Endothelial Function and Peripheral Vasomotion in the Brachial Artery in Neurally Mediated Syncope

BONPEI TAKASE, M.D., FACC, TAKASHI AKIMA, M.D., AKIMI UEHATA, M.D.,† SYUICHI KATUSHIKA, M.D.,† KAZUSIGE ISOJIMA, M.D.,† KIMIO SATOMURA, M.D., FUMITAKA OHSUZU, M.D., FACC, AKIRA KURITA, M.D., FACC*

National Defense Medical College, Internal Medicine-1, *Research Center, and †Self Defense Forces Central Hospital, Internal Medicine, Saitama, Japan

Summary

Background: Paradoxical peripheral vasodilation is one of the suspected mechanisms of neurally mediated syncope. Parasympathetic stimulation following sympathetic activation during orthostatic stress mainly contributes to this vasodilation.

Hypothesis: Since endothelial function modulates peripheral vascular tone, this study aimed to determine whether endothelial function and inappropriate peripheral vasomotion has a significant role in the pathogenesis of neurally mediated syncope.

Methods: To investigate whether endothelial function is augmented or whether abnormal peripheral vasomotion exists, flow-mediated dilation (FMD, endothelium-dependent vasodilation) and sublingual glyceryl trinitrate-induced dilation (0.3 mg, GTN-D, endothelium-independent vasodilation) were measured in the brachial artery in 16 patients with neurally mediated syncope, aged 33 ± 10 years, by using high-resolution ultrasound. All patients underwent positive head-up tilt testing. These measures were compared with those in 16 control subjects matched with the patients by age, gender, and coronary risk factors. For FMD, percent diameter changes were obtained from baseline to hyperemic conditions (1 min after 5 min occlusion of the forearm artery). There were five

smokers in both the patient and the control groups, but there was no structural heart disease in either group.

Results: Baseline brachial artery diameters were comparable (3.8 ± 0.6 vs. 3.8 ± 0.7 mm, NS). Flow-mediated dilation in patients with neurally mediated syncope had a normal value of $9.8 \pm 5.0\%$ despite the inclusion of five smokers. Flow-mediated dilation and GTN-D in patients with neurally mediated syncope were significantly greater than those in controls (9.0 ± 5.0 vs. $3.0 \pm 3.5\%$, $p < 0.05$; 18.4 ± 5.5 vs. $14.1 \pm 4.4\%$, $p < 0.05$).

Conclusions: Augmented endothelial function and/or abnormal peripheral vasomotion in peripheral arteries are important in patients with neurally mediated syncope in selected populations.

Key words: endothelial function, peripheral vasomotion, neurally mediated syncope

Introduction

Paradoxical peripheral vasodilation is one of the suspected mechanisms of neurally mediated syncope. Parasympathetic stimulation following sympathetic activation during orthostatic stress mainly contributes to this vasodilation.^{1,2} This vasodilation causes tilt-induced hypotension. However, neither antiparasympathetic nor antisymphathetic medications can completely abolish the tilt-induced hypotension.³ Other unknown mechanisms are suspected. Since endothelial function is reported to be associated with various cardiovascular disorders,⁴ augmented endothelial function and/or inappropriate peripheral vasomotion are possibly involved in the pathogenesis of neurally mediated syncope. The purpose of this study was to investigate whether endothelial function is augmented or whether abnormal peripheral vasomotion exists in patients with neurally mediated syncope. We studied both endothelium-dependent vasodilation (flow-mediated dilation) and endothelium-independent vasodilation measured by sublingual glyceryl trinitrate-induced dilation in the brachial artery of patients with neurally mediated syncope.

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Address for reprints:

Bonpei Takase, M.D., FACC
National Defense Medical College, Internal Medicine-1
3-2 Namiki, Tokorozawa
Saitama, Japan, 359-8513

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Methods

Study Population

The study population consisted of 16 patients (mean age 35 years, 7 women) with neurally mediated syncope, diagnosed by head-up tilt testing, and 16 normal controls. The controls were matched by age, gender, and coronary risk factors with the patients with neurally mediated syncope. Age, gender, and coronary risk factors are known to affect both endothelial function and peripheral vasomotion. Informed consent was obtained from all patients and controls.

Head-Up Tilt Testing

All patients were subjected to standard tilt testing in a dimly lit, quiet room. Patients were in a fasting condition at the time of the test and, whenever applicable, medications were withdrawn for at least five half-lives before the initiation of the test. A motor-driven tilt table with a foot board for weight support was used for an 80° passive tilt. Heart rate and blood pressure were measured at 1-min intervals using an automatic sphygmomanometer (BP 203, Nihon Colin, Inc., Tokyo, Japan). The lead II electrocardiogram (ECG) was continuously monitored throughout the study. The criteria for a positive test were defined as follows: syncope or presyncope induced during tilt following either a decrease in systolic blood pressure or a decrease in heart rate, compared with supine values.

The head-up tilt-testing protocol consisted of at least 6 min in supine position under stable and quiet conditions, followed by a 20-min period of control tilting. If necessary, a 20-min nitroglycerin (NTG) provocation test was performed.

For this test, NTG was intravenously administered at progressively increasing dosages, starting at 250 µg/h, with successive increments of 250 µg/h every 3 min up to a maximum of 1500 µg/h. At first, NTG infusion began at the rate of 250 µg/h in supine position. Patients were kept in supine position for at least 6 min after being stabilized. Then patients were tilted up under NTG infusion. If 3 min of 250 µg/h infusion during tilt did not induce symptoms, the NTG infusion rate was increased with 250 µg/h steps at every 3 min during tilting. Patients were kept in tilt-up position until either presyncope or syncope was induced. If syncope or clinical symptoms similar to a spontaneous episode developed in association with a systolic blood pressure and/or heart rate decrease, the patient was returned to supine position as soon as possible. When the repeat tilt testing with NTG infusion was continued for up to 20 min without inducing clinical symptoms similar to the spontaneous episodes of syncope, a total NTG infusion dosage of approximately 0.3 mg, which is identical to the dosage of NTG sublingual tablets in our country, was administered.

Brachial Artery Function Study

All studies were performed in a temperature-controlled room (25°C) with the subject in a fasting, resting, and supine

state. The study was performed on the day following the head-up tilt testing session at around 10 A.M. In the brachial artery endothelial function and vasomotion study, brachial artery diameter was imaged using a 7.5 MHz linear transducer ultrasound system with a YHP Sonos 2000 (Hewlett Packard, Sonos 1500, Andover, Mass., USA). To obtain the endothelium-dependent vasodilation (flow-mediated dilation), baseline measurements were recorded. Thereafter, a blood pressure cuff was inflated to occlusive pressure on the most proximal portion of the forearm for 5 min to induce reactive hyperemia. At 1 min after reactive hyperemia was achieved, the brachial artery diameter was imaged again to measure the diameter. To calculate flow-mediated dilation, percent diameter changes were determined as follows: (diameter after reactive hyperemia – baseline diameter)/baseline diameter × 100. Using the same method, we calculated endothelium-independent vasodilation by administering 0.3 mg of sublingual glyceryl trinitrate. The brachial artery was imaged before (baseline) and 5 min after the administration of sublingual glyceryl trinitrate. Endothelium-independent vasodilation was obtained as percent diameter changes by the following formula: (diameter after administering sublingual glyceryl trinitrate – baseline diameter)/baseline diameter × 100.

For the analysis of dilator responses in the brachial artery, the diameter of the brachial artery was measured using a commercial quantitative coronary angiography analysis computer (Kontron Elektronik, Cardio 500, Munich, Germany). For the brachial artery, images at peak systole, a maximal dilation, were identified and the diameter was digitized. As mentioned previously, the percent diameter changes from baseline in response to hyperemia or sublingual glyceryl trinitrate were calculated as a endothelium-dependent and -independent vasodilation.

Figure 1 illustrates the computerized analysis of brachial artery diameters. The mean diameter of the brachial artery was determined at baseline, after reactive hyperemia, and after sublingual glyceryl trinitrate administration. Brachial artery diameter analysis was performed in a blinded manner with respect to either the patient's background or the responses to tilt testing. Inter- and intraobserver variability of diameter measurements were < 3%.⁵

FIG. 1 Computerized analysis of brachial artery diameters. Mean diameter of brachial artery was determined at baseline, after reactive hyperemia, and after sublingual glyceryl trinitrate administration.

TABLE I Clinical characteristics of study patients

Patient No.	Age & sex	No. of syncopes	Nitro-provocation tilt testing	Dosage of nitro ($\mu\text{g/h}$)	Smoking	Other risk factors
1	25M	2	0	0	0	0
2	48M	2	+	750	+	0
3	32F	3	0	0	0	0
4	28M	2	+	750	+	0
5	23F	2	0	0	0	0
6	26F	2	0	0	0	0
7	28M	2	+	1,000	+	0
8	24F	2	+	250	0	0
9	40F	2	+	500	0	0
10	26F	2	0	0	+	0
11	49M	2	+	1,250	0	0
12	27F	2	+	750	+	0
13	52M	1	+	1,500	0	0
14	29M	2	+	750	0	0
15	45F	2	0	0	0	0
16	53M	1	+	750	0	0

Abbreviations: Nitro = nitroglycerin, M = male, F = female, No. = number.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation. Student's *t*-test was used for comparison between groups. Differences were considered significant if $p < 0.05$.

Results

Cardiac risk factors and selected clinical characteristics of the 16 patients with neurally-mediated syncope are listed in Table I. No patient had structural heart disease. Cigarette smoking was present in five patients. No other cardiac risk factors were noted. Six patients had syncope induced by control tilt testings. The remaining 10 patients needed the NTG provocation test. The median number of syncopal episodes was 2.0 and the median NTG dosage required to provoke syncope was 750 $\mu\text{g/h}$.

A comparison of vessel diameters of the brachial artery is shown in Figure 2. The mean diameter in neurally mediated syncope was 3.8 ± 0.6 mm and that in normal controls was 3.8 ± 0.7 mm. Both values were identical. Endothelium-dependent vasodilation values, shown as flow-mediated dilation, are compared in Figure 3. Mean value of flow-mediated dilation in patients with neurally mediated syncope was $9.0 \pm 5.0\%$. This value was significantly greater than that in control subjects, which was $3.0 \pm 3.5\%$. This result suggests that patients with neurally mediated syncope preserved brachial artery endothelial function despite the inclusion of five smokers, or augmented endothelial function.

Endothelium-independent vasomotion shown as glyceryl trinitrate-induced dilation is also compared in Figure 4. The mean value of endothelium-independent vasodilation in pa-

tients with neurally mediated syncope was $18.4 \pm 5.0\%$. This value was again significantly greater than that in the control subjects ($14.1 \pm 4.4\%$).

Since 10 of 16 patients in the present study needed the NTG provocation test, we compared the endothelium-dependent and -independent vasodilation in patients who did and did not undergo the NTG provocation test. As shown in Figure 5, the necessity for the NTG provocation test did not influence the dilation of brachial artery whether it was endothelium-dependent or -independent.

FIG. 2 Comparison of vessel diameter between patients with neurally mediated syncope and normal control subjects. NMS = neurally mediated syncope, CTRL = normal control subjects, SD = standard deviation.

FIG. 3 Comparison of flow-mediated dilation between patients with neurally mediated syncope and normal control subjects. Flow-mediated dilation was significantly greater in patients with neurally mediated syncope than in normal control subjects. Abbreviations as in Figure 2.

FIG. 4 Comparison of glyceryl trinitrate dilation between patients with neurally mediated syncope and normal control subjects. Glyceryl trinitrate dilation was significantly greater in patients with neurally mediated syncope than in normal control subjects. Abbreviations as in Figure 2.

Discussion

In this study we have demonstrated that endothelial function is augmented and that abnormal peripheral vasomotion exists in patients with neurally mediated syncope. Endothelium-dependent vasodilation in patients with neurally mediated syncope was normal despite the inclusion of five smokers. Both endothelium-dependent and -independent vasodilation in neurally mediated syncope were significantly greater than in control subjects.

Accentuated endothelium-dependent vasodilation reflects the augmentation of nitric oxide production in endothelial cells. This increased production of nitric oxide is caused by increased shear stress to the endothelial cells. Although the mechanisms are different, shear stress-induced augmentation of nitric oxide production suggests that receptor-mediated production such as acetylcholine stimulation is accentuated. The final pathway for bradycardia and hypotension in patients with neurally mediated syncope is increased vagal activity. In the vagal nerve, the postsynaptic transmitter is acetylcholine. The increased acetylcholine can cause vasodilation through nitric oxide production in patients with neurally mediated syncope.

In this study, sublingual glyceryl trinitrate-induced dilation was also intensified. Since glyceryl trinitrate is now considered to be similar to exogenous nitric oxide, responsiveness of brachial artery smooth muscle to nitric oxide is suggested to be greater in patients with neurally mediated syncope. This suggests that hypersensitivity of the vascular smooth muscle to nitric oxide exists, implying the presence of abnormal peripheral vasomotion in patients with neurally mediated syncope.

To the best of our knowledge, no study has measured either endothelium-dependent or -independent vasodilation in pa-

tients with neurally mediated syncope. However, Thomson *et al.*¹ reported that inappropriate forearm vascular resistance fall occurred in response to leg exercise in patients with neurally mediated syncope. This abnormal forearm vasomotion finding is consistent with our findings. Kaufmann⁶ suggested that nitric

FIG. 5 Comparison of endothelium-dependent and -independent dilation in patients with and without nitroglycerin provocation test. The left columns present the comparison of endothelium-dependent dilation such as flow-mediated dilation, and the right columns present endothelium-independent dilation, which is glyceryl trinitrate-induced dilation. The open column shows 10 patients who needed the nitroglycerin provocation test, and the closed column shows 6 patients who had syncope during control tilt. Nitro-test = nitroglycerin provocation.

oxide synthesis contributed to the pathogenesis of neurally mediated syncope. In contrast, Dietz *et al.*⁷ reported that NG-monomethyl-L-arginine, which has an antagonizing effect on nitric oxide production, could not prevent syncope in humans; however, their populations were all normal subjects; this discordance can be explained by the difference in study populations.

Nitroglycerin is an exogenous nitric oxide donor; therefore the necessity of an NTG provocation test might relate to the status of endothelial function and vasomotion in the brachial artery. Of 16 patients with neurally mediated syncope, 10 patients were diagnosed by the NTG provocation test. Since this test has been widely accepted as the diagnostic modality for neurally mediated syncope,^{8,9} we have recently introduced an NTG provocation test to the routine protocol for tilt testing. As shown in Figure 5, however, present results have shown that there were no significant relationships between either endothelial function or vasomotion and the requirement of an NTG provocation. The necessity of an NTG provocation is unlikely related to inappropriate endothelial function and/or peripheral vasomotion in patients with neurally mediated syncope.

Although the values for both flow-mediated and glyceryl trinitrate-induced dilation in the normal controls are less than those reported in earlier studies,^{10,11} the values in this study determined in our laboratory are representative for a healthy young population, since we previously reported that the normal values for flow-mediated dilation range from 5.3 to 9.7% and glyceryl trinitrate-induced dilation from 13.9 to 16.2%.¹² Several possible reasons for the fact that the values in the normal controls in the present study did not agree with those in the earlier studies may be considered. First, the normal controls in this study included five smokers who showed small values of flow-mediated dilation, which were all less than 2%, as shown in Figure 3. A second possible reason is that about 40% of the normal controls (7/16 subjects) had a relatively large brachial artery diameter (>4.0 mm), as shown in Figure 2. Since the degree of flow-mediated dilation is inversely related to baseline brachial artery diameter,⁵ the relatively large diameter can explain the small values of flow-mediated dilation in the normal controls in the present study. A third and, we believe, most likely reason for disagreement of our values of flow-mediated dilation with those of the earlier studies is that we measured flow-mediated dilation at a fixed time in the morning in all study populations. Our anecdotal evidence suggests that morning attenuation of flow-mediated dilation exists in the healthy subjects.¹² Regarding glyceryl trinitrate-induced dilation, several earlier studies^{13,14} agree with our findings.

Limitations

There are several limitations to our study. All patients with neurally mediated syncope had no structural heart disease. Patients with organic heart disease were not included, so our study patients had the pure type of neurally mediated syncope as reported by Grubb and Kosinski.¹⁵ Since many factors are involved in the overall pathogenesis of neurally mediated syn-

cope, augmented nitric oxide production and abnormal forearm vasomotion contribute one of these mechanisms.

Conclusion

According to these findings, we conclude that the augmentation of endothelial nitric oxide production and/or the hypersensitivity of the vascular smooth muscle to exogenous nitric oxide might play an important role in the pathogenesis of neurally mediated syncope. Augmented endothelial function and/or abnormal peripheral vasomotion in peripheral arteries are important in patients with neurally mediated syncope in selected populations.

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