Plasma Galanin Response to Head-Up Tilt in Normal Subjects and Patients With Recurrent Vasovagal Syncope

Marta Bondanelli, Paolo Alboni, Angelo Margutti, Paola Franceschetti, Maurizio Dinelli, Paolo Gruppillo, Paola Marchi, and Ettore C. degli Uberti

Neurohumoral factors may contribute to cardiovascular changes associated with vasovagal syncope (VVS). Galanin (GAL) is a neuropeptide, widely distributed in the central and peripheral nervous systems, that interacts with both sympathetic and vagal systems as well as with neurotransmitters, such as serotonin. We investigated the changes in plasma GAL and catecholamine levels during head-up tilt (HUT) test in patients with recurrent VVS. Twenty-two patients (11 women, aged 33.1 ± 4.2 years) with a history of VVS and 10 healthy subjects (5 women, aged 38.0 ± 5.8 years) underwent HUT test (60°, 45 minutes). GAL and catecholamine plasma levels were measured in the supine position, during HUT and, in patients with positive response, at presyncope, syncope, and after recovery of consciousness. Thirteen patients developed syncope during HUT, whereas no healthy subjects had a positive response. In healthy subjects, GAL did not change during HUT. By contrast, in patients with a history of VVS and a negative response to tilting (no syncope), GAL significantly ($P < .001$) increased in response to tilting (supine, 10.2 ± 0.6 pmol/L; tilting, 18.1 ± 1.1 pmol/L at 45 minutes) and correlated positively with the increases in blood pressure (BP) and heart rate (HR). In patients with a positive response, GAL did not change either before the loss of consciousness or during syncope. In patients with a positive response, norepinephrine (NE) significantly ($P < .001$) increased during tilting and then remained practically unchanged during syncope, whereas epinephrine (E) significantly ($P < .001$) increased during tilting and then showed further significant increases at presyncope and syncope. In conclusion, this study shows that circulating GAL levels progressively increase in correlation with the cardiovascular parameters during a negative HUT in patients with a history of VVS, whereas they remain unchanged in healthy subjects. Moreover, in the patients with tilting-induced syncope GAL does not change either before or during loss of consciousness. These data suggest a role for endogenous GAL in the adaptive responses to acute orthostatic stress preventing syncope in susceptible individuals.

VASOVAGAL SYNCOPE (VVS) is characterized by a withdrawal of sympathetic tone and often by activation of vagal tone, resulting in acute hypotension with or without bradycardia.[1,2]

It has been assumed that neurohumoral factors may exert a role in the regulation of neural pathways subserving cardiovascular changes associated with VVS. Some studies have suggested that serotonin (5-HT),[3,4] opioids,[5] nitric oxide,[6] arginine vasopressin (AVP),[7] and adenosine,[8] acting either independently or in concert, can produce a sympathetic inhibition leading to VVS in susceptible individuals.

Galanin (GAL) is a 29 (30 in humans) amino acid neuropeptide, widely distributed in the central and peripheral nervous systems, adrenal medulla, and pituitary, as well as gastrointestinal, genitourinary, and respiratory tracts.[9-11] GAL interacts with the endocrine system in the regulation of anterior pituitary function,[11-13] pancreatic hormone secretion,[11] and feeding behavior,[14] and has, moreover, been proposed to have a role in the central regulation of cardiovascular functions.[15,16] High concentrations of GAL-like immunoreactivity have been found in brain areas involved in cardiovascular control, where GAL coexists with catecholamines or 5-HT.[16] In animals, GAL can lower blood pressure (BP) and attenuate vagally induced slowing of the heart rate (HR).[17] Recent data report that GAL participates in the reduction of baroreceptor reflex sensitivity in the rat.[18] In humans, we found that the administration of GAL depresses the basal norepinephrine (NE) and the responses to both assumption of upright posture[19] and insulin-induced hypoglycemia.[20] Moreover, we demonstrated that GAL reduces the release of NE stimulated by pyridostigmine-induced enhancement of cholinergic activity.[21] To our knowledge, there are no studies that have examined the changes in plasma GAL during head-up tilt test (HUT) in patients with recurrent VVS. Similarly, there is no information that correlates temporal changes in plasma GAL levels with the development of syncope and with changes in catecholamine levels.

Therefore, GAL and catecholamine plasma levels were measured during HUT in patients with VVS, and the findings from patients with HUT-induced syncope (positive response) and with negative response were compared with those from a group of healthy subjects.

MATERIALS AND METHODS

Subjects

Twenty-two patients (11 women and 11 men; mean age, 33.1 ± 4.2 years [range, 18 to 66]; body max index [BMI], 23.0 ± 0.6 kg/m^2 [range, 18.5 to 27]) with a history of recurrent VVS and 10 healthy volunteers (5 women and 5 men; mean age, 38.0 ± 5.8 years [range, 19 to 64]; BMI, 23.2 ± 0.7 kg/m^2 [range, 19.2 to 27]) without history of syncope (control group) were studied. The patients referred for evaluation of syncope were included in the study if they had had more than 2 syncopal episodes in the preceding year with prodromal symptoms or signs suggestive of VVS (diaphoresis, nausea, abdominal discomfort,

From the Department of Biomedical Sciences and Advanced Therapies, Section of Endocrinology, University of Ferrara, Ferrara; Division of Cardiology, Hospital of Cento, Ferrara; and the Division of Internal Medicine, S. Anna Hospital, Ferrara, Italy.

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Address reprint requests to Ettore C. degli Uberti, MD, Department of Biomedical Sciences and Advanced Therapies, Section of Endocrinology, University of Ferrara, Via Savonarola, 9, 44100 Ferrara, Italy.

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pallor, feeling of cold, yawning). Other possible causes of syncope were excluded on history, physical examination, standard laboratory tests, orthostatic BP measurements, 12-lead electrocardiography, 24-hour Holter recording, echocardiography, carotid sinus massage, and neurological and pychiatric evaluation.[22] All subjects were nonsmokers and free from any medications, and they did not show any sign of cardiovascular, neurological, pulmonary, renal, hepatic, hematological, psychiatric diseases, systemic hypertension, or general disorders (diabetes mellitus, immunologic or neoplastic diseases).

Subjects were informed in detail about the nature and purpose of the experiments before consenting to participate in the study, the protocol of which was approved by the Local Ethical Committee of the University of Ferrara.

Methods

All subjects kept to a standard daily diet of 100 mmol sodium and 80 mmol potassium for at least 7 days before the study. The use of alcohol, tea, and caffeine-containing food was prohibited for 10 days before the study. HUT was performed between 9 and 11 AM, after an overnight fast, in a quiet room. Subjects were horizontally positioned on an electronically controlled tilt table with footboard for weight-bearing. An antecubital venous cannula was inserted 1 hour before the test for blood sampling with the subjects in supine position. Normal saline (0.9% NaCl) was infused to maintain the opening of the lines and to replace the blood volume. After blood samples had been taken in supine position at −15 and 0 minutes, the subjects were tilted at 60° for 45 minutes, or until syncope occurred, in accordance with Westminster protocol[23] and were then returned to supine position for a 30-minute recovery period. Blood samples were drawn at 3, 10, 15, 30, and 45 minutes after assumption of the head-up position in normal subjects (control group) and patients with a negative test. In the patients with a positive HUT, blood samples were taken at the appearance of presyncopal symptoms, during syncope, and 1, 5, and 30 minutes after recovery of consciousness. BP and HR were monitored noninvasively by means of an Ohmeda Finapress (Louisville, CO). The study procedures were attended by a nurse and a physician.

Analytical Procedures

Blood samples were drawn into precooled glass tubes containing EDTA (1 mg/mL) and aprotinin (Trasylol, Bayer, Milan, Italy; 500 kallikrein inhibitor units/mL) for GAL, and containing glutathione (1.2 mg/mL) and ethylene gylcol-bis (β-aminoethyl ether) N,N,N′, N′-tetraacetic acid (1.9 mg/mL) for catecholamine (NE and epineoherine [E]) determinations. They were promptly centrifuged at 3,000 × *g* for 15 minutes at 4°C, and then the plasma was frozen at −80°C until analysis. All samples for each hormone were processed in duplicate in the same assay.

Plasma NE and E were measured by a reverse-phase high-performance liquid chromatography (HPLC) with electrochemical coulometric detector (Model 5200 A, ESA, Chelmsford, MA), using materials supplied by ESA Laboratories. The intra- and interassay coefficients of variation for NE were 4.1% and 6%, respectively, at concentrations of 1,000 to 2,000 pmol/L in plasma. The intra- and interassay coefficients of variation for E were 4% and 5.3%, respectively, at concentrations of 100 to 200 pmol/L in plasma. The limits of detection for NE and E were 147 and 54 pmol/L, respectively.

Plasma GAL was measured by radioimmunoassay, using a commercially available kit (Peninsula Laboratories, Belmont, CA), after trifluoroacetic-acid extraction from EDTA plasma. The detection limit of the assay was 0.7 pmol/L. The intra- and interassay coefficients of variation were 11% and 10%, respectively.

Table 1. Clinical Characteristics, BP, and HR Values, Before and During HUT of the Healthy Subjects and Patients With a History of VVS

		VVS Patients	
	Healthy Subjects (n = 10)	With Negative HUT (n = 9)	With Positive HUT (n = 13)
Age (yr)	38.0 ± 5.7	32.2 ± 5.3	33.8 ± 4.3
Sex	5 F/5 M	4 F/5 M	7 F/6 M
BMI (kg/m^2)	23.2 ± 0.7	23.2 ± 0.8	22.9 ± 0.8
SBP (mm Hg)			
Supine	119.8 ± 4.0	112.6 ± 3.8	109.3 ± 4.5
Tilting (3 min)	128.7 ± 4.6*	127.1 ± 3.5‡	126.9 ± 3.3*
DBP (mm Hg)			
Supine	70.6 ± 3.9	67.3 ± 5.0	58.3 ± 2.8§
Tilting (3 min)	80.3 ± 2.0†	83.3 ± 4.0*	78.8 ± 2.7*
HR (beats min)			
Supine	68.0 ± 2.4	68.3 ± 3.2	69.9 ± 3.8
Tilting (3 min)	77.7 ± 3.3*	89.2 ± 3.4*¶	93.1 ± 7.2*

NOTE. Values are mean ± SEM.

*$P < .001$, †$P < 0.02$, and ‡$P < .05$ *v* supine.

§$P < .02$, and ¶$P < .05$ *v* healthy subjects.

Abbreviations: VVS, vasovagal syncope; HUT, head-up tilt; SBP, systolic blood pressure; DBP, systolic blood pressure; HR, heart rate; BMI, body max index.

Statistical Analysis

Unless otherwise indicated, the values are expressed as means ± SEM. The results were compared within each group and between groups by using analysis Student's paired or unpaired *t* test. Values of $P < .05$ were considered significant. Relationships between variables were analyzed by linear regression analysis. The basal hormone levels were obtained from the mean of the 2 values determined in the supine position.

Definitions

Spontaneous VVS was defined as transient loss of consciousness with inability to maintain postural tone and with spontaneous recovery, preceded by typical premonitory signs and symptoms. Presyncope was defined as premonitory signs and symptoms of imminent loss of consciousness. A positive response to HUT was defined as reproduction of syncope, in association with hypotension, bradycardia, or both. A positive response in which syncope occurred as a result of an asystolic pause (>3 seconds) or bradycardia (<40 beats/min) for at least a 10 second duration was defined as cardioinhibitory. A vasodepressor response was defined as a decrease in BP at time of syncope, where heart rate was maintained at greater than 60 beats/min.[24]

RESULTS

Of the 22 patients with spontaneous vasovagal syncope, 13 had syncope after 15.5 ± 2.7 minutes of HUT (range, 4 to 32 minutes). None of the healthy subjects developed syncope in response to HUT. There were no significant differences between individuals with a positive and negative HUT regarding age, sex, BMI, number of syncopal attacks in patients with a history of syncope, or supine BP (Table 1).

No significant differences in supine GAL and NE concentrations were observed between healthy subjects (Fig 1) and patients (Figs 2 and 3), whereas basal E levels were slightly ($P = .06$) higher in patients with positive response in comparison with healthy subjects. No significant differences in supine GAL

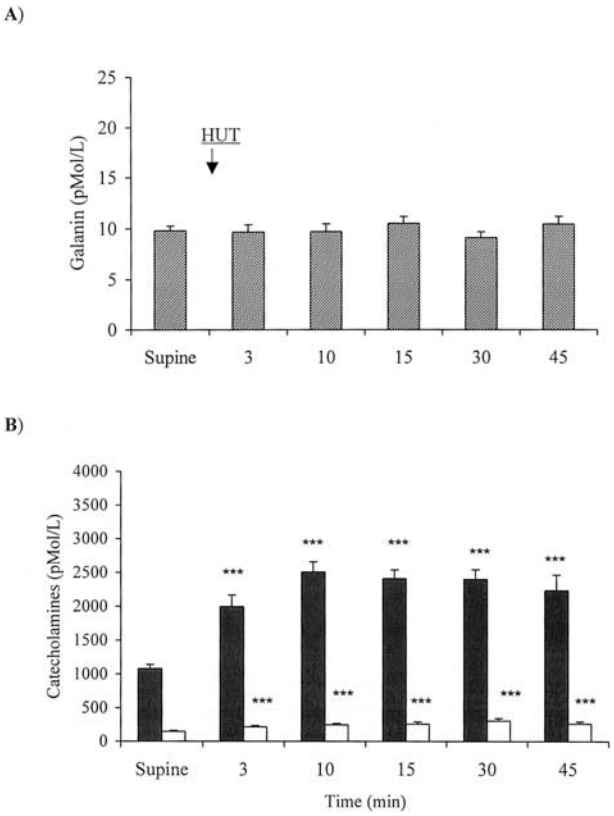

Fig 1. Plasma (A) GAL and (B) catecholamine (□, epinephrine, E; ■, norepinephrine, NE) changes during HUT in healthy subjects. Data are expressed as mean ± SEM. ***$P < .001$ *v* supine.

levels were observed between patients with positive and negative responses.

Plasma GAL Changes During HUT

In healthy subjects, GAL was unchanged from supine (9.8 ± 0.5 pmol/L) to tilting position (peak, 10.5 ± 0.7 pmol/L) (Fig 1A).

In patients with VVS and a negative response, GAL displayed a prompt rise (Fig 2A) in response to HUT, which attained statistical significance ($P < .001$) compared with that observed in healthy subjects (Fig1A), increasing from a mean basal value of 10.2 ± 0.6 to 13.9 ± 1.52 pmol/L at 3 minutes ($P < .001$ *v* supine) and remaining elevated (18.1 ± 1.1 pmol/L) up to 45 minutes ($P < .001$ *v* supine).

In patients with a positive response, GAL did not show significant changes in response to HUT (baseline 10.9 ± 0.5 pmol/L, tilting 10.5 ± 1.0 pmol/L at 3 minutes), similarly to that observed in healthy subjects. No change in GAL levels was observed before the loss of consciousness, during syncope, and during the recovery phase (Fig 3A).

Plasma Catecholamine Changes During HUT

In healthy subjects, HUT resulted in a significant ($P < .001$) increase in NE and E levels (Fig 1B). NE and E increased from the supine values of 1,078.6 ± 61.6 pmol/L and 146.9 ± 14.4 pmol/L to the maximum of 2,503.9 ± 143.7 pmol/L (at 10 minutes) and 304.3 ± 35.5 pmol/L (at 30 minutes), respectively, and remained significantly ($P < .001$) elevated up to 45 minutes.

In patients with a negative response, HUT resulted in a significant ($P < .001$) increase in NE and E levels (Fig 2B). NE and E increased from the supine values of 1,237.8 ± 218.9 pmol/L and 170.7 ± 24.6 pmol/L to the maximum of 2,922.2 ± 317.1 pmol/L (at 45 minutes), and 515.8 ± 73.8 pmol/L (at 3 minutes), respectively, and remained significantly ($P < .01$) elevated up to 45 minutes. The increase in E levels was significantly ($P < .05$) higher than that observed in healthy subjects (Fig 1B).

In patients with a positive response, NE significantly ($P < .001$) increased from the supine value of 1,137.0 ± 96.5 pmol/L to the value of 2,340.0 ± 175.5 pmol/L during the tilting position (at 3 minutes), with no significant differences compared to healthy subjects. NE levels did not show further significant changes during presyncope and syncope, and then returned to supine levels during the recovery phase. By contrast, E significantly ($P < .001$) increased from the supine value of 210.8 ± 28.1 pmol/L to the value of 384.4 ± 36.2 pmol/L during the tilting position (at 3 minutes) and showed further significant increases during presyncope and syncope, rising to the maximum values of 4,249.3 ± 1,131.3 pmol/L during loss of consciousness, and starting to return to supine levels during the recovery phase (Fig 3B). The increase in E levels during

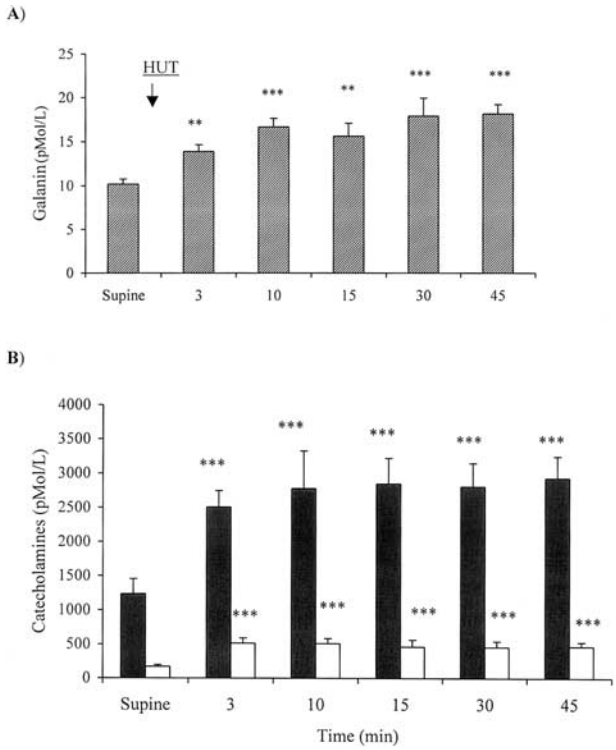

Fig 2. Plasma (A) GAL and (B) catecholamine (□, epinephrine, E; ■, norepinephrine, NE) changes during HUT in VVS patients without HUT-induced syncope (negative response). Data are expressed as mean ± SEM. **$P < .02$ and ***$P < .005$ *v* supine.

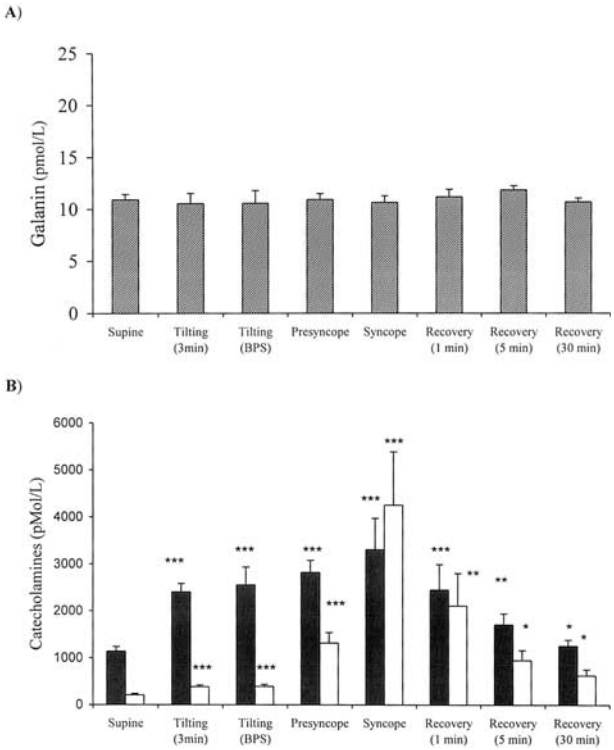

Fig 3. Plasma (A) GAL and (B) catecholamine (□, epinephrine, E; ■, norepinephrine, NE) changes during HUT in VVS patients with HUT-induced syncope (positive response). Data are expressed as mean ± SEM. *$P < .05$, **$P < .01$, and ***$P < .001$ *v* supine. Tilting (BPS) = last sample during tilting (9.7 ± 1.8 min) before presyncope (BPS).

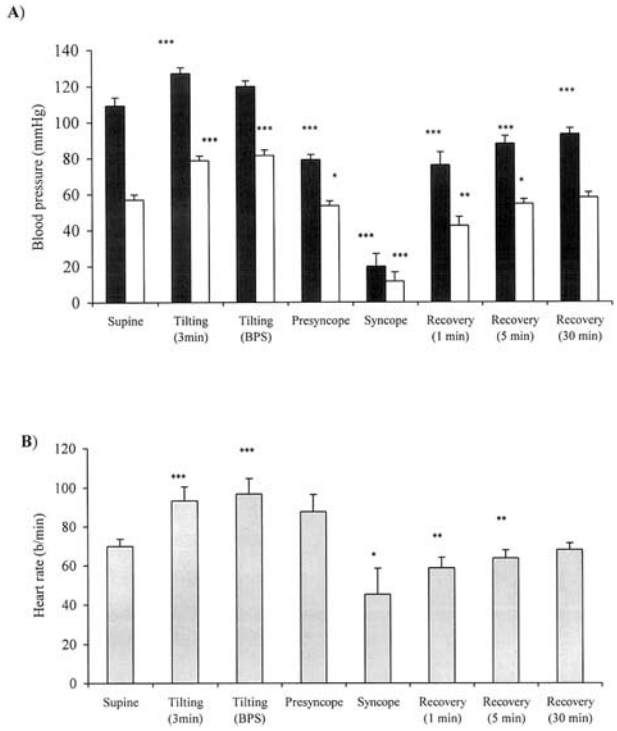

Fig 4. (A) BP and (B) HR changes during HUT in patients with HUT-induced syncope (positive response). Data are expressed as mean ± SEM. *$P < .05$, **$P < .005$, and ***$P < .001$ *v* supine. Tilting (BPS) = last sample during tilting (9.7 ± 1.8 min) before presyncope (BPS). (■) Systolic BP; (□) diastolic BP.

tilting was significantly higher ($P < .05$) than that observed in healthy subjects (Fig 1B).

Changes in BP and HR

In healthy subjects and in patients who did not develop syncope, systolic and diastolic BPs and HR significantly increased after 3 minutes of HUT (Table 1) and then remained practically unchanged. In the 13 patients who developed syncope, systolic and diastolic BPs significantly increased during tilting and then significantly decreased at the beginning of presyncope and during loss of consciousness (Fig 4A). HR significantly increased after 3 minutes of HUT, failed to change significantly at the beginning of presyncope, and significantly decreased during loss of consciousness (Fig 4B). Of these 13 patients, 6 had a cardioinhibitory response, 4 had a vasodepressor response, and 3 had a mixed response.

Linear regression analysis (Table 2) failed to detect any statistically significant correlation between GAL levels and the cardiovascular parameters studied at each time of tilting, both in the healthy subjects and in VVS patients with a positive response to HUT.

In patients with a negative response, GAL showed a positive relationship with HR ($r = 0.884$, $P < .005$), and systolic ($r = 0.915$; $P < .002$) and diastolic ($r = 0.896$; $P < .005$) BP. In this group of patients, a positive significant ($P < .01$) correlation was also found between GAL and catecholamines during tilting.

DISCUSSION

To evaluate the possible involvement of GAL in the response to acute orthostatic stress, we measured the circulating levels of GAL during HUT in normal subjects and in patients with history of VVS. The main finding of the present study is the different secretory pattern of GAL during the tilting position in

Table 2. Correlations Between GAL Levels and the Cardiovascular Parameters at Each Time of Tilting in the Healthy Subjects and Patients With a History of VVS

	Healthy Subjects (n = 10)		VVS Patients: With Negative HUT (n = 9)		VVS Patients: With Positive HUT (n = 13)	
GAL and	*r*	*P*	*r*	*P*	*r*	*P*
HR	+0.369	NS	+0.884	.005	+0.350	NS
SBP	+0.459	NS	+0.915	.002	+0.414	NS
DBP	+0.352	NS	+0.896	.005	+0.503	NS
NE	+0.291	NS	+0.928	.002	+0.05	NS
E	+0.151	NS	+0.776	.01	−0.336	NS

Abbreviations: GAL, galanin; VVS, vasovagal syncope; HUT, head-up tilt; HR, heart rate; SBP, systolic blood pressure; DBP, systolic blood pressure; NE, norepinephrine; E, epinephrine; NS, not significant.

healthy subjects and in patients with history of VVS. In fact, GAL did not change during the 45 minutes of tilting in healthy subjects, whereas it significantly and progressively increased in patients with a negative response. Moreover, in patients who developed syncope, GAL did not show any significant increase before the loss of consciousness. These results indicate that GAL activity is altered in patients with history of VVS.

The pathogenesis of VVS is not fully understood. Normally, pooling of blood in the lower extremities on the assumption of an upright posture (as in HUT) is associated with a sympathetic activation and withdrawal of parasympathetic tone. In patients with VVS this compensatory response is interrupted after a few minutes and is associated with a paradoxical withdrawal of sympathetic activity and often with an increase in parasympathetic activity, resulting in severe hypotension, bradycardia and loss of consciousness.[1,2,25]

The present data demonstrate a pronounced increase in plasma E levels associated with a moderate elevation of NE preceding syncope, confirming the presence of dissociation between the noradrenergic and adrenomedullary response during VVS that may play an important role in the hemodynamic events of this syndrome.[2,25,26]

It has been suggested that neurohumoral factors may participate in the genesis of cardiovascular events associated with VVS, such as 5-HT,[3,4] opioids,[5] nitric oxide,[6] AVP,[7] adenosine,[8] and calcitonin gene–related peptide,[25] by acting either independently or in concert to produce a sympathetic inhibition leading to syncope in susceptible individuals. However, the neurohumoral mechanisms involved in the hemodynamic responses induced by orthostatic stress in patients with good tolerance to HUT versus those with positive response still remain unknown, as does the reason for the random expression of the spontaneous VVS.[1,27] It is possible that some factors may exert a protective action on the onset of syncope and GAL could be one of them. In fact, GAL increases during HUT in relationship to cardiovascular changes in patients with a negative response, whereas it does not change before the loss of consciousness in patients developing syncope. These data suggest that GAL could prevent the development of VVS in susceptible individuals through its involvement in the integrated cardiovascular and endocrine responses to acute orthostatic stress.

Demonstration of the widespread distribution of GAL within the central and peripheral autonomic nervous system, and the discovery of its coexistence with other neuroactive substances, including catecholamines and 5-HT, support a role for GAL in the regulation of neural pathways subserving cardiovascular changes associated with VVS.[15,16] Several mechanisms of GAL action may be involved in the modulation of the autonomic homeostasis in patients with VVS. In fact, GAL is a neuroendocrine peptide, with very different biological activities, which is locally released from nerve fibers directly into target organs and also delivered into the systemic circulation.[28] Several studies in animals have demonstrated a central control of the peripheral GAL release as well as a peripheral modulation of the central GAL amount.[28-30] So far, in humans, there is no clear evidence that the evaluation of changes in peripheral GAL levels may be a suitable measure of the central galaninergic system, although the recent demonstration, in rats, of a negative-feedback mechanism, in which circulating GAL autoregulates its own synthesis at hypothalamic level, suggests a strong relationship between central and peripheral galaninergic activity.[31]

We have previously demonstrated that administration of hGAL decreases supine and upright-stimulated release of NE in healthy man.[19] However, the demonstration that the reduced sympathetic activity in VVS patients with positive response to HUT is not associated with a significant change in GAL levels excludes that the protective role of GAL, observed in VVS patients with negative response, could be mediated through an inhibition of sympathetic activity.

Moreover, an inhibitory effect of GAL on vagal activity has been shown, both in animals[15,17] and in humans.[21,32] We found that GAL, administered to healthy subjects, reduces the release of NE stimulated by piridostigmine-induced enhancement of cholinergic activity.[21] Carey et al have demonstrated that intravenous injection of human GAL decreases sinus arrhythmia, an effect which is consistent with inhibition of vagal activity.[32] A recent report in rats demonstrated that gastric peripheral GAL may produce an inhibitory effect on the nucleus tractus solitarius neurons receiving gastric vagal input, thus suggesting that GAL can activate the peripheral terminals of vagal afferents and modulate neuronal activity at the levels of the brainstem.[29] In addition, it has been proposed that GAL released from sympathetic nerves inhibits Ach release and cardiac vagal activity by an interaction with peripheral GAL receptors, particularly expressed in the heart.[33] In accordance with this, we suggest that the increased GAL level in VVS patients with a negative response can protect these patients against the reflex of bradycardia through an inhibitory effect on vagal activity. The exact mechanism and site of GAL action in humans as well as the GAL receptor subtypes underlying the adaptive response remain to be clarified; however the lack of GAL modulation on vagal activity during tilting could account for the development of syncope in susceptible individuals.

In the periphery GAL has been predominantly associated with sympathetic nerve and released with NE upon sympathetic nerve stimulation. However, the absence of a vascular effect in dogs[34] makes a vasoconstrictor action of GAL, released from sympathetic nerve during tilting in humans, unlikely.

Central 5-HT neurons may participate in BP regulation, with both stimulating and inhibiting effects on the sympathetic nervous outflow in different areas in the CNS,[35,36] and functional alterations in 5-HT system may be involved in the pathogenesis of VVS.[2-4] A high responsiveness of the central 5-HT system in patients with history of VVS has been assumed.[4] It has been demonstrated that the administration of 5-$HT_{1\&2}$ receptor antagonists markedly attenuates the tilt-induced changes in plasma NE, prolactin, β-endorphin, and plasma renin activity, suggesting that central 5-HTergic mechanisms may participate in the integrated cardiovascular and endocrine responses to central blood volume depletion.[37] Electrophysiological data have shown that GAL (1-29) or GAL fragments (1-15, 1-16) reduce the central 5-HT_{1A}-mediated transmission through an antagonistic action on the 5-HT_{1A} receptor at the pre- and postjunctional level.[38] Our demonstration of an increase in circulating GAL levels during negative HUT in patients with recurrent VVS suggests that GAL enhancement could prevent

the development of syncope through an inhibitory action on the central 5-HT system.[38,39] On the other hand we reported in a previous work that plasma and platelet serotonin levels did not significantly change during HUT-induced syncope, but this cannot exclude an involvement of central serotonergic mechanisms, which could not be detected by the measurement of peripheral serotonin.[40]

In conclusion, the present study shows a difference in the secretory pattern of GAL during the 45 minutes of tilting between patients with a history of VVS and healthy subjects. Circulating GAL levels progressively increase in correlation with the cardiovascular parameters during a negative HUT in patients with a history of VVS, whereas they remain unchanged in healthy subjects. Moreover, in the patients with HUT-induced syncope, GAL does not change before loss of consciousness. These data indicate that the galaninergic system may be altered in patients with VVS, and suggest that endogenous GAL may exert a role in the adaptive response to orthostatic stress by preventing VVS in susceptible patients, perhaps through an inhibitory action on vagal activity and /or on the central 5-HT system.

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