Circulatory galanin levels increase severalfold with intense orthostatic challenge in healthy humans

Helmut G. Hinghofer-Szalkay,1,2 Andreas Rössler,1,2 Joyce M. Evans,3 Michael B. Stenger,3 Fritz B. Moore,4 and Charles F. Knapp3

1Institute for Adaptive and Spaceflight Physiology, Wormgassee 9, and 2Institute of Physiology, Center for Physiological Medicine, Medical University, Graz, Austria; 3The Center for Biomedical Engineering, University of Kentucky, Lexington Kentucky; and 4National Aeronautics and Space Administration Ames Center for Biological and Gravitational Research, Moffett Field, California

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Hinghofer-Szalkay, Helmut G., Andreas Rössler, Joyce M. Evans, Michael B. Stenger, Fritz B. Moore, and Charles F. Knapp. Circulatory galanin levels increase severalfold with intense orthostatic challenge in healthy humans. J Appl Physiol 100: 844–849, 2006. First published December 1, 2005; doi:10.1152/japplphysiol.01039.2005.—The purpose of this study was to test the hypothesis that plasma galanin concentration (pGal) is regularly increased in healthy humans with extensive orthostatic stress. Twenty-six test persons (14 men, 12 women) were brought to an orthostatic end point via a progressive cardiovascular stress (PCS) protocol consisting of 70° head-up tilt plus increasing levels of lower body negative pressure until either hemodynamically defined presyncope or other signs of orthostatic intolerance occurred (nausea, clammy skin, excessive sweating, pallor of the skin). We further tested for possible gender, gravitational, and muscular training influences on plasma pGal responses: PCS was applied before and after 3 wk of daily vertical acceleration exposure training on a Human Powered Centrifuge. Test persons were randomly assigned to active (with bicycle work) or passive (without work) groups (seven men, six women in each group). Resting pGal was 26 ± 3 pg/ml in men and 39 ± 15 pg/ml in women (not significant); women had higher galanin responses (4.9-fold increase) than men (3.5-fold, P = 0.017) to PCS exposure. Overall, PCS increased pGal to 186 ± 5 pg/ml (P = 0.0003), without significant differences between presyncope vs. orthostatic intolerance, pre- vs. postcentrifuge, or active vs. passive gravitational training. Increases in pGal were poorly related to synchronous elevations in plasma vasopressin. We conclude that galanin is regularly increased in healthy humans under conditions of presyncopal orthostatic stress, the response being independent of gravity training but larger in women than in men.

blood pressure stability; syncope; hormones; gender differences

POSTURAL CHANGES IN A GRAVITATIONAL field evoke counterregulatory responses that importantly contribute to cardiovascular fitness. Lack of gravitational stress on the long axis of the body for several days leads to “deconditioning” and reduced orthostatic competence, i.e., decreased stability in the maintenance of brain perfusion (14, 22, 27).

Although a multitude of hormonal factors contributing to cardiovascular regulation during gravitational stress are well recognized, it appears that there are more to be investigated and understood. In an earlier study, we demonstrated that plasma adrenomedullin, as well as catecholamines, rise in a commensurate manner with the degree of passive head-up tilt (29). This was surprising because one would expect a vasodilatory and natriuretic hormone to be suppressed rather than stimulated under conditions of reduced cardiac output. Obviously, the hormonal regulation of cardiovascular stability is more complex than originally anticipated and merits further study.

Galanin, a multifunctional neuropeptide, qualifies as a potential candidate for a role in cardiovascular homeostasis because it has been shown to influence central cardiovascular regulation (11, 28) and to lower plasma norepinephrine in humans (10). A previous study claimed that venous galanin levels rise in orthostatically competent patients with a history of recurrent vasovagal syncope, but not in healthy humans or in patients who are unsuccessful in finishing a tilt table test (1). These authors used 45 min of 60° passive standing, without aiming for a presyncopal end point, and concluded that galanin did not play a physiological role in cardiovascular regulation in healthy humans. We wanted to retest this hypothesis with a simple progressive cardiovascular stress (PCS) protocol in which the subjects were brought to an end point of orthostatic incompetence. This protocol added lower body negative pressure (LBPN) of increasing magnitude to the orthostatic stress of head-up tilt, which per se is not sufficient to guarantee such an end point in a reasonable experiment time.

This progressive cardiovascular stress to arrive at a presyncopal state was used before and after 3 wk of artificial gravity training on the National Aeronautics and Space Administration (NASA) Ames Research Center’s Human Powered Centrifuge (HPC) (13). Gravity training consisted of two types: vertical (G2) acceleration alone (passive training) or Gz acceleration training plus exercise (active training). The study design allowed testing for gender, overall training effects, and/or types of training effects on pGal responses to PCS.

We are first to report that pGal increases with presyncopal orthostatic stress in healthy people, which occurred without a single exception in our study. Our data do not support the assumption that the magnitude of this response was dependent on gravity training of either type. Furthermore, women had greater increases in plasma galanin and shorter PCS tolerance times than men, indicating gender-dependent responsiveness to orthostatic stress, which might be indicated by, and possibly linked to, the amount of galanin response as judged from plasma levels.

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PROCEDURES AND METHODS

The experimental protocol was approved by both the NASA/Ames Research Center Human Research Institutional Review Board (HR IRB 227) and the University of Kentucky (IRB 98-50299). Subjects were 26 healthy, untrained, nonobese, nonsmoking men (n = 14) and women (n = 12) without history of vasovagal syncope, who each gave informed consent and received a comprehensive medical examination (physician screening, complete blood count and chemistry panel, urinalysis, and exercise stress test) before participation.

In the 2 wk preceding the pretraining PCS test, subjects were familiarized with both the centrifuge training and orthostatic testing protocols. Each PCS started with a ≥30-min supine rest period, during which noninvasive instrumentation was placed to record continuous blood pressure (Finapres) and ECG (BoMed) and an antecubital indwelling catheter (Jelco) was placed. Control (supine) data were taken for 10 min before the tilt and, shortly before tilting, a control blood sample was drawn. Thereafter, the tilt table was brought to 70° head-up for 5 min, after which pressure in the LBNP chamber was reduced 20 mmHg below atmospheric pressure for 3 min; subsequent 10-mmHg reductions in pressure were made at 3-min intervals until the onset of presyncopal symptoms. A second blood sample was taken 1–2 min after the test subject was brought back to the supine position for one or several of the following reasons. PRES: A presyncopal point was defined by a sudden drop in blood pressure (decrease in systolic blood pressure >25 mmHg/min, decrease in diastolic blood pressure >15 mmHg/min), or a sudden drop in heart rate (decrease >15 beats/min). INT: The orthostatic stress was also terminated because of intolerance if the subject felt nauseated; displayed clammy skin, excessive sweating, or pallor of the skin; and requested and/or agreed to terminate (13).

Blood samples were collected in EDTA- and aprotinin-treated tubes to determine hormone concentrations. Samples were centrifuged for 15 min at 1,500 G and 4°C (Ivan Sorvall, RC2-B); plasma was separated into vials and stored at −80°C for later analysis.

During the gravity training sessions on the HPC, one subject powered the centrifuge (active subject), and the other rode passively with feet elevated to simulate a supine pedaling posture (passive subject). Passive and active pairs of subjects were trained simultaneously in a supine position with their heads toward the center of the 1.9-m-radius HPC. Both groups received an artificial gravity training profile that oscillated at 4-min intervals between 1 and 2.5 Gz for 35 min per day for 3 wk (13).

Hormone measurements. Plasma galanin was measured by using a commercially available RIA (Peninsula Laboratories) after trifluoroacetic acid extraction onto C-18 Bond Elute cartridges (Millipore-Waters, Milford, MA) that had been prewashed with methanol and saline (9 g/l). Concentrated eluates were dried under air and extracts were stored at −20°C until the day of assay. Before measurement, samples were reconstituted in RIA buffer and the clear solution was tested in a single assay. The antibody of this assay does not cross-react with secretin, VIP, insulin, substance P, or galanin message-associated peptide (1-41) amide. Samples were incubated overnight with a specific antibody reactive to human galanin at 4°C. Radioactively labeled [125I]galanin was added and incubated another 24 h at 4°C. Free and bound fractions were separated by adding a second antibody and were further spun at 3,000 rpm for 20 min at 4°C. Radioactivity was measured with a gamma counter; the minimal detectable concentration was 1.5 pg/ml (sensitivity was defined as the apparent concentration at three standard deviations from the counts determined from pure assay buffer). The interassay and intra-assay coefficients of variation were 7.5 and 5.9%, respectively.

AVP was determined by a competitive RIA (Vasopressin, Nichols Institute Diagnostics) after prior trifluoroacetic acid extraction, using the same procedure as applied for galanin pretreatment and described elsewhere (29). Recovery rate for the peptide after purification from binding proteins was more than 93%, assay sensitivity was 1.3 pg/ml, and interassay coefficient of variation was 6.1%.

TREATMENT OF DATA. Because the distribution of pGal values was highly asymmetrical (skewness 2.08), hypothesis testing was performed with Kruskal-Wallis ANOVA, with time during PCS as a dependent variable. Gender and training conditions were independent variables. The difference of galanin responses to presyncopal orthostatic stress was tested with a two-way Wilcoxon’s test. All nonparametric tests were done with StatSoft’s Statistica 4.0. The upper limit for acceptable type I errors was 5%. Variations are indicated as means ± SE.

Changes in orthostatic tolerance were tested using a three-factor (men/women, passive-active, pre/post training) ANOVA with repeated measures on one factor (pre/post training). Cardiovascular variables were tested using a two-factor ANOVA to examine differences between INT and PRES groups with respect to control and PSC values. Post hoc analysis used least significant difference and significance was accepted at P < 0.05.

RESULTS

Resting plasma galanin levels were 25.8 ± 2.7 (median: 22.9 pg/ml in men and 39.1 ± 14.6 (median: 16.5 pg/ml in women, with no significant difference between genders; two women had very high resting levels (183 and 380 pg/ml, respectively). At presyncope, galanin increased in all subjects (Fig. 1); average values rose from 26.0 ± 4.6 in control to 186.3 ± 5.3 pg/ml at presyncope (P = 0.0003). Galanin rose to 115.6 ± 33.5 (median: 50.4) pg/ml in men, and to 231.6 ± 41.8 (median: 146.3) pg/ml in women, with a significant gender effect (Fig. 2). Women increased their plasma galanin by 192 ± 37 pg/ml at presyncope; the rise in men was 107 ± 40 pg/ml (P = 0.007). The nonparametric ANOVA did not suggest an influence of HPC training or training conditions (active vs. passive) on resting or PCS-induced values of pGal. The pGal increase was not different between the PRES and INT groups.

HPC training did not influence basal resting (supine) AVP values, which were slightly higher in men (4.1 ± 0.1 pg/ml) than in women (3.6 ± 0.1 pg/ml, P < 0.008). During orthostatic stress, men increased their vasopressin level from 4.0 ± 1.2 to 12.6 ± 1.7 (P < 0.0001) before and from 4.2 ± 0.2 to 8.1 ± 1.2 (P < 0.001) after gravitational training. Resting levels were lower, and did not change much, in women, who went from 3.2 ± 0.2 to 5.1 ± 0.3 before and from 3.9 ± 0.2 to 4.0 ± 0.6 after training.
to $5.5 \pm 0.4$ pg/ml AVP after gravitational training (Fig. 3). Control-to-presyncope increases in plasma galanin are shown as a function of simultaneously sampled changes in plasma vasopressin (Fig. 4).

Hemodynamic data of the PRES and INT groups are shown in Table 1. Starting from similar control values, heart rate rose in both groups during the stress but was significantly lower in the PRES group at the end of PCS. Blood pressure was maintained in both groups until a few minutes before the end of PCS, at which time it dropped significantly in PRES subjects.

Artificial gravity training increased tolerance to PCS in the group of 26 subjects by 13% ($P = 0.02$). Men were more tolerant than women to the PCS ($P = 0.01$) (Table 2). The longer tolerance times in men indicate an even steeper presyncopeal rise in galanin in female, compared with male, test persons.

**DISCUSSION**

Frequent postural changes in normal earth gravity are a salient component of cardiovascular fitness. Without them, regulatory deconditioning and a decrease of orthostatic competence occur within a matter of days (14, 22, 27). Assumption of an upright position challenges venous return, cardiac output, and arterial perfusion and triggers neurohumoral reflex responses that are designed to counteract those disturbances and to stabilize cardiovascular function (2, 4, 15, 24, 25, 31). Catecholamines rise within seconds after assumption of an upright position, the renin-angiotensin-aldosterone system responds with considerable delay, and vasopressin secretion is acutely triggered with presyncope, i.e., when primary mechanisms fail to stabilize brain perfusion. Galanin is a potential candidate for a role in cardiovascular homeostasis because it contributes to both peripheral (10) and central cardiovascular regulation (11, 28).

Little is presently known about the involvement of peptides like galanin in the maintenance of cardiovascular stability during orthostasis and whether circulating levels are affected by varying loads of gravity. It is also unclear whether galanin plays a protective role in orthostatic hypotension, but present evidence points to this neuropeptide as a sympatholytic neurotransmitter, acting both centrally and peripherally. For methodological and ethical reasons, it appears problematic to pinpoint, in an applied human model, how much galanin is released by particular tissues in a particular experimental situation, but it seems reasonable to assume that plasma levels represent general spillover from the synaptic clefts. Circulatory galanin would therefore increase when the corresponding neuronal systems are most active.

The primary finding of this work is that intense orthostatic challenge (as induced by PCS) up to a point where it cannot be continued because of hemodynamic or other presyncopal symptoms produces a large increase in plasma galanin levels in healthy humans. pGal increased in every single case of 26 orthostatically fit persons with two PCS trials each, without exception. It is important to note that even when blood pressure did not indicate a reason to terminate PCS trials, but other symptoms did, pGal was significantly increased and may serve as a precursor to impending cardiovascular collapse.

Although at first glance it might look like it, this is not contradictory to observations reported by Bondanelli and coworkers (1), who found no tilt-induced increase in pGal in either healthy subjects (5 men, 5 women) or in 13 patients with a history of vasovagal syncope (VVS) who were unable to
finish a passive 60° head-up tilt test. Their remaining 9 VVS patients who finished the tilt test, however, had a significant increase in pGal. The authors concluded that galanin played a role in the adaptive response to acute orthostatic stress preventing syncope in susceptible individuals (1). Because they limited their head-up tilt challenge to 45 min, many of their healthy subjects did not reach a presyncope point, whereas their VVS patients who did not develop syncope were conceivably more challenged than their healthy counterparts and were just able to finish the test.

We offer the explanation that this group of persons was more pressured to fight the orthostatic challenge and finished close to their presyncope point, thereby activating the galanin response that we found in all of our subjects. The mean increase reported by Bondanelli et al. (1) was ~80%, much less than the sixfold rise we observed in our study. In addition, the combination of tilt with increasing LBNP, used to evoke presyncope in our subjects, provided a different stress to cardiovascular regulation than did the tilt of the Bondanelli study and could explain the greater galanin increase in our study. The supine control galanin values reported by Bondanelli et al. were 10–15 pM in all subjects, which corresponds well to our 26 ± 4.6 pg/ml range. In summary, whereas Bondanelli et al. used a limited orthostatic challenge, clearly leaving healthy participants in a stable cardiovascular condition, our subjects were forced to reach a presyncope condition by employing larger cardiovascular stress, i.e., lower body suction in addition to head-up tilt, eliciting galanin responses that the other model was too weak to evoke.

Thus, when hormonal systems were fully employed in regulating cardiovascular stability during orthostatic stress, pGal was dramatically increased both in healthy subjects (this study) and in VVS patients (1), who were, however, able to tolerate moderate head-up stress. In patients who lacked orthostatic competence and developed presyncope during head-up tilt, pGal seemingly was not activated (1). This might have contributed to these patients’ orthostatic instability, but whether galanin acted to prevent the development of syncope is not known.

We chose two particular protocol times for pGal comparison: The first blood sample was drawn during steady-state supine control conditions, representing an unstressed state within the cardiovascular system and basal galanin levels. The second was taken 1–2 min after the person finished PCS and was brought back to supine, i.e., after the galanin discharged into the peripheral vascular beds had mixed with the central circulation and advanced to the cephalic vein sampling point.

When examined as a function of development of cardiovascular (as opposed to other) symptoms of presyncope, heart rate and blood pressure were similar in the two groups at rest and in response to tilt. By the end of the stress, however, both blood pressure and heart rate had dropped significantly in the PRES group, indicating the onset of vasovagal syncope.

The observed pattern of galanin response must be seen within the broader frame of humoral responses to orthostatic stress. On assumption of an upright position, plasma catecholamines rose early, followed by a 10- to 20-min delayed increase in renin-angiotensin-aldosterone activity; finally, vasopressin release was triggered in case of presyncope (2, 19, 21, 23, 25, 26, 33). It has been suggested that, besides vasopressin, other neurohumoral factors such as adenosine, renin, and angiotensin II were involved in maintaining cardiovascular stability.
adrenomedullin, or calcitonin gene-related peptide participate in cardiovascular stabilization and might protect against syncope. Galanin could be one such neurohumoral stabilizer.

Human galanin, a 30-amino acid neuropeptide first isolated in 1983 (10), is primarily synthesized and stored within the hypothalamus and pituitary gland (30), where it is involved in the regulation of vasopressin (6) and seems to trigger growth hormone release (24). Nerve fibers are capable of delivering galanin directly into target organ tissues but also into the systemic circulation (12). Once in the bloodstream, galanin is rather short lived; its half-life has been calculated as 3–4 min (3, 18). No circadian rhythm and no significant variability in plasma galanin levels in humans during the daytime have been observed by others, even with pulsations of pituitary hormone levels (34). Therefore, it is conceivable to assume that supine (control) values as observed in this study were not subject to major variability over time.

Centrally administered galanin increases heart rate and evokes a weak vasodepressor response (11). It also seems to inhibit parasympathetic acetylcholine release by an interaction with peripheral galanin receptors, which are particularly numerous in the heart (1). Thus it might be assumed that galanin secretion is an important part of normal cardiovascular regulation in humans. However, our data and observations by Bondanelli et al. (1) suggest that standing per se does not increase galanin levels, but a more significant stress, like our PCS model that leads to presyncope, is needed to trigger a galanin response.

We observed that when blood samples were taken occasionally during the orthostatic stress experiment before presyncopal signs and symptoms were reached, there was no significant change in galanin plasma levels. From this we deduce that there is no substantial variability in galanin levels over time as long as the cardiovascular situation is stable (i.e., not reaching presyncopal state), which is in accordance with the findings reported by Bondanelli et al. (1). This indicates that our galanin results are solid and could not be due to a methodological problem.

Galanin acts as neuromodulator, which might be involved in the mechanisms of vasopressin release from the hypothalamic system, because galanin coexists with AVP in the same cell bodies of magnocellular neurons. AVP is released in response to decreases in cardiopulmonary or arterial baroreceptor input with decreases in blood volume or pressure (20) and increases during severe orthostatic stress (7). Our results are consistent with the literature as resting plasma AVP concentration was higher in men compared with women (9); we are unable to explain the seemingly larger AVP increase during orthostatic stress in men on the basis of our data.

Whereas PCS increased galanin in all experiments without exception, vasopressin was not always increased. Overall, the changes in galanin and vasopressin plasma levels in response to orthostatic stress were poorly correlated (Fig. 4). It is conceivable yet unproven that larger fluctuations occur in these hormone levels owing to burstlike secretion (32); in dogs, pulsatile secretion of vasopressin has recently been demonstrated (34).

Like galanin, catecholamines and plasma renin levels increased in response to PCS, but responses in this study were not different pre- and posttraining. We speculated that galanin might be regulated differently after HPC training, but the galanin response as seen in the present study was similar before and after active or passive training.

We also speculated that the pGal response to PCS might be gender specific in magnitude because in the present as well as in similar studies women demonstrated lower orthostatic competence than men (8, 36). Indeed, we found that the median rose more than eightfold in women but only about doubled in men. Our women not only had lower orthostatic competence but also engaged their galanin response to a larger extent than did men, which might indicate a greater challenge to cardiovascular stabilization on reaching a presyncope situation. A recent study found that basal galanin levels in men exceeded that in women (17), but because two of our female subjects displayed very high basal galanin, we cannot confirm such a possible sexual dimorphism in galanin plasma levels on statistical grounds.

**Limitations.** Our findings show that galanin is regularly and greatly increased during presyncopal events in healthy humans and might play an important adaptive role in cardiovascular regulation. Whether the pGal increase was secondary to orthostatic intolerance or was the reason for it, or both, cannot be determined from our observations because of experimental design restrictions in terms of ethically justifiable measures in healthy human subjects. Both the source and fate of circulating galanin remain poorly understood (5, 17).

In conclusion, plasma galanin concentrations increased severalfold with presyncopal conditions in humans. This rise was more pronounced in female subjects, who also reached presyncope earlier than did men under standardized conditions of orthostatic challenge. Basal supine galanin levels were not statistically different between genders. Galanin may constitute an important hormonal element in cardiovascular emergency situations.

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