Triggering Mechanism for Neurally Mediated Syncope Induced by Head-up Tilt Test
Role of Catecholamines and Response to Propranolol

Shuji Kikushima, MD, Youichi Kobayashi, MD, Haruyuki Nakagawa, MD, Takashi Katagiri, MD
Tokyo, Japan

OBJECTIVES
We studied the triggering mechanism for neurally mediated syncope.

BACKGROUND
Although increased transient sympathetic tone is thought to be necessary for the development of neurally mediated syncope, little is known about the triggering mechanism for neurally mediated syncope.

METHODS
Plasma epinephrine (EP) and norepinephrine (NE) levels were assessed in 20 syncope patients during tilt test (80°, 15 min) with and without isoproterenol (ISP, 0.01, 0.02 μg/kg/min). If syncope occurred, propranolol (0.1 mg/kg) was injected.

RESULTS
Eight patients experienced syncope during tilting alone, and 9 patients required ISP for syncope. In the negative response without ISP, NE showed a small statistical 1.7-fold increase at end of tilting and EP did not change during tilting. When syncope occurred during tilting alone, a significant 11.7-fold increase in EP at syncope was registered concomitant with a small 2.5-fold increase in NE. When patients experienced syncope during tilting with ISP, a significant 5.0-fold increase in EP at syncope was registered concomitant with a small 1.7-fold increase in NE. In patients without ISP, propranolol did not interrupt syncope. In patients with ISP, six of eight receiving propranolol responded to tilting negatively.

CONCLUSIONS
An increase of NE levels may result in inhibition of syncope and an EP surge may be a triggering mechanism for neurally mediated syncope. Comparatively low levels of EP may be enough to induce syncope during tilting with ISP compared with tilting alone. Propranolol is not effective in patients without ISP, but it frequently inhibits syncope in patients with ISP. Propranolol (0.1 mg/kg) may be insufficient to block the actions of high levels of circulating EP. (J Am Coll Cardiol 1999;33:350–7) © 1999 by the American College of Cardiology
tion of organic heart disease or used medication. Written informed consent was obtained from all patients.

Preparation. To obtain blood pressure (BP) and blood samples, a cannula (21-gauge) was inserted into the right brachial artery >30 min before the study, and heparinized saline solution (concentration, 10 U/ml) was infused (3 ml/h). A transducer for BP was fastened and calibrated in the midaxillary line over the fourth intercostal space with the patient in the supine position. Another cannula was placed in the left antecubital vein and saline solution was infused (60 ml/h). ISP solution (concentration, 2.0 \( \mu \)g/ml) was also given via this cannula, when necessary.

HUT. HUT was performed using a tilt table with a foot board. Each patient was tilted to an angle of 80° from horizontal until positive response or for a maximum of 15 min. A positive response was defined as syncope or presyncope associated with marked hypotension. If there was no positive response during this 15-min HUT, ISP infusion was started at 0.01 \( \mu \)g/kg/min and then HUT was repeated. If the result was again negative, ISP was increased to 0.02 \( \mu \)g/kg/min, and HUT was repeated again. There was an interval of 10 to 15 min between each HUT. Surface electrocardiogram (ECG) and BP were monitored and recorded at a paper speed of 25 mm/s. Hemodynamic data were obtained as the average measurement of 5-s periods.

Study design. The study design is shown in Figure 1. The protocol consisted of three steps to both eliminate unnecessary blood samples and evaluate the response to PROP, since the reproducibility of positive HUT on the same day has been reported to be high \((8,9)\). If a positive response occurred in the initial step, the protocol turned to second step immediately. In the second step, HUT was performed again under identical conditions. In the third step, the patient was rechallenged with HUT after an injection of PROP.

Blood sampling. When the BP monitoring was judged to be more important than blood sampling, it was given priority, because the same cannula was used for both. The blood sample was immediately stored in ice water, and the plasma was separated by centrifugation. The plasma concentrations of EP and NE were measured by an automated high-performance liquid chromatography analyzer (Tosho Co, Tokyo, Japan) \((10)\). In the initial step, the blood samples were obtained during HUT alone. We sampled blood at 3 min prior to upright posture (baseline) and every 5 min during upright posture. In the second step, the first blood collection during upright posture was started at 5 min before the time when the positive response occurred during the initial step, and then at every minute and at positive response or at the end of the 15-min tilting, in addition to the baseline sample. In the third step, blood was sampled at baseline (pre-PROP and post-PROP) and at positive response or at the end of the 15-min tilting.

Data analysis. Data are presented as mean ± SD. For the purposes of analysis, we classified the patients into two groups, an ISP-independent group and an ISP-dependent group, according to the need for ISP to induce a positive HUT response. The mean rate of increase in CAs for each group was calculated as \(\text{mean postvalue}/\text{mean prevalue} = \text{mean rate of increase (reported as a multiple of the mean prevalue)}\). The differences within the groups were analyzed by repeated-measures analysis of variance followed by Scheffé F test, and the differences between the two groups were analyzed by nonparametric Mann-Whitney U test and chi-square analysis. A p value of <0.05 was considered statistically significant.

RESULTS

Outcome of the initial step. In the initial step, eight patients had a positive response during HUT alone (ISP-independent group) and nine patients required ISP for a

### Abbreviations and Acronyms

- **BP** = blood pressure
- **CA** = catecholamine
- **ECG** = electrocardiogram
- **EP** = epinephrine
- **HR** = heart rate
- **HUT** = head-up tilt test
- **ISP** = isoproterenol
- **NE** = norepinephrine
- **NMS** = neurally mediated syncope
- **PROP** = propranolol

**Figure 1.** Study design. This study consisted of three steps. If a positive response occurred in the initial step, the protocol turned to second step immediately. In the second step, HUT was performed again under identical conditions. In the third step, the patient was rechallenged with HUT after an injection of PROP.
positive response (ISP-dependent group); the remaining three patients did not develop NMS despite ISP provocation. The patients in the ISP-independent group tended to be younger than the patients in the ISP-dependent group (28.3 ± 10.2 years old vs. 45.2 ± 13.6 years old, respectively, p = 0.05, Table 1).

HEMODYNAMICS. Control (without ISP) supine BP and HR tended to be lower in the ISP-independent group than in the ISP-dependent group: systolic BP, 122.4 ± 10.1 versus 131.9 ± 8.6 mm Hg (p = 0.05); diastolic BP, 66.8 ± 8.3 versus 73.9 ± 7.0 mm Hg (p = 0.07); HR, 67.3 ± 7.8 versus 71.3 ± 13.3 beats/min (p = 0.53) (ISP-independent group versus ISP-dependent group, respectively). In the ISP-dependent group, continuous infusion of ISP caused a significant increase in supine HR to 106.2 ± 19.7 beats/min (p < 0.01) and a small increase in supine systolic BP to 139.4 ± 16.6 mm Hg (p = 0.16), and a significant decrease in supine diastolic BP to 68.6 ± 8.0 mm Hg (p < 0.01). At positive response, ultimate BP was approximately the same in both groups (mean BP; ISP-independent group, 51.5 ± 8.6 mm Hg versus ISP-dependent group, 58.5 ± 5.9 mm Hg; p = 0.06), and ultimate HR was significantly lower in the ISP-independent group (64.1 ± 23.2 beats/min) than in the ISP-dependent group (97.2 ± 16.7 beats/min, p < 0.01).

CAS AND HEMODYNAMICS DURING NEGATIVE HUT ALONE. Control (without ISP) supine EP levels were similar in both groups (ISP-independent group, 0.11 ± 0.04 ng/ml vs. ISP-dependent group, 0.07 ± 0.03 ng/ml; p = 0.08). Control supine NE levels were significantly higher in the ISP-dependent group (0.22 ± 0.07 ng/ml) than in the ISP-independent group (0.12 ± 0.06 ng/ml, p < 0.01). Blood samples during negative HUT alone were obtained from nine negative response patients (ISP-dependent group). In these patients, BP and HR remained at the same level during the 15-min tilting (Fig. 2,A). EP did not change significantly (a mean rate of increase at 15 min tilting, 1.1-fold; baseline vs. at 15 min tilting, p = 0.36, Fig. 2,B). NE showed a small but statistical increase at 15 min of tilting (a mean rate of increase at 15 min tilting, 1.7-fold; baseline vs. at 15 min tilting, p < 0.01, Fig. 2,B).

Outcome of the second step. All 17 patients who had a positive response in the initial step experienced syncope in the second step.

HEMODYNAMICS. The time to positive response in the initial step was similar to that in the second step (Table 1). Changes in hemodynamics are shown in Figure 3. During upright posture, HR tended to increase until just before positive response (until -1 min) in both groups. At positive response, BP dropped to approximately the same level in both groups, but the ultimate HR was significantly higher in the ISP-dependent group than in the ISP-independent group. Regarding the pattern of sequential hemodynamic changes, positive HUT resulted in similar hemodynamic responses in both groups.

CAS DURING POSITIVE HUT ALONE OR WITH ISP. In the second step, 100 blood samples were to be collected accord-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age</th>
<th>Isoproterenol (µg/kg/min)</th>
<th>Time to Positive Response (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial Step</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>23</td>
<td>0</td>
<td>12.25</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>28</td>
<td>0</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>27</td>
<td>0</td>
<td>6.67</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>30</td>
<td>0</td>
<td>4.25</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>16</td>
<td>0</td>
<td>4.75</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>51</td>
<td>0</td>
<td>3.00</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>28</td>
<td>0</td>
<td>12.00</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>23</td>
<td>0</td>
<td>5.00</td>
</tr>
<tr>
<td>mean ± SD</td>
<td></td>
<td>28.3 ± 10.2</td>
<td></td>
<td>6.2 ± 3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age</th>
<th>Isoproterenol (µg/kg/min)</th>
<th>Time to Positive Response (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>F</td>
<td>49</td>
<td>0.01</td>
<td>6.00</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>43</td>
<td>0.01</td>
<td>2.47</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>57</td>
<td>0.01</td>
<td>4.00</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>55</td>
<td>0.01</td>
<td>3.33</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>48</td>
<td>0.02</td>
<td>5.50</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>25</td>
<td>0.02</td>
<td>5.00</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>49</td>
<td>0.02</td>
<td>3.50</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>21</td>
<td>0.02</td>
<td>5.50</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>60</td>
<td>0.02</td>
<td>3.00</td>
</tr>
<tr>
<td>mean ± SD</td>
<td></td>
<td>45.2 ± 13.6</td>
<td></td>
<td>4.3 ± 1.3</td>
</tr>
</tbody>
</table>

*p value = 0.054, ND, not done.
ing to the predetermined study design. We were not able to obtain 27 of these blood samples; however, all blood samples at baseline and at positive response were obtained. Changes in CAs are shown in Figure 4. Baseline EP levels tended to be higher in the ISP-independent group than in the ISP-dependent group ($p < 0.05$). In the ISP-independent group, EP began increasing significantly at $23$ min (a mean rate of increase, 4.1-fold) and showed a maximum increase at positive response (a mean rate of increase, 11.7-fold; range, 5.2- to 43.6-fold; baseline, $0.11 \pm 0.04$ ng/ml vs. at positive response, $1.29 \pm 0.90$ ng/ml; $p < 0.01$, Fig. 4,A). In the ISP-dependent group, EP showed a significant increase at positive response (a mean rate of increase, 5.0-fold; range, 2.4- to 13.4-fold; baseline, $0.07 \pm 0.04$ ng/ml vs. at positive response, $0.35 \pm 0.31$ ng/ml; $p = 0.02$, Fig. 4,B). The ultimate EP levels ($1.29 \pm 0.90$ ng/ml) in the ISP-independent group was significantly higher ($p < 0.01$) than that ($0.35 \pm 0.31$ ng/ml) in the ISP-dependent group. Baseline NE levels were significantly higher in the ISP-dependent group ($0.34 \pm 0.15$ ng/ml) than in the ISP-independent group ($0.12 \pm 0.06$ ng/ml, $p < 0.01$). In the ISP-independent group, NE levels showed a statistical increase at positive response (a mean rate of increase, 2.5-fold; baseline versus at positive response, $p < 0.01$, Fig. 4,C). In the ISP-dependent group, NE levels showed a small but statistical increase at positive response (a mean rate of increase, 1.5-fold; baseline vs. at positive response, $p = 0.02$, Fig. 4,D).

**Outcome of the third step.** Among patients in the ISP-independent group, two patients refused PROP therapy. The remaining six patients participated in the PROP test. Although PROP did not interrupt a positive response in any of the six patients, the time to positive response was prolonged significantly compared with that of the initial ($p < 0.01$) and second steps ($p < 0.01$, Table 1). In the ISP-dependent group, eight patients participated in the third step. After PROP, a positive response did not develop in six of these eight patients (Table 1). The PROP therapy was more effective in ISP-dependent group than in ISP-independent group ($p < 0.01$).

**HEMODYNAMICS.** In the ISP-independent group, PROP significantly decreased supine HR ($64.8 \pm 7.5$ to $57.0 \pm 5.8$ beats/min, $p < 0.01$) and supine systolic BP ($125.5 \pm 8.3$ to $117.3 \pm 12.5$ mm Hg, $p = 0.02$), but it did not change supine diastolic BP ($67.8 \pm 9.2$ to $73.2 \pm 8.3$ mm Hg, $p = 0.17$). In the ISP-dependent group, PROP caused a significant decrease in supine HR ($109.3 \pm 18.7$ to $79.0 \pm 13.0$ beats/min, $p < 0.01$), a significant increase in supine diastolic BP ($67.9 \pm 8.3$ to $75.0 \pm 9.3$ mm Hg, $p < 0.01$) and a small decrease in supine systolic BP ($139.4 \pm 17.7$ to $129.5 \pm 9.6$ mm Hg, $p = 0.05$). At positive response, BP

![Figure 2](image1.png)  
**Figure 2.** Sequential changes in hemodynamic and CAs during negative tilt testing without ISP. In patients negative for the HUT alone, mean BP and HR were stable (A). Although EP did not change statistically, NE showed a small but significant increase (B). *$p < 0.01$ when compared with baseline values.

![Figure 3](image2.png)  
**Figure 3.** Sequential hemodynamic changes during positive tilt testing in the second step. HR increased during upright posture, although not significantly, until just before positive response (until $-1$ min). HR throughout tilt testing was significantly higher in the ISP-dependent group than in the ISP-independent group. Mean BP dropped suddenly to the same level in both groups. The pattern of sequential changes was similar in both groups. †$p < 0.05$ when compared between groups. $\dagger p < 0.05$ when compared with baseline values.
and HR were approximately the same as in the initial and second steps in each patient.

**CAS IN THE ISP-DEPENDENT GROUP.** After PROP, supine EP levels did not change significantly (0.12 ± 0.12 to 0.07 ± 0.05 ng/ml, p = 0.13) and supine NE levels tended to decrease (0.31 ± 0.18 to 0.21 ± 0.07 ng/ml, p = 0.05). In the six PROP responders, EP levels showed a small increase at the end of HUT (a mean rate of increase, 2.2-fold; range, 1.3 to 5.2-fold; baseline versus at the end of HUT, p = 0.05, Fig. 5,B) and NE levels showed a small but statistical increase at the end of HUT (a mean rate of increase, 1.7-fold; baseline versus at the end of HUT, p = 0.04). The mean rate of increase for EP at the end of negative HUT in the third step was significantly greater than in the negative HUT in the initial step (p = 0.04). The two PROP nonresponders (patients 11 and 14) showed a

**Figure 4.** Sequential changes in CAs during positive HUT alone or with ISP provocation. Baseline EP levels were similar in the two groups. In the ISP-independent group, EP levels increased gradually during upright posture and showed a significant increase at -3 min and a maximum increase at positive response (A). In the ISP-dependent group, EP levels increased gradually, and the difference reached the significance level at positive response (B). In both groups, there was statistical increase in NE levels at positive response (C, D). *p < 0.05. †p < 0.01.

**Figure 5.** Changes in EP after PROP. In the ISP independent group, EP showed a significant increase (A). In the ISP-dependent group, PROP responders had a mean 2.2-fold increase in EP levels at 15 min of tilting. Two PROP nonresponders showed a marked increase (29.2- and 11.7-fold) in EP levels (arrow) (B).
marked increase (29.2- and 11.7-fold, respectively) in EP levels (shown by arrow in Fig. 5,B) at positive response.

**DISCUSSION**

**Supine NE.** Control (without ISP) supine NE levels were significantly higher in the ISP-dependent group than in the ISP-independent group; however, the ISP-dependent group tended to be older than the ISP-independent group. Age-related increases in plasma NE levels have been reported among normotensive individuals (11–13); it has been reported also that plasma EP levels do not change with age (11,14). In our study, supine NE levels increased significantly by continuous ISP infusion and tended to decrease after PROP. Goldstein et al. (12) reported that intravenous ISP increases plasma NE levels, probably by augmenting NE release from sympathetic nerve endings.

**Role of NE in HUT.** Arterial baroreflexes affecting especially skeletal muscle sympathoneural vasomotor tone are the important components in the maintenance of postural normotension. Skeletal sympathoneural system activity increases instantaneously in response to orthostasis, and plasma levels of NE double within a few minutes (15). In our study, small but statistical increases in NE levels (1.7-fold) at 15 min of negative HUT alone were registered concomitant with unchanged EP levels. These results are similar to those of previous studies (16,17), and may suggest that activation of the sympathoneural system results in inhibition of the development of NMS. However, a report of severely reduced cardiac and renal NE spillover in patients who fainted during cardiac catheterization is in support of a sudden decrease in cardiac and renal sympathoneural activity during NMS (18). Plasma concentrations of NE are governed by a balance between the release of NE from sympathetic nerve endings, reuptake into the endings and catabolism of the amines (4). The reduction of cardiac output during NMS may cause a reduction in plasma CA clearance and lead to an elevation of plasma CA concentration (19). In the present study, small but statistical increases in brachial artery NE levels at positive response were registered. This suggests that an increase in NE release, rather than a lack of clearance, may be responsible for the raised concentration in this study.

**Triggering Mechanism for NMS.** Two different triggering mechanisms for NMS, central and peripheral, have been proposed (20). The central pathway descends from cortico-hypothalamic centers to medullary cardiovascular centers: emotional events can evoke NMS. The peripheral pathway is postulated to originate from various sites in the cardiovascular system. The assumed peripheral mechanism is as follows: 1) upright posture reduces venous return leading to a decrease in left ventricular filling pressure, cardiac output and arterial BP; 2) occasionally, the combination of reduced left ventricular filling pressure and raised sympathetic tone results in vigorous ventricular contraction, which can para-

doxically stimulate ventricular mechanoreceptors, thereby 3) eliciting the Bezold-Jarisch reflex (21). Accordingly, the raised transient sympathetic tone is thought to be necessary for development of NMS (2,3). In this study, increasing HR before positive response was demonstrated in both groups. This relative tachycardia is thought to indicate raised transient sympathetic tone.

EP has been suggested as the humoral vasodilator in NMS (22,23). In addition, EP can enhance the activity of ventricular mechanoreceptors directly and via vigorous contraction (24). When patients developed NMS during HUT alone, the gradual increases of EP reached significant levels at -3-min (4.1-fold) and maximum levels at positive response (11.7-fold). This EP surge may contribute to a vigorous ventricular contraction, which triggers the Bezold-Jarisch reflex, as well as dilation in both skeletal muscle and splanchnic resistance vessels. Calkins et al. (25) compared the effects of ISP and EP on NMS using HUT. They showed ISP to be associated with a significantly greater sensitivity for NMS. They employed continuous administration EP (maximum dose = 100 ng/kg/min) because they estimated that EP (100 ng/kg/min) infusion results in systemic levels of 0.484 ± 0.0069 ng/ml (26). The present study demonstrated that the peak EP surge in the brachial artery was 1.29 ± 0.90 ng/ml in the ISP-independent group and 0.35 ± 0.31 ng/ml in the ISP-dependent group. It follows from this that the dose of EP infused by Calkins et al. (25) may have been too low to induce NMS. In addition, continuous infusion may not be suitable. Because the surge of EP may occur after taking a upright position (Fig. 4,A and B), nearly bolus injections are thought to be better than low-dose continuous administration.

**Use of ISP.** Many investigators have found ISP to be useful in increasing the susceptibility to NMS during HUT (3,27), probably for the following reasons. Nonselective beta-agonist, ISP, has positive inotropic action, which may augment the cardiac mechanoreceptors, as well as positive vasodilation action. In the present study, ISP significantly increased HR and decreased diastolic BP. At positive response, the increase in EP levels in the ISP-independent group was significantly higher (p = 0.01) than that in the ISP-dependent group. In other words, when patients developed NMS during HUT plus ISP provocation, a small increase in EP levels was observed. The comparatively low EP surge may have been enough to induce NMS during HUT plus ISP provocation compared with HUT alone, since exogenous ISP can raise basal sympathetic tone.

**Responses to PROP.** In ISP-independent patients, PROP could not prevent NMS. Although the statistical difference was not significant (p = 0.06), these patients had greater ultimate increases (a mean rate of increase, 20.3-fold) in EP levels at positive response in the third step than in the second step (a mean rate of increase, 11.7-fold). The reason for this may be that PROP can attenuate the effects of released EP but not the release itself. In positive patients,
the levels of released EP may be strongly dependent on the tilting time, because the time to positive response was prolonged significantly compared with that of second step (p < 0.01).

In the ISP-dependent group, two PROP nonresponders showed a marked increase in EP levels (29.2- and 11.7-fold). Even with PROP pretreatment, when EP surges in an amount sufficient to act as a stimulus for NMS, the NMS may occur. PROP responders in the ISP-dependent group had higher levels of EP (2.2-fold) at the end of 15-min HUT compared with negative HUT in the initial step (1.1-fold, p = 0.01). It is likely that the effects of these 2.2-fold EP levels (0.13 ± 0.09 ng/ml) are almost diminished by 0.1 mg/kg of PROP. In these circumstances, if patients are maintained in the tilting position over 15 min, some will develop a positive response.

Aging effects. Nearly every aspect of the autonomic system maintenance of BP can be altered with age (28). Other investigators reported that patients showing NMS during HUT alone were younger than patients needing ISP for NMS (3,29). Newman et al. (11) reported that EP levels tended to rise with HUT in their younger healthy volunteers but not in their elderly healthy volunteers. Our ISP-dependent patients were older than our ISP-independent patients; the difference did not reach the significance level (p = 0.05). This difference in age between the two groups is thought to play a role in the differences in endogenous CAs between the two groups, but the difference in age was a result of our study. If we investigated a large number of patients, similar results could be observed.

Conclusions. An EP surge may be a triggering mechanism for NMS. Comparatively low levels of EP are thought to be enough to induce NMS during tilting with ISP compared with tilting alone because exogenous ISP can raise basal sympathetic tone. PROP frequently inhibits NMS, which is dependent on ISP, but it is not effective in inhibiting NMS, which is independent of ISP. It is presumed that PROP (0.1 mg/kg) is insufficient to block the actions of these high levels of circulating EP.

Study limitations. Our measurement of the plasma CA levels in the brachial artery is influenced by many organs. Likewise, direct measurement of peripheral muscle sympathetic bursts is useful to evaluate the sympathoneural activity (30). Although we concluded that PROP failed to block NMS that is independent of ISP, further study using a higher dose of PROP is needed to strengthen this conclusion.

REFERENCES
