Endogenous Opioids and Epinephrine in Nitroglycerin Provocation Tilt Test in Patients with Neurally Mediated Syncope

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SUMMARY

Endogenous opioids and catecholamines are involved in autonomic activity. Nitroglycerin provocation tilt is a useful modality for evaluating neurally mediated syncope. Endogenous opioids and epinephrine might play an important role in nitroglycerin provocation tilt. To investigate whether or not opioids and catecholamines are involved in the pathogenesis of nitroglycerin provocation tilt, we measured the temporal changes of the plasma levels of β-endorphin, norepinephrine, and epinephrine in 64 patients with syncope of unknown etiology, and compared the findings with those of 16 patients who underwent isoproterenol provocation tilt (1-3 µg/min) test with a positive response. We performed a 20 minute control tilt (80°) followed by a nitroglycerin provocation tilt of 20 minutes with the intravenous infusion of nitroglycerin. Nitroglycerin infusion was started at 250 µg/h, and was increased by 250 µg/h every 3 minutes up to 1500 µg/h during the tilt test. β-endorphin, norepinephrine, and epinephrine were measured in peripheral venous blood in the supine position 2, 10, and 20 minutes after the start of the tilt test, and also at the onset of syncope. Twenty-six patients had a positive response to the control tilt (group 1), and 22 patients had a positive response to nitroglycerin provocation tilt (group 2). The remaining 16 patients had a negative response to both control tilt and nitroglycerin provocation tilt (group 3), compared with isoproterenol provocation tilt patients (group 4). β-endorphin and epinephrine only significantly increased in groups 1 and 2 (β-endorphin; from 7.3 ± 3.3 pg/mL to 19.9 ± 17.7 pg/mL, in group 1, P < 0.05; from 7.3 ± 2.9 to 16.5 ± 10.7 pg/mL, in group 2, P < 0.05: epinephrine; from 42 ± 58 pg/mL to 157 ± 161 pg/mL, in group 1, P < 0.05; from 33 ± 25 to 202 ± 252 pg/mL, in group 2, P < 0.05), but not in groups 3 and 4. β-endorphin and epinephrine might participate in the pathophysiology in conventional tilt-induced as well as nitroglycerin provocation tilt-induced syncope in patients with neurally mediated syncope. (Jpn Heart J 2003; 44: 493-503)

Key words: Opioids, Epinephrine, Syncope, Nitroglycerin

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Received for publication September 18, 2002.
Revised and accepted November 21, 2002.
ENDOGENOUS opioids and catecholamines are involved in autonomic activity. Recent studies have shown that the plasma levels of epinephrine and one of the endogenous opioids, β-endorphin, significantly increased in patients with tilt-induced syncope. These findings suggest that either β-endorphin or epinephrine plays a significant role in the pathogenesis of neurally-mediated syncope.

Nitroglycerin provocation tilt is a useful modality for diagnosing neurally-mediated syncope. In addition, isoproterenol infusion during the head-up tilt test has been proposed to be a useful adjunctive method with which to induce neurally mediated syncope in patients with syncope of unknown etiology. Although both provocation tests have been utilized, the mechanism of the nitroglycerin provocation test might be different from that of the isoproterenol test.

In the isoproterenol provocation test, an earlier report revealed that the increase in the plasma levels of β-endorphin or epinephrine was relatively small, compared with that of the head-up tilt test without provocations. However, so far few reports have studied the effect of a nitroglycerin provocation test on the plasma levels of β-endorphin and epinephrine.

To investigate whether or not β-endorphin and epinephrine play important roles in the nitroglycerin provocation test, the temporal changes in the plasma levels of β-endorphin, norepinephrine, and epinephrine were measured during a head-up tilt test in patients with syncope of unknown etiology. The findings were then compared with patients who underwent an isoproterenol provocation tilt test with a positive response.

**METHODS**

**Patients population:** Eighty patients, aged 15-73 years (average, 34 ± 14 years, mean ± SD), were selected from among patients with syncope of unknown etiology, who were referred to either the Self Defense Forces Central Hospital or the National Defense Medical College Hospital. Among these, 38 patients underwent the nitroglycerin provocation test if no symptoms were induced during the 20 minute control head-up tilt test. The remaining 16 patients served as controls, who had a negative 20 minute control head-up tilt test response and a subsequent positive response to the isoproterenol provocation test. Informed consent was obtained from all subjects.

**Head-up tilt test protocol:** All patients underwent the standard tilt test. The patients were all in a fasting condition and medication was withdrawn for at least five half-lives before initiation of the test. A motor driven tilt table with a foot board for weight-support was used for an 80° passive tilt. An intravenous catheter was inserted in an antecubital vein for emergency medication should any be necessary. The head-up tilt test protocol consisted of at least 6 minutes in the supine
position under stable and quiet conditions followed by a 20 minute period of tilting. If syncope occurred, the patient was returned to the supine position as soon as possible to restore consciousness and to avoid any adverse sequela. The heart rate and blood pressure were measured at one minute intervals using an automatic sphygmomanometer. The lead II electrocardiogram was continuously monitored throughout the study. The criterion for a positive test was defined as follows: syncope or presyncope induced during tilt following either a decrease in the systolic blood pressure or a decrease in the heart rate, in comparison to the supine values. If a 20 minute tilt test did not induce clinical symptoms similar to a spontaneous episode of syncope, then the patient was returned to a supine position for at least 15 more minutes. A second tilt test was performed thereafter following the continuous intravenous infusion of nitroglycerin. As controls for a positive tilt response, 16 patients underwent an isoproterenol test.

**Nitroglycerin test:** A nitroglycerin test was also performed according to a method described in a previous report. Briefly, intravenous nitroglycerin at progressively increasing doses was administered starting at 250 $\mu$g/h with successive increments of 250 $\mu$g/h every 3 minutes up to a peak of 1500 $\mu$g/h. Nitroglycerin infusion was begun at 250 $\mu$g/h with the patients in the supine position, and then the patients were tilted. If three minutes of 250 $\mu$g/h infusion during tilt did not induce symptoms, the nitroglycerin infusion was increased by 250 $\mu$g/h every 3 minutes while the patients were kept in a tilt-up position until either presyncope or syncope was induced. If the repeat tilt test with nitroglycerin infusion was continued for up to 20 minutes without inducing any clinical symptoms, the test was classified as ‘negative’. If syncope or clinical symptoms developed, the test was labeled as ‘positive’.

**Isoproterenol test:** An isoproterenol test was performed according to the method described in a previous report. Briefly, intravenous isoproterenol infusion began at 1 $\mu$g/min and the infusion rate of isoproterenol was gradually increased (up to 3 $\mu$g/min) until the heart rate reached 20% above the baseline supine level. If the repeat tilt testing with isoproterenol infusion was continued for up to 20 minutes without inducing any clinical symptoms, the patient was returned to the supine position and the test was classified as ‘negative’. If syncope or clinical symptoms developed, the test was labeled as ‘positive’.

**$\beta$-endorphin and catecholamine assay:** In all patients and control patients, a plastic needle was inserted into an antecubital vein in order to draw blood. Fifteen milliliters of blood was obtained for the assay; 10 mL for $\beta$-endorphin assay and 5 mL for catecholamines. Blood samples were obtained from an antecubital vein as follows: for the control tilt; blood was obtained in a supine position, after 2 minutes of tilt, after 10 minutes of tilt, and after 20 minutes of tilt (at the end of the control tilt), and at the onset of clinical symptoms (presyncope or syncope).
For the nitroglycerin or isoproterenol tests, blood was obtained in a supine position, after 2 minutes of tilt, after 10 minutes of tilt, after 20 minutes of tilt (at the end of a negative test), and at the onset of clinical symptoms (presyncope or syncope). The plasma \( \beta \)-endorphin level was measured by radioimmunoassay according to a modification of the method of Furui, \textit{et al.}\(^7\) Briefly, after centrifugation at 3,000 rpm at 4°C for 5 minutes, the pellet was washed twice with an equal volume of cooled distilled water. The pellet was then extracted with 80% acetone in a 0.1 N solution of HCl by vigorous mixing for 5 minutes and centrifugation at 3,000 rpm for 5 minutes. The resulting supernatant was then decanted and the extract dissolved in 0.2 mL of a standard assay buffer. The \( \beta \)-endorphin content was then measured using \(^{125}\)I. The mean average recovery rate of \( \beta \)-endorphin was 97.7 ± 7.0%. The intra- and interassay coefficients of variation ranged from 4.7% to 5.3% and from 2.7% to 15.0%, respectively. For the catecholamine assay, blood was immediately transferred into chilled 10 mL polyethylene tubes containing ethylene diaminetetraacetic acid (EDTA). The samples were centrifuged at 4°C and 3,000 rpm for 10 minutes. Norepinephrine and epinephrine were then measured after absorption onto the alumina at pH 6.5 using high-pressure liquid chromatography (pg/mL).

**Study protocol:** The patients were allocated into four groups according to the response to control or pharmacological provocation tests. The patients who had a positive response to control tilt were labeled as group 1, and the patients who had a positive response to the nitroglycerin provocation test were labeled as group 2. The remaining patients who showed a negative response to both control tilt and the nitroglycerin provocation test were placed in group 3. The patients who had a positive response to the isoproterenol provocation test served as controls (group 4).

**Statistical analysis:** All data are expressed as the mean ± standard deviation. ANOVA was used for comparisons among the groups. The changes in each parameter throughout the study were compared using repeated measures of ANOVA. The subsequent alpha levels were corrected using the Scheffe method. The frequency distributions were compared using the chi-square test or Fisher's exact test. Differences were considered to be significant if \( P < 0.05 \).

**RESULTS**

Twenty-six patients had a positive response to control tilt and thus were classified as belonging to group 1. Twenty-two patients had positive responses to the nitroglycerin provocation test and were classified as group 2. Sixteen patients showed a negative response to both the control tilt and nitroglycerin provocation test, and they were labeled as group 3. The controls, group 4, consisted of patients
who had a positive response to the isoproterenol provocation test. Selected clinical characteristics of the four groups are shown in Table I. The age, sex, and number of comorbid conditions did not differ significantly among the four groups. The positive patients who belonged to group 1 terminated the tilt test at 13 ± 5 minutes because of syncope or presyncope. In the nitroglycerin test, the mean tilt-test duration was 14 ± 5 minutes for the positive patients (group 2), while 12 ± 4 minutes in the positive patients treated with isoproterenol (group 4). The mean infusion rate of nitroglycerin at the end of tilt in the positive patients was 1053 µg/h. The total mean dosage of nitroglycerin was 0.13 mg. The mean isoproterenol infusion rate was 1.5 µg/min. In addition, no side effects for either nitroglycerin or isoproterenol infusion were observed in groups 2, 3, or 4.

In the control tilt test (before nitroglycerin provocation), heart rate as well as systolic and diastolic blood pressure were not significantly different in the supine position. However, heart rate and systolic blood pressure at the onset of symptoms were significantly lower in group 1 than in the other three groups (heart rate, 61 ± 16 beats per minute at the onset of symptoms in group 1 vs 90 ± 14 beats per minute, 89 ± 16 beats per minute and 88 ± 11 beats per minute at the end of tilt in groups 2, 3, and 4, P < 0.05; blood pressure, 70 ± 24 mmHg at the onset of symptoms in group 1 vs. 104 ± 8 mmHg, 116 ± 10 mmHg, and 115 ± 15 mmHg at the end of tilt in groups 2, 3, and 4, P < 0.05).

In the pharmacological provocation tests, the heart rates in group 4 (isoproterenol provocation group) were significantly higher than those in groups 2 (nitroglycerin provocation group) and 3 in the supine position, at the onset of symptoms, and at the end of tilt. In addition, at the onset of symptoms or at the

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age ± SD</th>
<th>Male (%)</th>
<th>No. Syncope ± SD</th>
<th>Comorbid conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive response to control tilt (group 1)</td>
<td>26</td>
<td>30 ± 14</td>
<td>19 (73)</td>
<td>1.9 ± 0.7</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Nitroglycerin positive response (group 2)</td>
<td>22</td>
<td>35 ± 14</td>
<td>20 (91)</td>
<td>1.8 ± 0.7</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Nitroglycerin negative response (group 3)</td>
<td>16</td>
<td>43 ± 13</td>
<td>10 (73)</td>
<td>1.4 ± 0.8</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Isoproterenol positive response (group 4)</td>
<td>16</td>
<td>30 ± 11</td>
<td>9 (56)</td>
<td>1.7 ± 1.0</td>
<td>2 (13)</td>
</tr>
</tbody>
</table>

Mean ± SD; age, years; No. Syncope, mean number of syncopal spells; comorbid conditions, coronary artery disease, diabetes, heart failure or hypertension; percentage in parenthesis.
end of tilt, the heart rate in group 2 was significantly lower than those in groups 3 and 4 (heart rate, 62±13 beats per minute at the onset of symptoms in group 2 vs. 81±13 beats per minute and 68±9 beats per minute at the end of tilt in groups 3 and 4, *P* < 0.05). No significant difference in systolic blood pressure was seen in groups 2, 3, and 4 either in the supine position or during tilt before symptoms, whereas at the onset of symptoms or at the end of tilt, groups 2 and 4 had a significantly lower systolic blood pressure than group 3 (67±24 mmHg, in group 2; 95±13 mmHg, in group 3; 71±13 mmHg, in group 4; *P* < 0.05). The decreased mean values of the systolic blood pressure at the onset of symptoms did not differ among the three groups (groups 1, 2, and 4).

Both the β-endorphin and epinephrine levels at the onset of symptoms in the positive group with control tilt (group 1) or nitroglycerin treatment (group 2) were significantly higher than those at the end of tilt or at the onset of symptoms in the other two groups (Figures 1 and 2). The β-endorphin and epinephrine levels in groups 1 and 2 significantly increased at the onset of symptoms during the tilt test while they did not increase for groups 3 and 4. Both the β-endorphin and epinephrine levels increased significantly from those measured in the supine position to those at the onset of symptoms only in groups 1 and 2. In groups 3 and 4, neither the β-endorphin level nor the epinephrine level was increased signifi-

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**Figure 1.** Comparison of plasma beta-endorphin levels. Patients who had a positive response to control tilt were labeled as group 1 (G1), and those who had a positive response to the nitroglycerin provocation test were labeled group 2 (G2). The remaining patients who showed a negative response to both the control tilt and nitroglycerin provocation tests were placed into group 3 (G3). Patients who had a positive response to the isoproterenol provocation test were labeled as group 4 (G4).
significantly by the tilt test. In addition, neither the \( \beta \)-endorphin level nor the epinephrine level measured in the supine position and at any sampling point during the tilt test before the onset of symptoms showed any significant differences among the four groups. In contrast, norepinephrine increased similarly and significantly in the four groups during the tilt test (Table II).

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**Figure 2.** Comparison of plasma epinephrine levels. The format is the same as shown in Figure 1.

**Table II.** Summary of Catecholamines and Opioids

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>( \beta )-endorphin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive to control tilt (group 1)</td>
<td>288 ± 22/594 ± 368*</td>
<td>42 ± 58/157 ± 161*#</td>
<td>7.7 ± 3.3/19.9 ± 17.7*#</td>
</tr>
<tr>
<td>Nitroglycerin positive (group 2)</td>
<td>236 ± 120/765 ± 272*</td>
<td>33 ± 25/202 ± 252*#</td>
<td>7.3 ± 2.9/16.5 ± 10.7*#</td>
</tr>
<tr>
<td>Nitroglycerin negative (group 3)</td>
<td>224 ± 82/565 ± 143*</td>
<td>27 ± 12/33 ± 19</td>
<td>7.4 ± 3.4/7.8 ± 3.5</td>
</tr>
<tr>
<td>Isoproterenol positive (group 4)</td>
<td>241 ± 124/700 ± 283*</td>
<td>27 ± 21/55 ± 37</td>
<td>7.5 ± 3.1/7.6 ± 3.5</td>
</tr>
</tbody>
</table>

Mean ± SD; values in a supine position/values at the onset of symptoms or at the end of tilt; pg/mL; *\( P < 0.05 \) vs baseline, #\( P < 0.05 \) vs group 3 and group 4
**DISCUSSION**

The present study demonstrates that the plasma levels of $\beta$-endorphin and epinephrine increased along with a positive response to head-up tilt without pharmacological provocation (group 1) and also with the nitroglycerin provocation tilt test (group 2), but $\beta$-endorphin and epinephrine did not change in a negative response to the head-up tilt test (group 3) and in positive response to the isoproterenol provocation tilt test (group 4). The mean values of $\beta$-endorphin and epinephrine in the supine position and those values during tilt test prior to the onset of clinical symptoms demonstrated no significant differences among the four groups. In addition, the plasma levels of norepinephrine increased to a similar degree during the tilt test among the four groups and there were no significant differences in the plasma norepinephrine levels in the supine position throughout the study.

Although previous studies have reported increases in the $\beta$-endorphin and epinephrine levels in neurally-mediated syncope patients with a positive response to a head-up tilt test, there have been few clinical investigations regarding changes in the $\beta$-endorphin and epinephrine levels during a nitroglycerin provocation tilt test. Several investigators have previously reported that the plasma levels of $\beta$-endorphin and epinephrine increase during a positive response to a head-up tilt test in patients suspected of having neurally-mediated syncope. However, most of these studies were conducted during a head-up tilt test without pharmacological provocation. $^8\text{--}^{10}$ Perez-Paredes, et al. $^6$ measured the plasma levels of $\beta$-endorphin during an isoproterenol provocation tilt test and found no increase in plasma $\beta$-endorphin levels at the time of isoproterenol-induced syncope during a head-up tilt test. This previous observation correlated with our results. A nitroglycerin provocation tilt test is not a brand-new method with which to diagnose neurally-mediated syncope, $^4$ but nitroglycerin provocation is relatively new in comparison to the isoproterenol provocation tilt test. $^{11}$ In a search of the literature over the past 20 years, no reports have examined plasma $\beta$-endorphin levels during a nitroglycerin provocation tilt test. Our findings thus may be the first observation of $\beta$-endorphin levels in a nitroglycerin provocation tilt test.

Whether the rise of $\beta$-endorphin and epinephrine is a result or a cause of tilt-induced syncope was not clarified in this study. $\beta$-endorphin is reported to be an important chemical substance which regulates cardiovascular physiology. $^1,^{12}$ $\beta$-endorphin is mostly located in the hypothalamus and is related to the activity of the nucleus tractus solitarius $^{13}$ and the nucleus ambiguus $^{14}$ which contribute to autonomic nervous activity. Several reports have shown that either mental or physiological stress increased the production of $\beta$-endorphin. $^{15,16}$ Hypotension due to hemorrhaging or negative low body pressure also increased the plasma
levels of β-endorphin.\textsuperscript{16} According to these results, hypotension induced by a tilt test could cause a rise in β-endorphin levels. On the other hand, the increase in the plasma β-endorphin levels preceded the occurrence of tilt-induced hypotension and Wallbridge, \textit{et al}\textsuperscript{8} speculated that β-endorphin may thus be the causative substance for neurally-mediated syncope. However, naloxone, which is a selective β-endorphin inhibitor, could not prevent tilt-induced syncope.\textsuperscript{6} These results thus suggest that β-endorphin is not a causative substance. In the present study, the decrease in systolic blood pressure levels during tilt-induced syncope were identical among the three groups. Since the plasma β-endorphin levels were not reported to increase despite tilt-induced hypotension during an isoproterenol provocation tilt test,\textsuperscript{6} which correlates with our findings obtained in group 4 in this study, it is unlikely that hypotension and syncope resulted due to a rise in the β-endorphin level. However, further investigation is needed to clarify this question. In addition, the role of epinephrine in the pathophysiology of tilt-induced syncope also remains unclear. We previously reported that epinephrine plays a significant role in neurally-mediated syncope.\textsuperscript{17} In addition, Kikushima, \textit{et al}\textsuperscript{3} reported results similar to our findings. However, the results of many reports are conflicting.\textsuperscript{18} Whether the rise of epinephrine in tilt-induced hypotension is a result or a cause, therefore, still needs to be conclusively determined.

Nitroglycerin and isoproterenol provocation tilt testing may induce bradycardia and hypotension in neurally-mediated syncope by different mechanisms, especially, regarding the changes in β-endorphin and epinephrine levels. Since β-endorphin and epinephrine behaved similarly in groups 1 and 2 in this study, the nitroglycerin provocation test was thus found to be an effective physiological provocation regarding the changes in β-endorphin and epinephrine. Even if nitroglycerin-induced β-endorphin may cause hypotension with syncope through vagal stimulation and nitroglycerin may trigger tilt-induced syncope by increasing the epinephrine level, which generally causes β\textsubscript{2} -mediated peripheral vasodilation and increases cardiac contractility, the definitive roles of β-endorphin and epinephrine still need to be further investigated in future studies. However, according to our preliminary results, β-endorphin and epinephrine could be direct or secondary causative mediators to induce syncope so that they play a significant role in the pathophysiology in tilt-induced as well as nitroglycerin-induced syncope in patients with neurally-mediated syncope. In addition, we speculate that endogenous opioids and catecholamines seem to be more specifically involved in neurally-mediated syncope induced by both a head-up tilt test without pharmacological provocation and the nitroglycerin provocation test. However, these substances were less important in isoproterenol-induced syncope.

\textbf{Study limitations:} First, since there are several compounds in the opioid system other than β-endorphin, we should measure all of the opioid peptides to com-
pletely clarify the role of the opioid system in neurally-mediated syncope. However, in most previous human studies, \( \beta \)-endorphin was measured and found to be a representative substance among the opioid peptides. According to these previous studies, we measured \( \beta \)-endorphin levels. Second, \( \beta \)-endorphin is secreted from the central nervous system and therefore \( \beta \)-endorphin levels should be measured in the cerebrospinal fluid, however, an invasive method is necessary to obtain this result. An earlier report showed a good correlation between the plasma level of \( \beta \)-endorphin and that of the cerebrospinal fluid.\(^{19} \) The plasma level of \( \beta \)-endorphin could thus reflect the activity of the opioid system in the central nervous system. Finally, to compare the roles of \( \beta \)-endorphin and epinephrine between nitroglycerin and isoproterenol provocation tilt tests, a random crossover study design including testing with or without naloxone infusion might be necessary. To confirm our findings, we plan to perform such tests in the near future.

**Conclusion:** \( \beta \)-Endorphin and epinephrine may be important causative mediators in the pathophysiology of tilt-induced as well as nitroglycerin provocation tilt-induced syncope in neurally-mediated syncope. The nitroglycerin provocation tilt test might be an effective physiological provocation regarding the changes in endogenous opioids and catecholamines.

This study was presented in part at the 21th annual meeting of the North American Society of Pacing and Electrophysiology held in Washington, DC in 2000.

**REFERENCES**