Neurally mediated syncope is one of the most common types of syncope (1–3). Head-up tilt testing with or without drugs is the clinical tool of choice for the diagnosis of this syndrome (4).

Although a number of central mechanisms may play a role in neurally mediated syncope, no drug acting on the central nervous system has been tested as a challenge during head-up tilt testing. In a previous study, we found increases in plasma prolactin and cortisol after a vasovagal reaction during the head-up tilt test, suggesting participation of central serotonergic activation (5). In a recent study, we found that patients with a positive history of neurocardiogenic syncope had higher prolactin and cortisol responses to clomipramine infusion compared with normal subjects, indicating a more sensitive central serotonergic system in the former group (6). Acute clomipramine administration blocks the reuptake of serotonin (5-HT) in the synapse space and increases stimulation of the 5-HT receptors. Hypothalamic pituitary-adrenal axis hormones and prolactin secretion are in part regulated by 5-HT inputs, and their responses to early administration of 5-HT agents are mediated, at least in part, by 5-HT mechanisms (7,8).

The purpose of our study was to test the hypothesis that an increase of 5-HT in the central nervous system can facilitate syncope during head-up tilt testing in patients with a history of neurocardiogenic syncope. For this purpose, we used intravenous clomipramine administration during the head-up tilt test in an attempt to investigate the usefulness of this new test in patients with a history of neurocardiogenic syncope. To evaluate the central serotonergic responsiveness, we measured plasma prolactin and cortisol in samples taken during the test.

METHODS

Patients. The study group consisted of 77 subjects. Fifty-five of them (mean age 40 ± 17 years, 23 men) had a positive history of neurocardiogenic syncope (patient group). We considered as a positive history of recurrent neurocardiogenic syncope the presence of two or more
syncopal spells in association with symptoms of autonomic dysfunction such as pallor, nausea or sweating. The mean number of syncopal episodes during the last six months was 3.7 ± 2. All patients with a positive history were seen in the outpatient clinic for the evaluation of their syncopal episodes. A thorough clinical evaluation, 12-lead electrocardiography, echocardiography and electrophysiologic study, when needed, ruled out any structural heart disease, whereas neurologic diagnostic procedures (i.e., electroencephalography, computed tomography) performed when clinically indicated ruled out any neurologic disease in patients with neurocardiogenic syncope.

A group of 22 control subjects (mean age 46 ± 15 years, 12 men) with nonspecific symptoms, no history of syncopal attacks in their medical records and no evidence of structural heart disease served as the control group.

Protocol. All patients were tested with no medical treatment for at least one week. All patients underwent two consecutive head-up tilt tests, with a 24-h interval between the tests. The first basic head-up tilt test (B-HUT) was performed using the protocol currently used for the evaluation of neurocardiogenic syncope in our Cardiology Clinic (9), and the second head-up tilt test included intravenous clomipramine administration (Clom-HUT). All tilt table tests were performed between 8:00 AM and 1 PM, after the subjects had been fasting for at least 12 h. The subjects were connected to a standard electrocardiographic (ECG) monitor for continuous observation of heart rate and rhythm. Arterial blood pressure was also continuously monitored with a Finapress noninvasive blood pressure system. An automatic arterial blood pressure sphygmomanometer was also used to confirm the blood pressure measures every 5 min throughout the test and continuously during symptoms. Thirty minutes before the test, a venous cannula was inserted into a forearm vein.

The subjects were placed in the supine position for 10 min for baseline ECG and blood pressure recordings and then tilted to a head-up position at 60° for 30 min on a foot plate support (passive phase). If a positive response to the upright tilt test occurred during the initial upright tilt, patients were returned to the supine position and the test was terminated. If 30 min of passive tilt testing was completed without a positive response, patients were returned to the supine position for 10 min and upright tilting was repeated for 15 min with intravenous infusion of isoproterenol (infusion rate 2 μg/min, increased until the heart rate reached the target of 130 beats/min).

The second day, all subjects underwent a second test at 60° for 20 min, after a 10-min rest in the supine position. At the start of the tilt test, intravenous infusion of 5 mg of clomipramine was given over the first 5 min.

Blood samples from the venous cannula, for the estimation of cortisol and prolactin plasma levels, were taken at baseline and at 5, 10 and 20 min during Clom-HUT.

The hormone levels were estimated using commercially available radioimmunoassay kits (Serono Diagnostics [Rome, Italy] for prolactin and Diagnostic System Laboratories [Texas] for cortisol). The interassay and intra-assay coefficients of variation for all estimations were <5%.

A test was regarded as positive if it succeeded in reproducing the patient’s syncope or presyncope, associated with an abrupt fall in systolic blood pressure <80 mm Hg or concomitant bradycardia (heart rate <50 beats/min), or both. The test was regarded as negative if it was completed with no symptoms. Positive responses were classified into vasodepressor (defined as hypotension, without significant bradycardia) cardioinhibitory (bradycardia, without associated hypotension) and mixed type (hypotension, followed by bradycardia).

All subjects were informed of the experimental nature of the study and gave their written consent. Two of the authors were included in the control group. The Ethics Committee of the Hospital approved the study protocol.

Statistical analysis. Two-way analysis of variance with repeated measures was used for statistical evaluation of the hormone responses, followed by planned comparisons (STATISTICA, version 5.0). A value of $p < 0.05$ was considered significant.

RESULTS

B-HUT. Twenty-nine (53%) of the 55 subjects in the patient group had a positive response during B-HUT (19 during the passive tilt test and 10 during tilt testing with isoproterenol). The mean time to syncope was 15 ± 10 min (minimum 2 min, maximum 30 min) during the passive tilt test and 5 ± 2 min during tilt testing with isoproterenol (minimum 3 min, maximum 12 min). The type of the positive response was cardioinhibitory in 8 patients, vasodepressor in 8 and mixed in the remaining 13. None of the 22 control subjects had a positive B-HUT.

Head-up tilt test with clomipramine. Forty-four (80%) of the 55 subjects in the patient group had a positive response during Clom-HUT. The mean time to syncope in patients with a positive Clom-HUT response was 9 ± 3 min (minimum 5 min, maximum 17 min). The type of the positive response was cardioinhibitory in 9 patients, vasodepressor in 13 and mixed in the remaining 22. One subject of the 22 in the control group had a positive Clom-HUT response of the vasodepressor type.

Comparison between the two test responses. A comparison of the results of the two tests in the patient group is shown in Figure 1. Twenty-one control subjects had a negative response during both B-HUT and Clom-HUT.
Comparison between the types of responses in the positive tests. Sixteen (64%) of 25 subjects in the patient group reproduced positive responses in both tests, with the same response modality (2 [25%] of 8 patients with a cardioinhibitory type, 9 [69%] of 13 with a mixed type and 5 [63%] of 8 with a vasodepressor type).

Hormonal responses during the clomipramine test. During the clomipramine test, blood samples for cortisol and prolactin plasma levels estimations were drawn from 61 subjects (41 from the patient group and 20 from the control group). The remaining subjects refused blood sampling.

The patients who had syncope (n = 32) during Clom-HUT showed a significant increase in plasma levels of both hormones, as compared with the control subjects with a negative basic tilt test (10–14). Twenty-five (86%) of the 29 patients with a positive basic tilt test also reproduced a positive response with the clomipramine test. The sensitivity and specificity of this test in evaluating neurocardiogenic syncope, compared with existing tests, is worth studying in future large-scale trials with greater numbers of patients and control subjects.

Central serotonergic activity and syncope. The central nervous system plays a major role in the homeostasis of the cardiovascular system. Medullary nuclei contain the major excitatory (pressor) and inhibitory (depressor) regions (15). The functional role of the nucleus tractus solitarius and other brain stem nuclei is significant in the cardiovascular control of peripheral vascular resistance and heart rate.
Serotonergic receptors are found in the nucleus tractus solitarius, in the raphe nuclei and within the ventrolateral area (16–18).

In humans, drugs that enhance central serotonergic activity, such as clomipramine, fenfluramine and 5-hydroxytryptophan, have been shown to increase the plasma levels of prolactin and cortisol and have been used as a probe to assess the reactivity of the system (19–23). It has been proven that as low as 10 mg of clomipramine administered intravenously leads to an increase in plasma prolactin and cortisol concentrations, suggesting that even small doses can be used as a useful probe of serotonergic function in humans (24). In our study, we administered only 5 mg intravenously, because we assumed that the increase of 5-HT in the central nervous system, along with orthostatic stress, would be sufficient to provoke syncope in patients with neurocardiogenic syncope.

In a previous study, we have shown that plasma levels of cortisol and prolactin increased during the tilt test in subjects who developed syncope (5). In the present study, the statistically significant increased plasma levels of cortisol and prolactin during syncope confirmed the findings of our previous study. In another study, we found that after an intravenous infusion of 25 mg of clomipramine in patients in the supine position, those with a history of recurrent neurocardiogenic syncope had higher cortisol and prolactin during syncope confirmed the findings of our previous study. In another study, we found that after an intravenous infusion of 25 mg of clomipramine in patients in the supine position, those with a history of recurrent neurocardiogenic syncope had higher cortisol and prolactin during syncope confirmed the findings of our previous study.

**Conclusions.** The results of our study show that central serotonergic activation is a major component of neurocardiogenic syncope. Clomipramine infusion during head-up tilt testing can reliably reproduce syncope by enhancing serotonergic activity in the central nervous system. The head-up tilt test with clomipramine may prove to be a valuable, time-saving test for the evaluation of patients with neurocardiogenic syncope.

**References**