Clinical Research

Hormonal Responses During Neurally Mediated Syncope as an Indication of Central Serotonergic Activity

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Introduction: The role of central serotonergic activity in neurocardiogenic syncope has not been completely investigated especially in humans. Changes of cortisol and prolactin plasma levels are good, although indirect, indicators of the central serotonergic activity. The purpose of our study was to evaluate the participation of central serotonergic activity in neurocardiogenic syncope by comparing the cortisol and prolactin plasma levels in patients with positive and negative tilt test.

Methods: Forty-six patients with history of neurocardiogenic syncope underwent tilt test at 60° for 30 minutes. Blood samples for cortisol and prolactin plasma evaluation were drawn from a peripheral vein at 0 (before), 10, 20 and 30 minutes after the start of tilt test (blood samples 1, 2, 3 and 4) in case of a negative tilt test. In case of a positive tilt test the patient was returned to supine position and blood samples were drawn at 5 and 10 minutes after the development of syncope (blood samples 3 and 4).

Results: Eighteen of the 46 patients had a positive tilt test result while the remaining 28 completed the test without symptoms. The mean time of syncope in the positive group was 15 ± 4 minutes. Cortisol plasma levels were similar in the positive and negative tilt test group before the start of the tilt test. Cortisol plasma levels increased significantly in the positive group compared to the negative tilt test group: 123 ± 44 ng/ml 5 minutes after syncope versus 91 ± 44 at the 20 minute in the negative group, (blood sample 3, $p<0.05$) και 142 ± 44 ng/ml 10 minutes after syncope versus 93 ± 45 at the 30 minute in the negative group, (blood sample 4, $p<0.05$). Prolactin plasma levels were similar in the two groups before the start of the tilt test. Prolactin plasma levels increased significantly in the positive group compared to the negative tilt test group: 16.9 ± 9.8 ng/ml 5 minutes after syncope versus 7.7 ± 3.5 at the 20 minute in the negative group, (blood sample 3, $p<0.05$) και 19.4 ± 9.0 ng/ml 10 minutes after syncope versus 7.6 ± 3.9 at the 30 minute in the negative group, (blood sample 4, $p<0.05$).

Conclusions: Cortisol and prolactin plasma levels increased significantly after syncope in patients with a positive tilt test result. This might be an indication of increased central serotonergic activity during tilt test induced syncope.

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The pathophysiology of neurally mediated syncope (NMS) is not well known. Both peripheral (Bezold-Jarish reflex) and central nervous system (CNS) activation have been proposed to explain the paradoxical activation of the inhibitory cardiac vagal afferent neuronal activity and withdrawal of the sympathetic activity that occurs during NMS[1]. Among the latter, central serotonergic mechanisms have been received little attention.

By the early 1990's a lot of research work was published on the participation of serotonin (5-hydroxytryptamine (5-HT)) of the CNS in the cardiovascular homeostasis[2-4] and provocation of the NMS[5-6]. Application of serotonin directly

to the rat brain results in hypotension and bradycardia similar to that of NMS[7]. The administration of 8-OH-DPTA (8-hydroxy-2-di-n-propylamine-tetriline, a 5 HT1A CNS receptor agonist) in cats caused a rapid decrease in heart rate and renal sympathetic activity[8].

In humans, the participation of the central serotonergic mechanisms in the provocation of NMS is less well established. Drugs that enhance the central serotonergic activity, like fluoxetine hydrochloride in adults[9] and sertaline in children[10], proved to be effective in more than 50% of patients with refractory to the "classic" NMS therapy. The authors hypothesized that the beneficial effects of these drugs may be attributable to the enhancement of serotonergic transmission and the down regulation of the serotonin receptors in the CNS. At present, with the current techniques, the direct estimation of the central serotonergic activity in humans is impossible. A less direct method would be the calculation of the serotonin in the cerebrovascular fluid (CBF). The need for serial serotonin CBF levels estimation necessitates multiple CBF paracenteses, which is technically difficult and unethical in experimental studies for humans. It is well known that the release of serotonin from projections of the raphe nuclei to the hypothalamus results in prolactin and adreno-corticotropic hormone (ACTH) secretion from the pituitary[11]. Subsequently the release of ACTH results in cortisol secretion from the adrenals. The purpose of our study was to investigate the participation of the central serotonergic activity in humans with NMS by comparing the changes of cortisol and prolactin plasma levels in patients with positive and negative tilt test.

Patients and methods

Forty-six patients (46) referred to the outpatient clinic of the 2nd Cardiologic Department of Onassis Cardiac Surgery Center, with a history of recurrent unexplained syncope, were included in the study. Syncope was defined as the sudden and complete loss of consciousness with concomitant loss of postural tone. Before tilt test all patients underwent neurologic and cardiologic evaluation with:
• complete history and clinical examination
• 12-lead electrocardiography
• echocardiography
• 24-hour ambulatory electrocardiography monitoring

No neurologic or cardiac disease was found in any of the patients. No patients were undergoing any

medical treatment. All tilt tests were performed between 11.00 and 15.00 hours with standard conditions of temperature and dim light. All patients had fasted for at least 12 hours. Heart rate and rhythm was continuously observation with a 2-lead monitor during the tilt test. Blood pressure was measured with an automatic pressure manometer whilst a standard mercury pressure manometer was used when symptoms appeared. All tilt tests were done according to the following protocol:

Thirty minutes before the tilt test, a venous cannula was inserted into a peripheral vein, in order to obtain blood samples for cortisol and prolactin plasma levels estimations and to administrate drugs or normal saline in case of syncope. The patency of the venous cannula was maintained by slow infusion of normal saline (<50 cc throughout the tilt test).

All patients were placed in supine position for 10 minutes for baseline recordings of blood pressure and heart rate. Blood samples were collected for baseline estimations of cortisol and prolactin plasma levels.

After 10 minutes in supine position patients were tilted in head-up position at 60° for 30 minutes or until syncope occurred.

We considered an episode of syncope or pre-syncope associated with a sudden drop of systolic blood pressure <80 mmHg with or without bradycardia (<50 beats per minute) as a positive tilt test. Blood samples for cortisol and prolactin plasma level estimations were collected before (baseline), 10, 20 and 30 minutes after the beginning of the tilt test (samples 1, 2, 3 and 4). If syncopal symptomatology developed, the patient was returned to the supine position and blood samples were taken 5 and 10 minutes after the event (samples 3 and 4).

Blood samples were centrifuged at 3000 rads/minute and the plasma stored at −7^{0}C until estimations were obtained using commercially available radioimmunoassay kits (Shono Diagnostic Coisins Switzerland for prolactin and Diagnostic System Laboratories Webster Texas for cortisol). The analysis of variance for repeated measures (ANOVA) was used for the statistical analysis of the results.

Results

Eighteen (18) of the 46 patients had a positive response at the 15±4 minute of the tilt test (minimum 6th, maximum 25th minute). The remaining 28 patients completed the tilt test without symptoms.
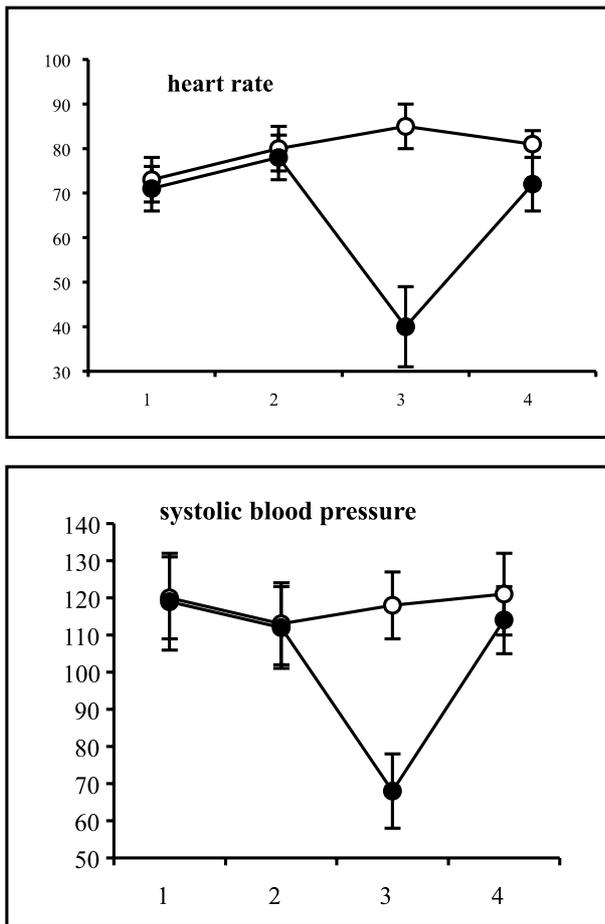
Figure 1. Heart rate (beats per minute) and systolic blood pressure (mm Hg) in patients with positive (filled circles) and negative (open circles) tilt test.

The patients of the two groups (Group A with negative tilt test and Group B with positive tilt test) were of similar ages (42±17 years in Group A and 35±16 years in Group B, p=0.15).

At baseline there were no significant differences in the mean systolic blood pressure values (120±11 mm Hg in group A vs 119±13 in group B, p=0.66) and heart rate (73±6 bpm in group A vs 71±5 in group B, p=0.25) between groups. Tilting until the 10th minute, provoked a similar increase in heart rate and similar drop in systolic blood pressure in the 2 groups (Figure 1). During syncope both variables were significantly reduced in Group B.

Plasma levels of cortisol and prolactin are shown in Table 1 and the results of the statistical analysis in Table 2. Baseline cortisol and prolactin plasma levels were similar in the two groups. In both groups tilting caused a small increase in cortisol plasma levels. The cortisol plasma level increases were more pronounced in Group B after syncope than in Group A: 123±44 ng/ml 5 minutes after syncope in Group B vs 91±44 at the 20th minute in Group B (sample 3, p<0.05) and 142±44 ng/ml 10 minute after syncope in Group B vs 93±45 at the 30th minute in Group A, (sample 4, p<0.05), (Figure 2). Baseline prolactin plasma levels were similar in the 2 groups. In Group A prolactin remained stable throughout the tilt test. In Group B prolactin increased significantly after syncope: 16.9±9.8 at the 5th minute after syncope in Group B vs 7.7±3.5 at the 20th minute in Group A (sample 3, p<0.05) and 19.4±9.0 at the 10th minute after syncope

Table 1. Cortisol and prolactin plasma levels (mean ± SD) in ng/ml.

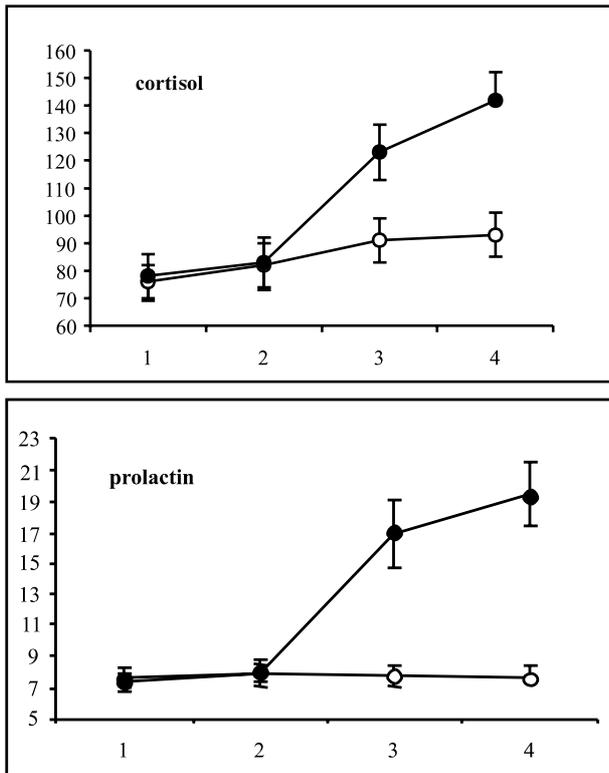| **Cortisol plasma levels**				
Tilt table result	1	2	3	4
Negative N=28	76±32	2±41	91±44	93±45
Positive N=18	78±35	83±37	123±44	142±44
Prolactin plasma levels				
Tilt table result	1	2	3	4
Negative N =28	7.6±3.2	7.9±3.4	7.7±3.5	7.6±3.9
Positive N =18	7.3±2.6	7.9±3.2	16.9±9.8	19.4±9.0

Sample 1: baseline (before tilting).
Sample 2: 10 minutes after tilting.
Sample 3: 20 minutes after tilting in patients with negative tilt test and 5 minutes after syncope in patients with positive tilt test.
Sample 4: 30 minutes after tilting in patients with negative tilt test and 10 minutes after syncope in patients with positive tilt test.

Figure 2. Cortisol and prolactin plasma levels (in ng/ml, mean values ± standard error) in patients with positive (filled circles) and negative (open circles) tilt test.

in Group B vs 7.6±3.9 at the 30th minute in Group A (sample 4, p<0.05). The differences in cortisol and plasma levels secretion were statistically significant (ANOVA, F=21.4, p<0.0001 for cortisol and F=42.4, p<0.0001 for prolactin, Table 2). The differences become significant at the 5th and 10th minute after syncope from baseline (planned comparisons, Table 2).

Discussion

Increases in plasma levels of cortisol and prolactin were found, after syncope, during tilt test. This hor-

monal profile during a positive tilt test is similar to that found in humans after administration of drugs that increase central serotonergic activity. Increases in cortisol and prolactin were observed in healthy volunteers after intravenous infusion of m- chloro-phenylpiperazine, a drug that releases serotonin and blocks its uptake[12]. Prolactin and cortisol increases were also found after administration of the specific serotonin releaser d-fenfuramine[13]. The similar hormonal response pattern of a positive tilt test to that obtained by central serotonergic agents supports the hypothesis that there is a transient activation of the central serotonergic system that leads to 1) hypotension through its influence on the sympathetic system and 2) stimulation of pituitary hormone release like prolactin and ACTH that subsequently leads to cortisol release. Increased levels of cortisol and prolactin 5 and 10 minutes after syncope are an indication of central serotonergic stimulation.

All patients with a positive tilt test had significantly elevated cortisol and prolactin plasma levels 5 and 10 minutes after syncope. CNS serotonin increased significantly enough to provoke syncope, exclusively in patients with a positive tilt test (in these patients alone). Positive tilt test response's mean time was 15±4 minutes from the beginning of the test. CNS serotonin activation beginning time differs among patients with syncope. In order to prove the central serotonergic participation in neurocardiogenic syncope, blood samples were drawn 5 and 10 minutes after syncope, associating the increase of cortisol and prolactin plasma levels with CNS activation. This participation seems to have a similar pathway between all patients with positive tilt test, but time of occurrence differs, depending on syncope's beginning.

Lack of any changes in TSH levels after syncope, reported in our previous work[14], indicates that the stimulatory input in the pituitary during syncope is

Table 2. Statistical evaluation results.

	Cortisol		Prolactin	
	F	p	F	p
Results	3.487	0.0685	15.677	0.0003
Time	58.720	<0.0001	40.662	<0.0001
Result × Time	21.400	<0.0001	42.442	<0.0001
Planned comparisons				
1st vs 2nd Sample	0.084	0.77	0.65	0.42
1st vs 3rd Sample	13.27	0.0007	31.86	<0.0001
1st vs 4th Sample	28.00	<0.0001	58.53	<0.0001

specific and that the TRH which stimulates the release of prolactin and TSH from the pituitary is not involved in the release of prolactin during syncope. In other published works during intravenous fenfuramine infusion, peak plasma levels of prolactin and cortisol appear between 15 and 30 minutes from the beginning of the infusion[12,15].

The same results have been reported by Matzen et al[16] who investigated the plasma levels of prolactin, ACTH, cortisol, beta-endorphin, noradrenaline and renin in 50° head-up tilt test, in normal men. They reported increased levels of pituitary hormones during increased vagal activity. Treatment with serotonin receptor antagonist (5HT1 and 5HT2) with cardiovascular variables but attenuated the response of noradrenalin, prolactin, beta-endorphin and renin. The 5-HT3-receptor antagonist ondansetron abolished the adrenomedullary response to hypotension without affecting cardiovascular tolerance or the activity of the pituitary-adrenal axis. The authors concluded that serotonergic mechanisms may be involved in the integrated cardiovascular and endocrine responses to central blood volume depletion during neurocardiogenic syncope in humans. The type of serotonergic receptors being stimulated in the central nervous system during neurocardiogenic syncope are not well known. It seems that more than one receptor type is involved in the stimulation of cortisol and prolactin in humans at the three levels of serotonin neural activity in CNS: the raphe nuclei, the hypothalamus and the pituitary. Most of the available data indicate participation of 5HT1A, 5HT1C, and 5HT2 receptors[17]. Activation of 5HT1A serotonin receptor with the selective agonist 8-OH-DPTA (8-hydroxy-2-di-n-propylamine-tetriline) lowers blood pressure and heart rate[18]. Although many of 5HT1A agonists exhibit affinity for a1 adrenoreceptors, hypotension seems to result from their action on central 5HT1A receptors rather than by a1 adrenorecptor blockade[19]. Beta blocking agents like propranolol and pindolol decreases serotonin synthesis, whereas acute administration of salbutamol, a b2 adrenoreceptor agonist increases brain levels of 5-hydroxyindoleacetic acid, the main serotonin metabolite, an index of central serotonin turnover[20].

On a clinical level, treatment with fluoxetine hydrochloride in adults[9] and sertaline in children[10] prevented neurocardiogenic syncope, refractory to "classic" therapy, in more than 50% of patients. In a randomized, double-blind, placebo-controlled study

serotonin reuptake inhibitor paroxetine hydrochloride prevented vasovagal syncope in patients resistant to or intolerant of previous traditional therapies[21]. During follow-up spontaneous syncope was reported in 17.6% of patients in the paroxetine group as compared to 52.9% in the placebo group (p<0.0001). Only 2.9% of patients asked for the the drug to be discontinued because of severe side effects. The authors concluded that paroxetine was found to significantly improve the symptoms of patients with vasovagal syncope unresponsive to or intolerant of traditional medications and was well tolerated by patients. The therapeutic effects of these drugs may be due to the "down regulation" of serotonin receptors after chronic treatment. Different mechanisms may be responsible for neurocardiogenic syncope in the same patient and this may explain the fact that although the therapeutic response to serotonin reuptake inhibitors is among the highest in the literature there are patients who do not seem to benefit at all. The higher success rate of propanolol than other b-blockers which do not penetrate the blood brain barrier, may be understood on the basis of its 5HT1A receptor antagonistic activity[20]. On a clinical level the increased cortisol and prolactin plasma levels during tilt test would be an indication for a better therapeutic approach of patients with neurocardiogenic syncope with serotonin reuptake inhibitors but this hypothesis needs further investigation.

Conclusion

The increased cortisol and prolactin plasma levels after syncope are an indication of increased serotonergic activity. This may be useful for further approach in this category of patients providing new pathophysiological and possibly therapeutic insights into this common clinical problem.

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