Adrenomedullin and Galanin Responses to Orthostasis in Older Persons

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**Key terms:** Adrenomedullin; galanin; gender differences; orthostatic challenge; elderly.

**Abstract**

**Background:** Neuroendocrine responses to orthostasis may be critical in the maintenance of mean arterial pressure in healthy individuals. A greater reduction in orthostatic tolerance with age may relate to modulation of hormonal responses such as adrenomedullin and galanin. Thus, we investigated 1) whether adrenomedullin and galanin concentrations increase during orthostatic challenge in older subjects, 2) whether adrenomedullin and galanin concentrations are higher in older females compared with older males when seated and during orthostatic challenge, and 3) whether postural changes in plasma concentrations of galanin are correlated with levels of adrenomedullin in either older females or males.

**Materials and Methods:** Subjects (n=18; 12 ♂; 55-80 years old) performed a sit-to-stand test in a 25°C sensory-minimised environment, with blood samples collected after 4 mins of being seated and then when standing. Plasma adrenomedullin and galanin concentrations were determined.

**Results:** Baseline plasma concentration of adrenomedullin (5.35 ± 0.74 (n=12, females) vs. 7.40 ± 1.06 pg/ml (n=5, males)) and galanin (64.07 ± 9.05 vs. 98.99 ± 16.90 pg/ml, respectively) did not significantly differ between genders. Furthermore, plasma adrenomedullin and galanin concentrations were not significantly affected by adoption of the upright posture in either gender, and were not correlated in females or males.

**Conclusions:** Adrenomedullin and galanin concentrations were similar between genders and did not change following adoption of the standing posture. To further clarify the roles these hormones play in orthostatic intolerance, adrenomedullin and galanin concentrations should be assessed in participants who show presyncopal symptoms during an orthostatic challenge.
Key terms: Adrenomedullin; galanin; gender differences; orthostatic challenge; elderly.

Introduction

Orthostasis challenges the cardiovascular system to maintain cardiac preload, stroke volume and mean arterial pressure [1-3]. Compensatory hormonal responses to orthostatic challenge include rapid elevation of plasma adrenaline and noradrenaline [4-8] and, after a 10- to 20-minute delay, renin-angiotensin system activation, leading to elevated plasma renin activity, angiotensin II and aldosterone [4, 7-10]. Presyncope, caused by a failure to restore cerebral perfusion, is associated with vasopressin secretion [6, 8, 9], stimulating subsequent secretion of adrenocorticotropic hormone and cortisol [7, 11].

More recently, elevated plasma levels of the 52-amino acid peptide adrenomedullin and the 30-amino acid neuropeptide galanin have been identified during orthostatic challenge [8, 9, 12]. Paradoxically adrenomedullin and galanin are both vasodilatory hormones [13, 14], and therefore reduced plasma concentrations are expected during orthostatic challenge [8]. However, recent research found plasma adrenomedullin and galanin were unchanged during orthostatic challenge in young males [7, 14], indicating the complexity of understanding hormonal regulation of blood pressure. Furthermore, we are not aware of any study that has examined the effects of orthostatic challenge on adrenomedullin in older persons.

Studying the etiology of falls in older persons is important, as un-intentional falls in the population aged over 60 costs the UK approximately £1 billion/year, with 50% and 41% of this figure accounted for by resultant hospitalisation and institutionalisation, respectively [15]. In addition, falling and fall-related injuries are more common in older females in comparison with age-matched males [16, 17]. Gender influences hormonal responses to orthostatic challenge. Hormones which are modulated by gender include catecholamines [4, 6, 18], adrenomedullin [19] and galanin [8]. Differential hormonal responses to orthostatic challenge across gender presumably relate to a modulatory effect of sex hormones [20], with assessment of such differences complicated by...
hormonal changes during the normal female menstrual cycle [21-24]. Thus, it is likely that the depletion of plasma oestradiol associated with menopause [25] will significantly modulate the hormonal responses to orthostatic challenge in women [6]. The role of neuroendocrine regulation in risk for falls and, in particular, its potential contribution to this increased risk for falls in older females, however, has not received much attention.

This study therefore investigated 1) whether plasma adrenomedullin and galanin concentrations increase from seated baseline levels during orthostatic challenge in older subjects; 2) whether plasma adrenomedullin and galanin levels are higher in older females compared with older males when seated and during orthostatic challenge; and 3) whether postural changes in plasma concentrations of galanin are correlated with levels of adrenomedullin in either older females or males.

Methods

Subjects

Eighteen (12 post-menopausal females and 6 males) age-matched non-smoking healthy volunteers (55-80 years old) gave written informed consent having been recruited from the local area by the Department of Neurology, University Hospital of Graz. Subjects had no history of alcoholism, cardiovascular disease (or prophylactic medication such as beta blockers) or mental illness. Each subject underwent a comprehensive neurological examination prior to participation.

The study received ethical approval from the Medical University of Graz Ethics Committee and was conducted in accordance with ethical principles stated in the World Medical Association Declaration of Helsinki (2013). Subjects were requested to refrain from exercise and other stressful activity for 48 hours before test sessions. In addition, subjects were requested to abstain from coffee and other stimulants for 24 hours prior to test sessions.
Study Design

This was a prospective, observational study. Reporting conforms to the STROBE statement and the broader EQUATOR guidelines [26].

After a light breakfast and urinary bladder voiding each subject performed a sit-to-stand test, during which haemodynamic parameters were measured continuously (Finometer® PRO, Finapres Medical Systems B.V., Amsterdam, Netherlands) and venous blood samples were collected once in the seated position and again when standing. The sit-to-stand test was performed between 7-10 am within a sensory-minimised environment provided by a dimly lit room with minimal ambient noise in order to negate any diurnal effects upon orthostatic and/or stress responses [27]. Room temperature was maintained at 25°C in order to negate any temperature effects upon orthostatic and/or stress responses [27].

The sit-to-stand orthostatic challenge test was employed rather than a more aggressive head-up tilt (HUT) + lower body negative pressure (LBNP) protocol in order to minimise the risk of injurious syncope in our older (albeit healthy) subjects [14]. Provision was made to terminate the protocol in the event of presyncope (defined herein as reported dizziness, nausea, pallor, stomach awareness, sweating or visual fading) during the sit-to-stand test. However, all subjects were able to successfully complete the protocol. Plasma hormone measurements were conducted in a single-blinded manner, such that subject gender and posture of each sample was unknown to the technician who performed the assays.

Experimental Protocol

An antecubital intravenous cannula was inserted into the left arm in order to facilitate direct collection of an 8 ml intravenous blood sample into a pre-chilled (4-8°C) aprotinin-/EDTA-treated tube in the 4th minute of seated rest and standing with the use of a tourniquet. In order to minimise in
vitro haemolysis, tourniquet compression time was less than 2 minutes and the collecting tube was not shaken vigorously [28, 29].

Continuous arterial blood pressure measurements, derived from a pressure trace acquired using a digital artery cuff placed on the middle finger with the hand held at heart level assisted with an arm sling, were obtained in the sitting and standing positions.

Each subject sat quietly with arms relaxed by their sides for 5 minutes, whereupon instruction was given to adopt an upright standing position for a further 6 minutes with assistance provided (if appropriate) from a researcher. During standing the subject was not supported and was instructed to keep their eyes open, maintain gaze at eye-level and not alter feet placement unless absolutely necessary. Upon completion of 6 minutes standing, a seated position was re-adopted (with assistance) for a 5-minute recovery period.

Blood Sample Analysis

Each blood sample was centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 1500 g for 15 minutes at 6°C in order to separate plasma. The plasma was stored at -70°C for subsequent hormone analysis.

Plasma adrenomedullin and galanin concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (E90220Hu and E91084Hu, Uscn Life Science Inc. Wuhan, Hubei, PRC) as the antibodies do not significantly cross-react with adrenomedullin and galanin analogues. Absorbance was measured with a Wallac 1420 VICTOR²™ Multilabel Counter (PerkinElmer, Inc., Waltham, MA, USA); the minimum detectable concentration was 1.43 pg/ml (sensitivity was defined as the apparent concentration at 2 standard deviations from the optical density counts determined from pure assay buffer) for adrenomedullin and 4.85 pg/ml for galanin. The intra-assay and inter-assay coefficients of variation were <10% and <12% respectively for both adrenomedullin and galanin.

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Statistical Analysis

Physical characteristics of age-matched female and male groups were compared using unpaired t-tests. Mean arterial pressure measurements in 10s epochs were assessed at different time points: Baseline: final 10s of sitting; during standing: 10-20 sec, 20-30 sec, 180-190 sec (to assess orthostatic hypotension risk), last 10 sec of standing (290-300 sec); and during recovery: similar epochs as in standing. Due to uneven number of males and females, mean arterial blood pressure across age-matched female and male groups were compared using Welch t-tests. Plasma hormone concentrations during the sit-to-stand tests were compared between groups using two-way analysis of variance (ANOVA) with factors of gender (male vs. female) and posture (seated vs. standing). Subsequent post-hoc multiple comparisons were performed using the Šídák method. The relationship between changes in plasma concentrations of adrenomedullin and galanin upon standing was assessed using the Pearson product-moment correlation in female and male groups independently. All statistics were performed using GraphPad Prism 6.0f (GraphPad Software Inc, La Jolla, CA, USA) with significant differences defined as a p<0.05.

Results

The female subjects were significantly shorter and lighter than the age-matched males, although body mass index (BMI) did not differ between groups (Table 1). Mean arterial pressure was significantly higher in females compared with males at baseline (111 ± 3 mmHg vs. 98 ± 4 mmHg; p = 0.0488), 190s standing (115 ± 4 mmHg vs. 103 ± 2 mmHg; p = 0.0272) and 300s recovery (114 ± 3 mmHg vs. 101 ± 4 mmHg; p = 0.0178), but no gender difference was observed at other time epochs during the sit-to-stand test (Table 2). Mean arterial pressure was not significantly changed from baseline during the sit-to-stand test in females or males.
Blood samples from 1 male subject when standing and another both when seated and standing were haemolysed, rendering subsequent plasma hormone concentrations unreliable [29]. As such, hormone data are presented for all 12 female subjects in the seated and standing positions, but from only 5 males in the seated position and 4 when standing.

Baseline plasma adrenomedullin concentration was not significantly different in females compared with males (5.35 ± 0.74 pg/ml vs. 7.40 ± 1.06 pg/ml; p = 0.4001), and adoption of the upright posture failed to induce significant differences in either females (p = 0.9761) or males (p = 0.6665). Consequently, plasma adrenomedullin concentration was not significantly different in females compared with males (5.60 ± 1.12 pg/ml vs. 5.70 ± 1.28 pg/ml; p = 0.9979) during orthostasis. However, increased plasma adrenomedullin concentrations were observed upon standing in 7 of the 12 female subjects and in only 1 of 4 male subjects (Figure 1).

Baseline plasma galanin concentration was not significantly different in females compared with males (64.07 ± 9.05 pg/ml vs. 98.99 ± 16.90 pg/ml; p = 0.0561), and adoption of the upright posture did not cause significant changes in either females (p = 0.6992) or males (p = 0.2304). Consequently, plasma galanin concentration was not significantly different in females compared with males (55.21 ± 6.39 pg/ml vs. 68.66 ± 11.42 pg/ml; p = 0.6632) during orthostasis. However, it was observed that plasma galanin concentration decreased upon standing in some participants (in 9 of the 12 female subjects, and in 3 of 4 male subjects) (Figure 2).

Plasma galanin concentration changes were not significantly correlated with plasma adrenomedullin concentration changes in females (r = 0.0673; p = 0.8353) or in males (r = 0.8351; p = 0.1649) (Figure 3).
Discussion

The main findings of this study were that baseline plasma adrenomedullin and galanin concentrations did not differ between genders, and there were no significant changes in hormonal concentrations induced by standing in healthy older subjects. Plasma galanin concentration changes were not significantly correlated with plasma adrenomedullin concentration changes in either females or males. Mean arterial pressure was unaltered by standing in either females or males although a gender difference in mean arterial pressure was observed intermittently when seated and standing.

Adrenomedullin responses

Whilst plasma adrenomedullin has been identified as a vasodilatory peptide, some studies involving younger subjects have identified a paradoxical increase in its concentration during orthostatic challenge [8, 9, 12]. Furthermore, plasma adrenomedullin levels have also been reported to increase in proportion to the tilting level [9]. However, we found no such change in adrenomedullin levels during orthostatic challenge, in our older subjects. Our data are in agreement with other studies in younger subjects that demonstrated no significant plasma adrenomedullin changes in response to orthostatic load [7, 14, 30].

In addition, a strong correlation between plasma noradrenaline and adrenomedullin in healthy subjects during orthostatic challenge has been suggested. This suggests an important role of sympathetic nervous system activation in the regulation of adrenomedullin secretion [9]. These findings appear to point to a symmetric function of adrenomedullin and catecholamines in terms of quick endocrine responses to the baroreceptor stimulation in younger persons; this has, however, been challenged [30]. In our study, involving older subjects, increasing barosensory vessel wall stiffness and decreasing cardio-vagal autonomic control effectiveness with advancing age [31] could play important additional roles in blood pressure regulation.
Slower [volume-regulating hormone] angiotensin II production may have also regulated adrenomedullin production [8], as evidenced by concentration-dependent increases in adrenomedullin mRNA expression and adrenomedullin-secreting cell numbers following 4 hours of human aortic endothelial cell incubation with angiotensin II [20]. However, whilst plasma angiotensin II concentration was not measured in this study, the 6-minute standing period is unlikely to be of sufficient duration to induce significant plasma angiotensin II increases and thus invoke plasma adrenomedullin increases [8].

The absence of an effect of gender in our study is in contrast with recent research demonstrating a marked gender difference in plasma adrenomedullin concentration within an older age group: Kawano et al identified significantly (p<0.01) lower plasma adrenomedullin levels in females [19]. The observations of Kawano et al are in line with the suggestion that sex steroids have a regulatory role in adrenomedullin production by human endothelial cells [20, 32]. All our female subjects were post-menopausal and characteristic plasma oestradiol depletion and plasma luteinising hormones elevation at this stage of the female life cycle are associated with a higher male-like level of plasma adrenomedullin [25]. In addition, our subjects were overweight: plasma adrenomedullin positively correlates with BMI, especially in females [19].

_Galanin responses_

As with adrenomedullin, data regarding the plasma galanin response to orthostatic challenge is equivocal. We could find only 2 published studies and they reported contradictory results [8, 14]. Our data agree with the finding that there is no significant plasma galanin change during a 80° HUT + graded LBNP orthostatic challenge [14]. Significantly increased plasma galanin was observed at presyncope in response to a more aggressive short-duration 70° HUT + incrementally increased LBNP protocol [8]. As the rapid plasma galanin increase coincided with the onset of presyncope symptoms prior to haemodynamic changes, it was hypothesised by these researchers that galanin may have a protective role in orthostatic hypotension as a central and peripheral sympatholytic

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neurotransmitter, which causes presyncope and is a marker of impending cardiovascular collapse [8, 14]. However, Hinghofer-Szalkay et al also observed that the plasma galanin levels were relatively unchanged during progressive HUT + LBNP until the point of postural instability/ presyncope. As we only used a simple sit to stand test of six minutes, which did not allow the development of presyncopal signs and symptoms, we cannot confirm whether galanin would increase at presyncope. Similarly, the failure in our study to observe gender difference in plasma galanin concentrations is in agreement with the observations of O’Shea et al [14]. Furthermore, whilst Hinghofer-Szalkay et al found that resting plasma galanin concentration was not significantly different between young females (39 ± 15 pg/ml) and males (26 ± 3 pg/ml), females had a significantly increased galanin response (4.9-fold increase) compared with males (3.5-fold increase) to a 70° HUT + incremental LBNP orthostatic challenge [8]. However, once again such increases were only observed at the point of postural instability in the Hinghofer-Szalkay study [8], which was never reached in our study.

**Adrenomedullin—galanin relationship**

Although some studies have demonstrated changes in plasma adrenomedullin and galanin during orthostatic challenge [8, 9], no study has investigated the relationship between plasma adrenomedullin and galanin. Due to their strong vasodepressor and hypotensive effects [13, 14], we had hypothesised that there would be strong positive correlations in these hormones in females and males. However, we could not identify such relationships in our study.

During orthostatic challenge, plasma hormone concentration change depends on its specific kinetics (distribution volume, fractional clearance, incretion rate, total mass) [7]. Adrenomedullin has been shown in younger subjects to increase in an intensity-dependent manner during the first minutes of orthostatic challenge [9]: such relatively rapid plasma hormone concentration changes are generally indicative of altered release rates into the circulation [7], supported by a long circulating half-life of 22 ± 1.6 minutes [33]. In contrast, significant galanin surges have been identified after some delay, possibly due to general spillover from the synaptic clefts of activated neuronal systems [8], and is

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rapidly eliminated resulting in a half-life of 3.7 ± 0.4 minutes [34]. Increased plasma galanin may be a response to the attainment of a plasma adrenomedullin threshold, promoting vasodilation and thus, hypotension leading to presyncope.

Ideally, continuous sampling- and longer periods of orthostatic loading - are required to evaluate the specific time course of responses when the orthostatic challenge is sufficient to induce such responses (e.g. until the point of presyncope/ end point of cardiovascular stability). In our study, in which orthostatic challenge was applied for only six minutes, other rapid neuroendocrine responses were probably responsible for ensuring that mean arterial pressure remained unaltered upon standing [12]. Yet concentration measurements alone do not facilitate discrimination between altered hormone release and clearance. Herein unmeasured plasma volume changes may impact plasma hormone concentrations, with orthostatic central hypovolaemia in conjunction with increased concentration reflecting only minimal change in the circulating hormone pool [7].

Limitations

A lack of change in adrenomedullin and galanin may have resulted from methodological issues such as ELISA cross-reactivity and reduced sensitivity compared to radioimmunoassay kits used in previous research [35]. Furthermore, no protocol repeats were immediately performed; the size of the study sample, and especially the male subject group, was originally small and was further diminished by the 3 blood samples that underwent in vitro haemolysis—possibly owing to excessive aspiration force where subjects had small or superficial veins [29].

Future directions

Future research should investigate the effects of orthostatic loading (HUT + LBNP vs. sit-to-stand test), time (across 1 year), ageing and gender on plasma concentrations of adrenomedullin, galanin and adrenocorticotrophic hormone, brain natriuretic peptide and pituitary adenylate cyclase-
activating peptide, which also have blood pressure regulatory roles [3, 36, 37]. In addition to hormonal responses, the modulation of endothelial function, cardiovascular autonomic function and cerebral blood flow should be assessed. All these aspects are important in understanding how they could contribute to falls in older persons. Recently, for example, Rodriguez and colleagues [38] have shown that there is continued autonomic dysfunction after recovery from ischemic stroke in older persons, with potential attenuation of the cardiovascular response to standing, which could predispose older persons with histories of stroke to orthostatic intolerance and falls.

**Conclusions**

In this study, plasma adrenomedullin and galanin concentrations were unchanged during orthostatic challenge in older subjects, possibly due to the insufficient duration or magnitude of the orthostatic challenge provided by 6 minutes of standing (given that presyncope was not induced). Thus, further work is required with a more aggressive orthostatic challenge leading up to presyncope. Furthermore, adrenomedullin and galanin levels were not different between genders either at rest or when standing. This failure to observe any differences between the two groups may be related to the fact that our female subjects were post-menopausal and had a high BMI. We also did not identify a linear adrenomedullin-galanin relationship in older females or males.

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Disclosure

James Broadbent and Joel Rodriguez participated in the study as part of their dissertations for the Space Physiology & Health MSc, King’s College London, UK.

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Figure 1. Bar graph and overlaid before-after graphs, showing plasma adrenomedullin (ADM) concentration in the seated and standing postures across gender. Seated bars are means ± SEM of 12 females and 5 males; standing bars are means ± SEM of 12 females and 4 males. Symbols represent plasma ADM concentrations of each individual in a subject group. Lines depict the change in plasma ADM concentration upon transition from the seated position to the standing position.

Figure 2. Bar graph and overlaid before-after graphs, showing plasma galanin concentration in the seated and standing postures across gender. Seated bars are means ± SEM of 12 females and 5 males; standing bars are means ± SEM of 12 females and 4 males. Symbols represent plasma galanin concentrations of each individual in a subject group. Lines depict the change in plasma galanin concentration upon transition from the seated position to the standing position.

Figure 3. Relationship between changes (Δ) from sitting to standing positions in plasma concentrations of adrenomedullin (ADM) and galanin, with corresponding Pearson correlation coefficient for (A) females (n=12) and (B) males (n=4).
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Table 1. Physical characteristics of the study population in relation to gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>12</td>
<td>63±7</td>
<td>164±7</td>
<td>72±13</td>
<td>26.8±5.9</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>62±7</td>
<td>176±6**</td>
<td>87±14*</td>
<td>28.0±3.1</td>
</tr>
</tbody>
</table>

Age, height, mass and body mass index (BMI) are presented as means ± SD for n=18 subjects.
Significant differences from females are indicated by * (p<0.05) and ** (p<0.01).
**Table 2.** Mean arterial pressure of the study population during the sit-to-stand test

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Mean arterial pressure (mmHg)</th>
<th>Base</th>
<th>Standing</th>
<th>Recovery</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0-10s 10-20s 20-30s 290-300s 0-10s 10-20s 290-300s</td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>12</td>
<td>111±3 102±5 106±4 115±4 115±4 114±4 115±4 114±4</td>
<td>3 5 4 5 4 4 3 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>98±4 90±5 93±5 96±5 103±2 106±4 105±4 103±4 104±4 101±4</td>
<td>* * 3 2 4 4 *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean arterial pressure is presented as means ± SEM for n=18 subjects. Mean arterial pressure measurements in 10s epochs were assessed at different time points: Baseline: final 10s of sitting; during standing: 10-20 sec, 20-30 sec, 180-190 sec (to assess orthostatic hypotension risk), last 10 sec of standing (290-300 sec); and during recovery: similar epochs as in standing. Due to uneven number of males and females, mean arterial blood pressure across age-matched female and male groups were compared using Welch t-tests. Significant differences from females are indicated by * (p<0.05).
A
Female

Δ Plasma Galanin (pg/ml)

Delta Plasma ADM (pg/ml)

r = -0.06731; p = 0.8353

B
Male

Δ Plasma Galanin (pg/ml)

Δ Plasma ADM (pg/ml)

r = 0.8351; p = 0.1649

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