CLINICAL STUDY

# Role of the peripheral serotoninergic system in the pathogenesis of vasovagal syncope

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**Abstract**

***Objective:*** **To evaluate the role of peripheral serotoninergic system in the pathogenesis of vasovagal syncope.**
***Background:*** **Increased central serotoninergic activity was suggested to play a role in sudden inhibition of sympathetic activity responsible for the genesis of vasovagal syncope. There is good correlation between the central serotoninergic activity and the plasma levels of serotonine.**
***Methods:*** **In twenty-two patients (mean age 48±19 years, 10 men, 12 women) with suspected vasovagal syncope head-up tilt test (HUT) was performed. Passive HUT (60 degrees, 20 minutes) was followed, if negative, by nitroglycerine stimulated HUT (400 μg sublingually, 15 minutes). Blood samples were obtained at baseline (in supine position), in 5 minute, 15 minute of HUT and finally at syncope or end of the test. Plasma levels of serotonine were measured by enzyme-immunoassay (EIA) method.**
***Results:*** **HUT was positive in fifteen patients and negative in seven patients. In all HUT positive patients syncope developed after nitroglycerine stimulation. Mean duration of nitroglycerine phase was 4.8±1.2 min. In 5 min of HUT serotonine level was significantly lower in HUT positive patients HUT (102.40±43.11 vs 160.85±43.71 ng/ml, p=0.01). At the time of syncope no significant differences were observed between HUT positive and HUT negative patients (184.26±118.72 vs 196.57±88.91 ng/ml, p=0.40).**
***Conclusion:*** **In patients with vasovagal syncope lower level of plasma serotonine were observed during early HUT when compared to controls. No differences in serotonin activity were observed at the time of syncope *(Tab. 1, Fig. 1, Ref. 24)*.**
**Key words: vasovagal syncope, head-up tilt test, pathogenesis serotonin, nitroglycerine.**

Serotonin, 5-hydroxytryptamine (5-HT) is a biogenic monoamine involved in the regulation of a variety of physiological functions. Serotonin is present in significant concentrations in neurons of the central nervous system (CNS) and enteric neurons. Another important pool of serotonin outside of central nervous system represent enterochromaffin cells of the gut and blood platelets (1). However peripherally synthetized serotonin does not effect activity of CNS, because is not able to cross blood-brain barrier. It is synthesized centrally in neurons and peripherally in enterochromaffin cells from L-tryptophan by the enzyme tryptophan hydroxylase (TPH), which has two isoforms encoded by two different genes – TPH1 (non-neuronal) and TPH 2 (neuronal) (2).

Serotonin peripheral synthetized in enterochromaffin cells is released into the circulation. Subsequently it is taken up by platelets by the activity of serotonin transporter (SERT or 5-HTT). Therefore platelets are the most important reservoir of serotonin in the periphery. Plasma level of free serotonin is low. Serotonin is released from platelets mainly upon platelet activation and plays a role in haemostatic function. Platelets adhesion is stimulated by serotonin (1). In addition, peripheral serotonin modulates a number of cardiovascular functions including vasoconstriction, vasodilation and positive inotropic,

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lusitropic and chronotropic effects in the heart. Free plasma serotonine is degraded by the action of monoamine oxidase (MAO) and can be also taken up by sympathetic neurons a and vascular endothelial cells (1). Peripheral serotonin mediates its effects through five types of receptors: 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$ and 5-$HT_7$.

Altered peripheral serotoninergic function was suggested to play a role in genesis of arrhythmias. Excess of circulating serotonin stimulates atrial 5-$HT_4$ receptors and triggers atrial fibrillation (4). Overexpression of 5-$HT_{2B}$ receptor in the heart results in cardiac hypertrophy. Increased serotonin level was also associated with cardiac valve abnormalities and primary pulmonary hypertension (5, 6). Serotonin in gastrointestinal neurons participates in regulation of gastrointestinal motility and may play a role in irritable bowel syndrome (7).

Serotonin in CNS acts as neurotransmitter and modulates a variety of behavioral functions like appetite, mood, sexual behavior, sleep and wakeness. Abnormal serotoninergic function has been implicated in psychiatric diseases, including depression, anxiety, eating disorders, compulsive disorder (8–11). Central serotonin is involved also in cardiovascular regulation. Serotoninergic neurons in raphe nuclei innervate autonomic areas of brainstem and spinal cord and in this way they modulate sympatho-parasympathetic balance (11). Central serotonin acts throught three types of receptors: 5-$HT_{1A}$, 5-$HT_2$ and 5-$HT_3$. Pharmacological studies have shown that activation of central 5-$HT_{1A}$ receptor causes inhibition of sympathetic tone and increase in parasympathetic tone whereas activation of central 5-$HT_2$ receptor causes sympathoexcitation leading to rise in blood pressure and tachycardia. 5-$HT_3$ receptors are located on afferent vagal nerve endings and their stimulation results in Bezold-Jarisch reflex with abrupt bradycardia (1). It has been also shown that stimulation of 5-$HT_3$ receptors is involved in mechano-electric transduction – their activation decreased the mechanical sensitivity of the afferent cardiac nerves (12).

Pathogenesis of vasovagal syncope is not fully elucidated. Most important hemodynamic change represents arterial hypotension due to sudden sympathetic withdrawal. Given the fact that central serotoninergic activity influences autonomic balance it is hypothetized that serotonin might play a role in pathogenesis of vasovagal syncope.

It is difficult to measure the central serotoninergic activity in vasovagal patients. Plasma level of serotonin represents the extracellular compartment of newly synthesized serotonin released from platelets as well as. There is a good correlation between the central and peripheral serotoninergic activity as showed by the analysis of serotonin concentrations in the blood and cerebrospinal fluid. Although central and peripheral serotonin pools are separated by the blood – brain barrier, genetically determined mechanisms involved in the regulation of the serotonin level in the extracellular space are probably similar (14). Thus the central serotoninergic activity can be inferred from peripheral serotonin levels.

The aim of the study was to investigate the role of serotoninergic system in the pathogenesis of vasovagal syncope, by assessing the plasma levels of serotonin during vasovagal syncope induced by head-up tilt test.

## Methods

Twenty-two patients with suspected vasovagal syncope were included into study (mean age 48±19 years, 10 men, 12 women). Vasovagal syncope was suspected when: 1) patients have prodromal symptoms typical for vasovagal syncope, 2) no overt organic heart disease was present and 3) diagnostic work-up for other etiology of syncope was negative. Standard diagnostic algorithm included: history, physical examination, electrocardiogram, 24 ambulatory ECG monitoring, echocardiography, transoesophageal electrophysiology, carotid sinus massage and neurological evaluation .

Patients were instructed to refrain from smoking, alcohol and caffeine-containing beverages for at least 3 days before planned head-up tilt test and to discontinue any cardioactive and psychoactive drugs for the same period of time. Informed consent was obtained from all patients and the study was approved by the local ethical committee.

*Head-up tilt test (HUT)*

The tilt-table testing was performed between 9.00 and 12.00 hours, with subjects in fasting state. An intravenous cannula was inserted into antecubital vein and subjects were placed in the supine position for 30 minutes. Subjects were then positioned upright at an angle of 60 for 20 minutes. Blood pressure was measured in 5 minute intervals during HUT and in 1 minute intervals during presyncopal phase. Blood pressure measurement was performed by conventional mercury sphygmomanometer. ECG was monitored continuously and heart rate was determined from ECG records. If syncope with hypotension and/or bradycardia developed during the tilt patient was lowered immediately to supine position and the test was terminated. If passive tilt table test was negative, it was followed by nitroglycerine stimulated HUT without lowering the patient to supine position. Nitroglycerine stimulated HUT was performed for additional 15 minutes after sublingual administration of 0.4 mg nitroglycerine in a form of spray.

*Blood sampling*

Four blood samples were taken into tubes containing heparin at following times: in supine position prior to tilt (0 minute), at 5 and 15 minute in 60° upright position, and finally at the onset of syncope or (if syncope not developed) at the end of the test (35 minute).

All samples were immediately centrifuged, and plasma was frozen and stored at -30 °C until estimation. Time until estimation was less than 3 months.

*Hormonal assays*

The plasma levels of serotonin was determined by enzyme immunoassay (ELISA) method by commercionally available kit (DRG Instruments GmbH, Germany).

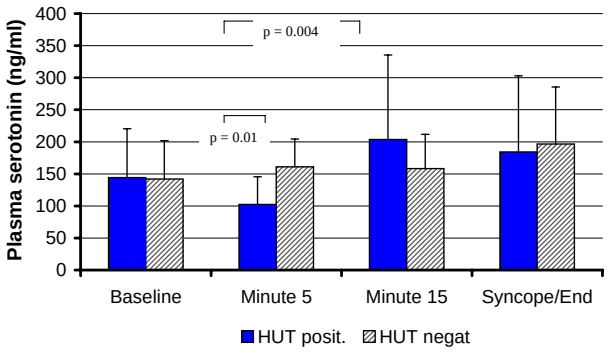


**Fig. 1. Plasma serotonin levels during HUT in patient with positive and negative tilt test. HUT posit. – patients with positive head-up tilt test, HUT negat. – patients with negative head-up tilt test.**

*Statistical analysis*

Differences in plasma serotonin levels between groups were compared using unpaired Student t-test. Analysis of variance (ANOVA) was used for comparison of differences within the group. Differences with p values •0.01 were considered significant. Results are shown as mean standard deviation.

## Results

HUT was positive in 15 patients (6 men, 9 women, mean age 53±17 years) and negative in 7 patients (4 men, 8 women, mean age 37±10 years). Mean age and gender ratio was not significantly different between two groups. Baseline heart rate tended to be lower in HUT-positive patient although the difference did not reach statistical significance (76.5±16.7 $min^{-1}$ vs 98.6±19.8 $min^{-1}$, p=0.02). There was no difference in baseline blood pressure values between two groups of patients (SBP 140.6±20.5 mmHg vs 122.6±8.1 mmHg, p=0.12, DBP 88.0±9.5 mmHg vs 80.0±6.3 mmHg, p=0.07)

In all HUT-positive patients unmedicated tilt was negative and syncope developed after pharmacological stimulation with nitroglycerine. Mean duration of nitroglycerine phase until development of syncope was 4.8±1.2 minutes. At the time of syncope heart rate as well as systolic and diastolic blood pressures were significantly lower in HUT-positive patients in comparison to HUT-negative patients (HR 62.4±13.2 $min^{-1}$ vs 89.0±14.68 $min^{-1}$, p=0.005, SBP 72.9±9.9 mmHg vs 110.6±14.0 mmHg, p<0.001, DBP 53.0±9.0 mmHg vs 73.5±7.1 mmHg, p<0.001).

No differences in serotonin levels were observed in supine position before HUT between HUT-positive and HUT-negative patients. Similarly no differences were found during late HUT (15 minute) and at the development of the syncope (Tab. 1).

In early phase of passive HUT, significantly lower serotonin level was observed in patients who eventually developed syncope in nitroglycerine stimulated phase of HUT compared to patient with negative HUT (102.40±43.11 vs 160.85±43.71 ng/ml, p=0.01). In addition, in HUT-positive patients serotonin levels rose significantly between minute 5 and minute 15 of passive tilt (102.40±43.11 vs 203,60±131,73 ng/ml, p=0.004). In HUT-negative patients no significant change in serotonin levels was observed during tilt test (Fig. 1).

## Discussion

Direct intracerebral administration of serotonin in experimental animal leads to decreased sympathetic activity of renal nerves and increases adrenal sympathetic activity. This experimental situation resembles vasovagal syncope (15). It is believed that central serotoninergic stimulation may trigger vasovagal reaction. However, the evidence supporting this hypothesis is only indirect and comes mainly from the pharmacological manipulation of the serotonin level in CNS.

Matzen examined the influence of three different serotonine receptor antagonists on hemodynamic and neurohumoral response during head-up tilt test. Administration of 5-$HT_{1+2}$ antagonist methysergide attenuated tilt- induced change in noradrenaline, prolactine, beta-endorphin and renin activity but did not affect heart rate and blood pressure response. Blockade of 5-$HT_3$ receptor by ondrasetron abolished tilt induced hypotension without influencing humoral response to tilt. Ketanserin (5-$HT_2$ antagonist) prevented rise in blood pressure in early phase of tilt-test. He suggested that serotonin plays a role in integrated cardiovascular and endocrine response to central hypovolemia in humans (16).

Theodorakis and coworkers have used clomipramine, a drug enhancing central serotoninergic activity by inhibiting 5-HT reuptake, to study the serotoninergic response during HUT in patients with vasovagal syncope. They showed higher levels of prolactine and cortisol levels during HUT in patients with history of vasovagal syncope. This was interpreted as an evidence of higher serotoninergic responsiveness in vasovagal patients (17). However this evidence is not persuasive. Presyncopal phase in the development of vasovagal syncope represents a stressful situation for the patient and an increase in cortisol and prolactin can be explained in this way.

In another study by the same group of authors clomipramine administration during HUT increased sensitivity of tilt test without loss of specificity and improves its diagnostic value (18).

Another indirect evidence for the role of serotoninergic system in the development of VVS comes from chronic administration of selective – serotonin reuptake inhibitors (SSRI). While acute administration of SSRI increases concentration of serotonin in synaptic cleft and increases serotoninergic activity, chronic

**Tab. 1. Serotonin level (ng/ml) during head-up tilt test in patients with positive and negative test.**

| | HUT positive | HUT negative | P value |
|---|---|---|---|
| Baseline | 144.13±76.14 | 142.00±59.85 | 0.47 |
| 5 minute | 102.40±43.11 | 160.85±43.71 | 0.01 |
| 15 minute | 203.60±131.73 | 158.28±53.37 | 0.20 |
| Syncope/End | 184.26±118.72 | 196.57±88.91 | 0.40 |

HUT – head-up tilt test

administration has an opposite effect. Chronic SSRI administration leads to down-regulation of central serotoninergic receptors due to their long term exposition to increased serotonin levels and finally leads to diminished serotoninergic activity. Various SSRI were shown to be effective in the treatment of VVS.

Fluoxetine and sertraline were shown to be effective in non-randomized trials (19, 20). Paroxetine was superior to placebo in double-blind randomized trial, although the number of included patients was small (21).

Results from above mentioned studies suggest, but do not prove that serotonin plays a role in pathogenesis of VVS. In another study paroxetin failed to prevent neurally mediated syncope induced by lower body negative pressure (22).

First study directly measuring serotonin in patients with vasovagal syncope was published by Alboni and coworkers (23). They measured platelet and plasma serotonin in fifteen patients with vasovagal syncope during nitroglycerine-stimulated HUT. No significant change in serotonin levels was observed during passive HUT and at the time of syncope neither in patients who developed VVS nor in patients who did not develop VVS. No differences in serotonin response to tilt were noted between tilt positive and tilt negative groups of patients. In patients who developed syncope during nitroglycerine-stimulated HUT no change in serotonin was observed after drug administration and at syncope. Authors concluded that serotoninergic system does not play a role in pathophysiology of vasovagal syncope.

Our results differ from above mentioned observations in some aspects. We observed a significantly lower serotonin levels in early phase of passive HUT in patients who eventually developed syncope in nitroglycerine stimulated phase of tilt test when compared to patient with negative HUT. In addition, in HUT-positive patients serotonin levels rose significantly between minute 5 and minute 15 of passive tilt. In HUT-negative patients no significant change in serotonin levels was observed during tilt test.

Pathogenetic mechanisms of vasovagal syncope are probably not uniform. By using a method of heart rate variability two different patterns in the cardiac autonomic changes preceding vasovagal reaction were recognized by Furlan. One is characterized by increased sympathetic modulation before syncope, the other by a progressive sympathetic inhibition up to the onset of syncope (24). It could be speculated that different serotonine responses found in our study and in the study of Alboni might explain this pathogenic heterogenity. However, it is very difficult to find a clear correlation between serotoninergic activity and autonomic tone. Increased cerebral serotonin level can be associated with sympatho-excitation as well as with sympatho-inhibition depending on the type of stimulated serotoninergic receptor (1). For further studies on the role of serotonin in vasovagal syncope an assesssment of autonomic tone changes during head- up tilt test ( e.g. by the method of heart rate variability or baroreflex sensitivity) in addition to serotonin level measurement could be usefull. Absence of such an assessment in the present study should be declared as a major limitation. Another limitation is a small number of patients included into a study.

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