Central Serotonergic Responsiveness in Neurocardiogenic Syncope
A Clomipramine Test Challenge

George N. Theodorakis, MD; Manolis Markianos, PhD; Efthimios G. Livannis, MD; Elias Zarvalis, MD; Panagiota Flevari, MD; Dimitrios T. Kremastinos, MD

Background—Central serotonergic mechanisms appear to participate in the pathogenesis of recurrent neurally mediated syncope. The aim of the study was to investigate the responsiveness of the central serotonergic system by measuring the prolactin and cortisol responses to intravenous administration of the serotonin reuptake inhibitor clomipramine.

Methods and Results—Twenty subjects free of any medical treatment were tested. Twelve had a history of recurrent syncopal attacks and positive tilt test (patient group, mean age 47 ± 18 years, 8 men); 8 subjects without syncope and a negative tilt test result served as control subjects (mean age 49 ± 10 years, 5 men). Twenty-five milligrams of clomipramine was administered intravenously within 15 minutes, and blood samples were taken at 0, 15, 30, 45, and 60 minutes. Two days later, a tilt test was performed at 60 degrees for 30 minutes and blood samples were taken at 0, 10, 20, and 30 minutes. During the clomipramine challenge, plasma prolactin levels increased in both groups. The levels at 30 minutes were higher in the patient group compared with the control group (17.3 ± 7.2 vs 9.3 ± 7.6 ng/mL, P = 0.05). Similar results were observed for cortisol at 30 minutes (172 ± 15 vs 118 ± 21 ng/mL, P = 0.04) and at 45 minutes (189 ± 20 vs 116 ± 23 ng/mL, P = 0.03). The tilt test was positive in 8 (67%) out of 12 of the patient group and negative in all control subjects. In the samples taken during the tilt test, significant increases in prolactin and cortisol were observed only in the subjects with positive tilt test results.

Conclusions—Patients with a history of neurocardiogenic syncope show a higher responsiveness of the central serotonergic system to clomipramine challenge. The results support the view that central serotonergic mechanisms are involved in the pathophysiology of the syndrome. (Circulation. 1998;98:2724-2730.)

Key Words: syncope ■ brain ■ transplantation ■ nervous system

The exact cause and pathophysiological mechanism of neurocardiogenic syncope remain uncertain. Many mechanisms have been implicated, including central and peripheral participation. Some peripheral-central involvement such as the Bezold-Jarisch reflex explains some aspects of the pathogenesis, but this does not answer all the questions about the pathogenesis of the disease. In this respect, neurocardiogenic reaction in heart-transplanted patients with denervated heart is still unexplained.

A number of studies have shown that central serotonergic pathways participate in the regulation of blood pressure. Brain stem regions including the nucleus tractus solitarius and the anterior hypothalamic preoptic region are involved in the cardiovascular control and contain a dense population of serotonergic neurons.

Drugs that enhance the central serotonergic activity such as fenfluramine, clomipramine, 5-hydroxytryptophan, and flesinoxan have been shown to increase the plasma levels of prolactin and cortisol and have been used to evaluate the reactivity of that system. Recently, we reported significant increases in prolactin and cortisol during vasovagal reaction induced by the tilt test, a fact that indicates participation of the central serotonergic system.

The aim of this study was to investigate the central serotonergic response in patients with neurocardiogenic syncope and in control subjects by measuring the plasma prolactin and cortisol responses to intravenous administration of clomipramine. Clomipramine, a drug that enhances serotonergic activity by inhibiting 5-hydroxytryptamine (5-HT) reuptake, has been used as a probe to study the serotonergic system responsiveness by measurement of hormonal responses to intravenous administration.

Methods

Patients
Twenty subjects were studied: 12 with a positive history of >2 syncopal spells in the last 6 months and positive tilt test results (patient group: 8 men, mean age 47 years, SD = 18) and 8 with a...
negative history of syncope and negative tilt test results (control group: 5 men, mean age 49 years, SD = 10).

Clinical examination, 12-lead ECG, and echocardiography study were performed in all subjects, and in 2 patients an electrophysiological study was done for evaluation of the syncopal attacks.

All subjects were tested without any medical treatment. Patients were asked to stop medical therapy for at least 7 days before the test.

Protocol
A venous cannula was inserted into a peripheral vein, and subjects were placed in supine position for 30 minutes. Continuous ECG monitoring was implemented. Noninvasive automated arterial blood pressure was performed (Dinamap, Critikon) and the blood pressure and heart rate were recorded at 5-minute intervals. After baseline measurements of the heart rate and arterial blood pressure were made, a blood sample of 5 mL was taken. For each subject, 150 mL of normal saline solution containing 25 mg of clomipramine was administered over a 15-minute period beginning immediately after the collection of the baseline sample. Further, blood samples were taken at 15, 30, 45, and 60 minutes.

Forty-eight hours after this test, subjects were head-up tilted at 60 degrees for 30 minutes on a tilt table with a foot plate support according to the protocol we have previously described.14 It includes the heart rate and arterial blood pressure monitoring and a venous cannula insertion 30 minutes before the test. Blood samples were taken at baseline in supine position and thereafter every 10 minutes. If syncope developed, the subject was put in the supine position and an additional sample was taken after 5 minutes.

Tests were defined as positive if syncope or presyncope developed, preceded by bradycardia or asystole (cardioinhibition), hypotension, or mixed reaction with bradycardia and hypotension. During the tilt test, blood samples were taken from all control subjects and from only 9 of 12 subjects of the patient group because 3 of them, with positive tilt test results, refused the blood sampling. Plasma was separated by centrifugation stored at -30°C until estimations.

The hormone levels were estimated with the use of commercially available radioimmunnoassay kits (Serono Diagnostics for prolactin and Diagnostic System Laboratories for cortisol). The interassay and intra-assay coefficients of variation for all estimations were <5%. All subjects were informed about the experimental nature of the study and gave informed consent. Two of the authors were included in the control group. The study protocol was approved by the Ethics Committee of the Hospital.

Statistical Analysis
Two-way ANOVA with repeated measures was used for statistical evaluation of the hormone responses, followed by planned comparisons. A value of \(P<0.05\) was considered significant.

Results
Clomipramine Infusion
Five subjects from the 12 of the patient group and 3 of the 8 control subjects had gastrointestinal discomfort during clomipramine infusion that resolved after 2 to 3 hours. One subject who did not tolerate the infusion was not included in the study.

No changes in mean blood pressure were observed during clomipramine infusion from baseline or between the groups except for a significant increase in the patient group 10 minutes after the beginning of infusion (Figure 1).

Heart rate tended to be lower after infusion in both groups, with no significant changes from baseline or between the 2 groups (Figure 1).

The prolactin and cortisol response patterns are shown in Figure 2 and the results of the statistical analysis in the Table. Regarding prolactin, significant increases were observed in both groups, the response at 30 minutes being significantly higher in the patient group (planned comparison, \(P=0.05\)).

Cortisol also increased in both groups, but in the patient group the cortisol plasma levels were higher than in control subjects at 30 minutes (172±15 vs 118±21 ng/mL, \(P=0.04\)) and at 45 minutes (189±20 vs 116±23 ng/mL, \(P=0.03\)).

Tilt Testing
Eight (67%) subjects out of 12 of the patient group reproduced a similar episode of syncope during the tilt test. Two had a pure cardioinhibitory response and the rest had mixed reaction. Tilt testing was negative in all control subjects.

As mentioned in the “Protocol” section, blood samples during the tilt test were taken from the 8 control subjects and from 9 of the 12 patients (3 patients refused the blood sampling), 5 of whom had a positive tilt test result and 4 negative.

Prolactin in the patient group with positive tilt test results increased significantly (\(P<0.001\)) after syncope (20.5±8 ng/mL) compared with control subjects (5.7±3.3 ng/mL) and with the patient group with negative tilt test results (10.4±5.8 ng/mL, Figure 3).

Cortisol also showed a similar pattern of changes. Cortisol in the patient group with positive tilt test results increased during the test.
significantly (P<0.02) after syncope (170±65 ng/mL) compared with control subjects (127±67 ng/mL) and with the patient group with negative tilt test results (135±48 ng/mL, Figure 3).

Analysis of Clomipramine Infusion According to Tilt Test Result
The prolactin and cortisol responses to the clomipramine infusion were similar in the 8 patients who exhibited positive tilt results and the 4 patients with negative results (P=NS, Figure 4).

Discussion
This study shows that patients with a typical history of recurrent syncope have a different hormonal response to clomipramine challenge compared with control subjects. Hypothalamic pituitary-adrenal axis hormones and prolactin secretion are in part regulated by 5-HT inputs, and their responses to acute administration of 5-HT agents are mediated, at least in part, by 5-HT mechanisms. The acute clomipramine administration blocks the reuptake of the serotonin (5-HT) in the synapse space and thus increases stimulation of the serotonin receptors.

Central serotonergic neurons appear to be important in the central neural regulation of cardiovascular function. There are many studies that have suggested that the activity of central serotonergic nerves elevates arterial blood pressure, although others reported depressor effects.

Stimulation of 5HT_1A postsynaptic receptors by 5HT_1A agonists increase blood pressure and sympathetic nerve discharge. In contrast, the selective 5HT_1A receptor stimulation decreases blood pressure and heart rate by a centrally mediated decrease in sympathetic tone and an increase in vagal tone. Serotonergic receptors are found in the nucleus tractus solitarius in the raphe nuclei and into the ventrolateral area. In animals, most 5HT_1A receptors are found in the ventrolateral pressor area.

The exact serotonergic mechanism involved in the pathogenesis of neurocardiogenic syncope is unknown. From animal experiments, it appears that 5HT_1A postsynaptic receptors might be important. When serotonin synthesis in central nervous system in animals is blocked by p-chlorophenylalanine, the hypotensive phase after hemorrhage is attenuated. This hypotensive phase is induced by vagally mediated stimulation. Activation of 5HT_1A serotonin receptors with the selective agonist 8-hydroxy-2-(di-n-propylamino)tetrolan (8-OH-DPAT) lowers blood pressure and heart rate. Although many 5HT_1A agonists exhibit affinity for α_1-adrenoceptors, hypotension appears to result by their action on central 5HT_1A receptors rather than by α_1-adrenoceptor blockade. 5HT_1A receptors and other subtypes such as 5HT_2c are also involved in the stimulation of prolactin and cortisol in humans at the 3 levels of 5HT neural activity, the raphe nuclei, the hypothalamus, and the pituitary.

In rats it has been shown that acute administration of propranolol decreases synthesis of serotonin, whereas acute administration of salbutamol, a β-blocker agonist, increases brain levels of 5-hydroxyindoleacetic acid, the main serotonin metabolite, an index of central serotonin turnover. It is believed that β-blocker agents such as propranolol, pindolol, and penbutolol exhibit their antihypertensive properties partially by blocking central 5HT_1A receptors. These properties may be involved in the therapy of neurocardiogenic syncope. β-Blocking agents, penetrating or not penetrating the central nervous system, have been used to

Results of Statistical Evaluation of Prolactin and Cortisol Response Patterns to Intravenous Clomipramine Administration Between Groups With Positive or With Negative History of Neurocardiogenic Syncope

<table>
<thead>
<tr>
<th>Effect</th>
<th>Prolactin</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Group</td>
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<td>0.05</td>
</tr>
<tr>
<td>Time</td>
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<td>0.0001</td>
</tr>
<tr>
<td>Interaction</td>
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<td>0.15</td>
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</table>

Planned comparisons

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<tr>
<th>Time, 0 vs 15 min</th>
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<th>P</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.39</td>
<td>0.25</td>
<td>0.80</td>
<td>0.38</td>
</tr>
<tr>
<td>Time, 0 vs 30 min</td>
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<td>4.96</td>
<td>0.04</td>
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<tr>
<td>Time, 0 vs 45 min</td>
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<td>0.18</td>
<td>5.74</td>
<td>0.03</td>
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<tr>
<td>Time, 0 vs 65 min</td>
<td>0.82</td>
<td>0.37</td>
<td>3.87</td>
<td>0.06</td>
</tr>
</tbody>
</table>

ANOVA with repeated measures was applied followed by planned comparisons to identify time when significant differences occurred between groups.
treat these patients.\textsuperscript{14,28} It is unknown whether the therapeutic efficacy between $\beta$-blocking agents penetrating or not penetrating the central nervous system is different. Another aspect of the actions of serotonin on cardiovascular control has been explored by Lehnert et al.\textsuperscript{29} and Verrier.\textsuperscript{30} They showed that the increase of brain serotonin levels in cats suppressed the efferent sympathetic outflow. They also concluded that enhancement of central serotonergic neurotransmission can reduce the susceptibility to ventricular fibrillation mediated through a decline in sympathetic neural traffic to the heart.

The higher responsiveness to clomipramine challenge of patients with positive history of neurocardiogenic syncope indicates that these subjects have higher serotonergic reactivity to afferent inputs and thus they are more likely to have a syncopal event caused by excessive hypotension and bradycardia.

Head-up tilt testing, performed 48 hours after the clomipramine infusion, reproduced positive results in the patient group in 67\% (8 out of 12), whereas the tilt results remained negative in all the subjects of the control group. We do not think that clomipramine administration had any influence in the tilt test results, first because the half-time of clomipramine is $<$20 hours,\textsuperscript{10,31} and second because the acute serotonergic stimulation aggravates vasovagal reaction.\textsuperscript{32,33} The therapeutic effect of the serotonin reuptake inhibitors is seen after 4 to 6 weeks of therapy, when the postsynaptic serotonin receptors are downregulated because the continuous increase in extracellular serotonin concentration leads to a progressive decrease in postsynaptic receptor density.\textsuperscript{34,35}

This study also shows that even though part of the patient group reproduced syncope at tilt testing, the hormonal reactivity to serotonergic challenge in both positive and negative tilt test subgroups was the same. This finding supports the aspect that patient history of recurrent syncope might be more accurate than tilt table testing to evaluate these individuals. In a recent study, Fitzpatrick et al.\textsuperscript{36} showed that patients with recurrent syncopal episodes were more likely to have positive tilt test results than other groups of patients with a single episode or structural heart disease. Sheldon et al.\textsuperscript{37} reported that the probability of recurrence of syncope after positive tilt test results was directly related to patient history. The number of preceding syncopal spells was the most powerful predictor of recurrence of syncope. Both studies are in agreement with our findings that show that patients with recurrent syncope have specific hormonal characteristics, suggesting a central nervous system serotonergic involvement.

Hormonal response during tilt testing also showed a significant increase of prolactin and cortisol in the group who developed syncope. This was not found in the group with positive history but negative tilt test results who had the same response as the control group. We have found similar results in a previous study in which we showed that during vasovagal

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Prolactin (PRL) and cortisol (CORT) plasma levels during tilt test according to tilt test results: Eight subjects with negative history of neurocardiogenic syncope and negative tilt test results (control group, \(\bullet\)); 5 subjects with positive history and positive tilt test results (\(\bigcirc\)); and 4 subjects with positive history but negative tilt test results (\(\square\)).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Prolactin (PRL) and cortisol (CORT) plasma levels during clomipramine infusion in patient group according to tilt test results. Similar patterns of both hormones were found in patients who had negative tilt test results (\(\square\), 4 patients) and in patients with positive tilt test results (\(\bigcirc\), 8 patients). CMI indicates clomipramine infusion.}
\end{figure}
reaction the plasma levels of prolactin cortisol and growth hormone were increased in subjects who developed syncope. In that study, we also measured the thyroid-stimulating hormone plasma levels, which remained unchanged, suggesting that the observed hormonal release was specific because the hypothalamic thyrotropin releasing hormone, which stimulates the release of both prolactin and thyroid-stimulating hormone from the pituitary, was not involved in the release of prolactin during syncope.12

Matzen et al38 examined during 50 degree head-up tilt testing in normal men the hormonal levels of prolactin, adrenocorticotropic hormone, cortisol, β-endorphin, norepinephrine, and plasma renin activity during development of syncope. They found that the pituitary hormones were markedly increased during vasovagal reaction, as in our study. Administration of 5HT1 or 5HT2 receptor antagonists did not affect heart rate or blood pressure responses but did markedly attenuate tilt-induced changes in plasma noradrenaline, prolactin, β-endorphin, and plasma renin activity. Blockade of the 5HT1 receptors (with ondansetron) abolished the adrenomedullary response to tilt-induced hypotension without affecting cardiovascular tolerance or pituitary adrenal response. They concluded that central serotonergic mechanisms may be deeply involved in the integrated cardiovascular and endocrine responses to central blood volume depletion in humans.

Except for the central serotonergic system, the role of other parts of the central nervous system in the pathogenesis of the vasovagal reaction has been investigated by many authors. The excessive release of vasopressin or endothelin-1 from the hypothalamus during vasovagal reaction might be another aspect of the central nervous system involvement. The release of these hormones seems to be regulated by afferent signals from the peripheral receptors.39 Other investigators have shown that the vasopressin release is independent of the afferent cardiac signals.40

The role of δ-opiate receptors has been reported by other investigators. Using renal nerve activity as a sympathetic efferent activity in conscious rabbits, they found that opioid receptor blockade with naloxone reverses the hypotension and the depressed renal activity induced during hemorrhage.41,42

In a recent study, Wallbridge et al43 examined 24 patients with history of unexplained syncope and found an increase in plasma noradrenaline, prolactin, and the depressed renal activity induced during hemorrhage. They concluded that central serotonergic mechanisms may be deeply involved in the integrated cardiovascular and endocrine responses to central blood volume depletion in humans.

From all the above findings, questions have arisen whether the existence of the Bezold-Jarisch reflex is always essential in the development of the neurally mediated syncope. Fitzpatrick et al4 reported syncopal episodes during tilt testing in humans with recent transplantation in which total heart denervation was expected. However, it is believed that the activation of central nervous system activation is the main regulator that induces or does not induce vasovagal reaction.1 It is well known that emotional or painful stimuli can produce vasovagal reaction with sinus asystole or bradycardia with hypotension. Cortical inputs may interface into the hypothalamus and other places in the brain either to potentiate or to eliminate an afferent cardiac or noncardiac input. Cortical inputs may replace or mimic cardiac or peripheral afferent signals and thus introduce or not introduce a vagal reaction.

The relation of neurally mediated syncope to psychiatric illness has also been studied. In a study by Kapoor et al,47 174 patients with recurrent unexplained syncope episodes were subjected to a diagnostic interview schedule. The diagnostic interview schedule suggested a psychiatric diagnosis in 24%, with major depression in 12% and somatization, panic, or anxiety disorder in another 12%. Thus it could be indirectly postulated that there is a “vicious circle” in which syncope can lead to psychological distress and dysfunction and that on the other hand, syncope can apparently result from anxiety and depressive disorders.48

Central serotonergic systems have been evaluated with clomipramine and other challenge drugs in patients with psychiatric disorders such as major depression or panic disorders. Most of them have shown a significant increase in prolactin and cortisol in patients with panic disorders compared with normal control subjects as a response to drug challenge. This finding implies that increased serotonergic responsiveness exists in these situations.49,50

However, a wide range of disorders from the simple emotional stress to psychiatric illnesses can cause syncope. It is uncertain whether these findings indicate an association between recurrent syncope attacks and psychiatric illness.

Clinical Implications
Fluoxetine, sertraline hydrochloride, methylphenidate, and nefazodone have been used therapeutically in some cases of vasovagal patients resistant to conventional medical treatment.51–54 In the future, our findings can be used to investigate patients with recurrent syncope and negative tilt test results. The diagnostic tool currently used for identification of patients with neurocardiogenic syncope is tilt testing with or without drug challenge. Most series show a sensitivity ranging from 20% to 75%, according to the population subjected to this test.55–58 The combination of the history of recurrent syncope spells and the information taken from the serotonergic profile patients may increase the diagnostic yield and the ability to identify subgroups of patients. The use of serotonergic drugs as challenge agents during tilt testing to unmask negative tilt test results in patients is another potential application.
In conclusion, our results indicate that patients with a positive history of recurrent syncope exhibit a specific hormonal response during serotonergic challenge. This suggests that serotonergic activation is involved in the pathogenesis of neurally mediated syncope. These hormonal responses might be useful for further approach to this category of patients, providing new pathophysiologic and possibly therapeutic insights in this disease.

References

Serotonergic Activity and Neurocardiogenic Syncope

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_Circulation_. 1998;98:2724-2730
doi: 10.1161/01.CIR.98.24.2724

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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