>
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</table>

MAP indicates mean arterial pressure; SP, systolic pressure; DP, diastolic pressure; and HR, heart rate.

*Significant (P < 0.05 by paired t test) vs baseline.
Results

All subjects stayed normotensive during HUT and completed the protocol (Table 1). Before HUT, systolic blood pressure averaged 103.2±1.2 and diastolic 51.2±1.7 mm Hg (average of all subjects and tilt levels pooled; n=40).

The relative changes in plasma ADM concentration during and after different angles of HUT are shown in Figure 1. The mean plasma levels of ADM before stimulus averaged 4.5±0.2 pmol/L (mean±SEM), increased during sustained tilt, and returned within 50 minutes of recovery to pretilt baseline levels. With HUT ≥30°, norepinephrine, AVP, and ADM were significantly elevated; with HUT ≥53°, aldosterone, plasma renin activity, and epinephrine were elevated. Two minutes after HUT, plasma levels of ADM and catecholamines were already lower than during tilt (Table 2). Plasma ADM and catecholamines were linearly correlated (epinephrine, R=0.902; P<0.0001; norepinephrine, R=0.921; P<0.0001) with pooled data from all levels of orthostatic challenge (Figure 2). Diastolic pressure and tilt angle were directly correlated (Table 1), as were diastolic pressure and plasma ADM (R=0.887; P<0.0001) (Figure 2). With unchanged systolic pressure, this resulted in decreased pulse pressure at HUT 53° and 70° (−4% and −10%). Heart rate increased with degree of HUT, as did BD and PD; consequently, plasma volume declined as expected (Table 3).

The density of fluid shifted (FD) was 1008.4±3.1 g/L as found in earlier tilt table experiments.9 There was a significant correlation (R=0.995) between plasma volume loss and ADM increase. 50 minutes after HUT, all variables returned to preHUT levels.

Discussion

ADM, a recently discovered multifunctional peptide with slight homology to calcitonin gene–related peptide and amylin, is conceivably involved in circulatory control as a result of its vasodilator and natriuretic activities.1,2 ADM mainly originates from the adrenal medulla, lung, and kidney, and to a lesser extent from the heart, spleen, duodenum, and submandibular glands.1,2,13 Plasma ADM levels are elevated with strenuous exercise14 and with heart failure, hypervolemia, and

<table>
<thead>
<tr>
<th>Hormone, pmol/L</th>
<th>Baseline</th>
<th>Minute 2 HUT</th>
<th>Minute 27 HUT</th>
<th>2 Minutes Post-HUT</th>
<th>30 Minutes Post-HUT</th>
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<td>Epinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rest</td>
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<td>273±29</td>
<td>256±33</td>
<td>258±31</td>
<td>257±30</td>
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<td>12°</td>
<td>255±17</td>
<td>275±30</td>
<td>281±30</td>
<td>269±27</td>
<td>285±22</td>
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<td>30°</td>
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<td>290±21</td>
<td>356±39*</td>
<td>281±44</td>
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<tr>
<td>53°</td>
<td>236±40</td>
<td>327±47*</td>
<td>367±50*</td>
<td>273±28†</td>
<td>247±43</td>
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<td>70°</td>
<td>219±17</td>
<td>320±30*</td>
<td>375±48*</td>
<td>287±23†</td>
<td>277±14*</td>
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<td>Norepinephrine</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>1425±92</td>
<td>1445±117</td>
<td>1414±81</td>
<td>1485±79</td>
<td>1485±60</td>
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<tr>
<td>12°</td>
<td>1530±185</td>
<td>1598±232</td>
<td>1759±206</td>
<td>1551±166†</td>
<td>1627±242</td>
</tr>
<tr>
<td>30°</td>
<td>1324±76</td>
<td>1633±91*</td>
<td>1858±81*</td>
<td>1500±92†</td>
<td>1351±200</td>
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<tr>
<td>53°</td>
<td>1474±196</td>
<td>2485±380*</td>
<td>2678±485*</td>
<td>1983±245†</td>
<td>1439±225</td>
</tr>
<tr>
<td>70°</td>
<td>1242±102</td>
<td>2268±231*</td>
<td>2392±199*</td>
<td>1787±133†</td>
<td>1382±149</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>5.3±0.3</td>
<td>5.4±0.4</td>
<td>5.3±0.4</td>
<td>5.2±0.4</td>
<td>4.9±0.4</td>
</tr>
<tr>
<td>12°</td>
<td>4.8±0.4</td>
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<td>5.3±0.3</td>
<td>5.0±0.4</td>
<td>4.6±0.3</td>
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<tr>
<td>30°</td>
<td>4.4±0.6</td>
<td>5.0±0.7*</td>
<td>5.4±0.7*</td>
<td>4.6±0.7</td>
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<tr>
<td>53°</td>
<td>4.8±0.6</td>
<td>5.7±0.6*</td>
<td>6.6±0.5*</td>
<td>5.5±0.5*†</td>
<td>4.8±0.6</td>
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<td>70°</td>
<td>3.3±0.3</td>
<td>4.3±0.4*</td>
<td>5.6±0.5*</td>
<td>4.3±0.4†</td>
<td>3.5±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Degrees indicate level of HUT.

*Significant (P<0.05 by paired t test) vs baseline.

†Significant (P<0.05 by paired t test) vs minute 27.

Figure 1. Time course of relative plasma ADM concentrations with 4 different angles of HUT (minutes 1 through 30) and 1 control run with supine rest in 8 normotensive test subjects. HUT12 indicates HUT at 12°; HUT30, HUT at 30°; HUT53, HUT at 53°; and HUT70, HUT at 70°.
sympathetic activation; a counterregulatory role of ADM in terms of balancing peripheral vascular resistance elevation has been proposed. To our knowledge, only 1 study has yet examined the integrated hemodynamic and ADM time course during and after orthostatic stress in healthy humans. Tilting level and duration clearly influence the degree of thoracic blood volume, atrial diameter, and central venous pressure decrease in healthy young people, with concomitant increase in plasma catecholamines, renin-angiotensin-aldosterone, and occasionally vasopressin. Our results demonstrate that orthostasis elicits stimulus-dependent ADM increase as part of a “quick humoral response,” along with epinephrine and norepinephrine. Even at 30° HUT (sin, 0.5), the effects were significant within 3 minutes. Furthermore, the post-HUT decrease of these hormones was likewise significant within 2 minutes (Table 2), which can be attributed to the short half-life of ADM of several minutes. This might indicate a symmetric function of ADM and catecholamines in terms of quick endocrine response to baroreceptor stimulation, which results in a fine-tuning of vascular diameter after postural changes.

The results of the present study complement those presented by Mallamaci et al., who did not find significant increases in ADM with tilting. Nevertheless, they also observed a rapid ADM increase within the first minutes of orthostasis with elevated levels throughout the entire HUT period. Basal vasopressin plasma levels were unusually high in their study, with a tilt-induced response 3 times higher than is usually seen with orthostasis. Thus, their subjects conceivably were dehydrated or orthostatically not fully competent, a condition likely to blunt ADM responses with HUT. Thus, our data are the first to present significant ADM effects with passive tilting in obviously euhydrated, normotensive, and orthostatic stable subjects. The observed blood pressures of our subjects are in the lower range (Table 1), which is consistent with the 40-minute supine resting preHUT period established to reach blood pressure equilibrium and with the fact that the subjects were familiar with the experimental procedure from earlier, similar experiments.

Direct interactions of ADM with other hormones and autonomous nerve have been demonstrated. ADM inhibits renal sympathetic nerve activity while increasing renin output in a paracrine fashion, which influences both renin gene expression and secretion. Furthermore, ADM triggers adrenal catecholamine release and enhances cardiac contractility via cAMP-independent mechanisms. ADM may in fact buffer the sympathetic response to tilting, thereby damping baroreflective effects and counteracting further blood pressure elevation. It remains to be clarified which of the various cardiovascular effects of ADM prevail under physiological circumstances of orthostatic challenging.

In conclusion, plasma ADM rapidly changes during and after various degrees of HUT in humans, which suggests quick and sensitive baroreceptor-driven secretion. This complements findings of previous investigations and suggests that ADM plays an important role in cardiovascular regulatory stability in concert with other hormonal mechanisms.

**Acknowledgments**

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 References
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