Chapter 4. Sublingual nitroglycerine used in routine tilt testing provokes cardiac output mediated vasovagal response

J.Gisolf, B.E.Westerhof, N.van Dijk, K.H.Wesseling, W.Wieling and J.M.Karemaker, *J Am Coll Cardiol 2004 Aug 4;44(3):588-93*

Sublingual nitroglycerine can be used during routine tilt testing of patients with a history of vasovagal syncope, to enhance venous pooling and thereby increase the test sensitivity. In the following study we investigate the circulatory changes brought about by nitroglycerine during the tilt test, and we compare the response of tilt-positive patients (those who experienced a vasovagal response after nitroglycerine) to the response of tilt-negative patients.

Introduction

Recurrent syncope is a common medical problem. Head-up tilt testing with or without pharmacological intervention has been shown to be a useful diagnostic test to document a tendency for vasovagal faints [10; 11; 22; 77; 101; 113; 119]. Nitroglycerine (NTG) is commonly administered to increase the diagnostic yield of the procedure [1; 102; 103] because as potent venodilators [90], nitrates might facilitate vasovagal syncope (VVS) by enhancing venous pooling in the upright posture. NTG has been proposed to enter smooth muscle cells where it undergoes metabolic activation to nitric oxide (NO) [20; 68]. There is mounting evidence that NO-donors have pronounced central effects [133]. NTG is lipid soluble and readily crosses cell membranes. Animal studies suggest a direct effect of NTG on the central nervous system resulting in sympathetic inhibition [88]. We therefore hypothesized that NTG facilitates a vasovagal reaction in routine head-up tilt testing not only via venous vasodilatation, but also by acting centrally on circulatory control and inhibiting the baroreflex control of heart rate and arterial peripheral resistance, thus leading to syncope by dual pathways. The purpose of this study was to investigate the effect of NTG, as used in routine tilt testing in otherwise healthy patients with a history of VVS, on hemodynamic characteristics and baroreflex control of heart rate (HR) and systemic vascular resistance (SRV). We further sought to assess whether immediate cardiovascular response to NTG was related to test outcome.

Methods

Study population

The study group consisted of patients with suspected vasovagal syncope, referred for routine tilt-table testing to the Syncope Unit of the Academic Medical Center in the period of June 2002 to July 2003. Excluded were patients with a history of cardiovascular disease, carotid sinus syndrome or any disease that might affect the autonomic nervous system, and patients using medication that might affect the circulation or circulatory control. Subsequently we excluded patients who experienced a vasovagal episode before administration of nitroglycerine (2 patients). A total of 39 patients (18 females) were included in the study.

Abbreviations and Acronyms

NTG	nitroglycerine
VVS	vasovagal syncope
NO	nitric oxide
BRS	baroreflex sensitivity
SV	stroke volume
CO	cardiac output
HR	heart rate
MAP	mean arterial pressure
IBI	interbeat interval
SVR	systemic vascular resistance
SAP	systolic arterial pressure
DAP	diastolic arterial pressure
LF	low frequency
SNP	sodium nitroprusside

Tilt test protocol and measurements

The tests were done between 9:00 AM and 1:00 PM in a temperature controlled room (23ºC). A manually operated tilt table with a footboard was used. Blood pressure was measured continuously and non-invasively using Finapres Model 5 (TNO Biomedical Instrumentation, Amsterdam, the Netherlands). Beat-to-beat changes in stroke volume (SV) were estimated by modeling flow from arterial pressure (Modelflow, TNO Biomedical Instrumentation) [125 71 57]. The tilt-table test started with 5 minutes of supine rest followed by 20 minutes head-up tilt (60º). If no VVS developed, nitroglycerine was administered sublingually (0.4 mg) for an additional 15-minute tilt duration [21]. Oncoming syncope was aborted by means of tilt back or counter-maneuvers such as leg crossing [79], before loss of consciousness set in. The study was approved by the Medical Ethical Committee of the Academic Medical Center, University of Amsterdam, the Netherlands.

Data acquisition and analysis

The Finapres arterial pressure signal was analog/digital converted at 100 Hz and stored on hard disk for off-line analysis. Mean arterial pressure (MAP) was the true integral of the arterial pressure wave over 1 beat divided by the corresponding beat interval. HR was computed as the inverse of the interbeat interval (IBI) and expressed in beats per minute. Cardiac output (CO) was the product of SV and HR, and SVR was MAP at heart level divided by CO. Beat-to-beat values were computed and averaged per minute. SV, CO and

SVR were set at 100% (baseline) in the upright posture, 5 minutes prior to NTG, and variations were expressed as percentages of this baseline. Slopes were computed of the minute-averages of HR, systolic and diastolic arterial pressure (SAP and DAP respectively), MAP, SV, CO, and SVR over a 4-minute time frame starting at NTG administration, in all patients.

Of those patients who developed VVS during the tilt test, minute averages were calculated of beat-to-beat data up to a point where a drop in HR and/or arterial blood pressure preceding the vasovagal episode was detected. To analyze the hypotensive, presyncopal episode in tilt positive patients, the last 15 seconds prior to intervention such as tilt back were analyzed.

Baroreflex sensitivity

Beat-to-beat SAP and IBI time series were detrended and Hanning Windowed. Power spectral density and cross-spectra of SAP and IBI in the low-frequency (LF) band (0.06 – 0.15 Hz) were computed using Discrete Fourier Transform as described elsewhere [38]. For time-domain analysis of spontaneous baroreflex sensitivity (BRS) we used the cross-correlation method PRVXBRS that is now standard part of the software packages delivered with Portapres and Finometer products (FMS, Netherlands). The SAP and IBI time series were resampled at 1 Hz. In a 10 s window, the correlation and regression slope between SAP and IBI were computed. Delays of 0 to 5 s increments in IBI were computed, and the delay with the highest positive coefficient of correlation was selected. The slope between SAP and IBI was recorded as a BRS estimate if the correlation was significant at P=0.01.

Statistical analysis

Variables were tested for normality using the Kolmogorov-Smirnov test, and expressed as mean and SD, unless stated otherwise. Responses to sublingual nitroglycerine were analyzed using non-parametric tests for 2 related samples (Wilcoxon signed rank test), or paired t-tests where appropriate. Differences between groups were analyzed using non-parametric tests for 2 independent samples (Mann-Whitney U test), or t-test where appropriate. Pearson's correlation coefficient was computed for the correlation between the BRS results in the time and frequency domain. The association between data (computed slopes) and test outcome (time to faint), including censored data of those patients without vasovagal syncope during the test, was assessed using Cox regression analysis (SPSS for Windows, release 11.5.2). Significance of the Walt statistic was computed.

Results

Subjects

NTG induced presyncope in 22 (56%) of the 39 otherwise healthy patients included in the study. The average age (36±16 years), height (175±10 cm), weight (73±15 kg) and distribution of gender did not differ between the patients who experienced near syncope during the tilt test and those who did not. The time from administration of NTG to presyncope in the tilt-positive patients ranged from 2 min 50 s to 14 min 50 s. All vasovagal patients indicated prodromal symptoms such as light-headedness or nausea. Data recording stopped in 2 tilt-negative patients (in the 4th and 10th minute after NTG administration) for technical reasons.

Cardiovascular response to NTG

Hemodynamics during 4 to 1 minutes preceding, and 1 to 4 minutes following NTG administration in all patients are summarized in Table 4.1. Prior to NTG patients were asymptomatic and the average MAP was 87 mmHg (range 67 to 101 mmHg). One patient became symptomatic in the 3rd minute after NTG, another in the 4th minute. Systolic and mean Finapres blood pressure were well maintained after NTG, whereas the DAP increased. There was a reduction in SV and although HR increased, the CO diminished. SVR increased after NTG (P<0.001) (Fig. 4.1). During these periods there were no significant differences in hemodynamic characteristics between tilt-positive and tilt negative patients.

Table 4.1. Cardiovascular characteristics of all patients during periods of 3 minutes before and after GTN administration while tilted to 60º head-up tilt.

	4 to 1 min Before GTN	1 to 4 min After GTN	p Value
Hemodynamic data			
SAP (mmHg)	118 ± 12	117 ± 12	n.s.†
MAP (mmHg)	87 ± 10	87 ± 10	n.s.†
DAP (mmHg)	72 ± 10	75 ± 9	0.001†
HR (beats/min)	85 ± 14	95 ± 17	0.001‡
SV (%)	98 ± 6	84 ± 10	0.001‡
CO (%)	99 ± 5	93 ± 8	0.001†
SVR (%)	103 ± 8	111 ± 11	0.001‡
Frequency domain analysis			
SAP LF power ($mmHg^2$)*	18 ± 15	24 ± 21	n.s.‡
IBI LF power (ms^2)*	819 ± 673	1095 ± 866	0.05‡
BRS LF gain (ms/mmHg)*	4.9 ± 2.5	3.8 ± 2.4	0.01‡
Coherence*	0.66 ± 0.16	0.65 ± 0.16	n.s. ‡
Time domain analysis			
BRS (ms/mmHg)*	8.0 ± 3.1	6.1 ± 2.8	0.001‡

*Results of 38 of 39 patients. †Paired samples t-test. ‡Wilcoxon signed rank test. Data are presented as mean value ± SD. BRS, baroreflex sensitivity; CO, cardiac output; DAP, diastolic arterial pressure; HR, heart rate; IBI, interbeat interval; LF, low frequency; MAP, mean arterial pressure; NTG, nitroglycerine; SAP, systolic arterial pressure; SV, stroke volume; SVR, systemic vascular resistance.

Power spectral density and baroreflex sensitivity

After excluding one tilt-positive patient from spectral analysis due to frequent extrasystolic beats, for the remaining 38 of the 39 patients the IBI LF power density increased after NTG (P<0.05). The SAP LF power also tended to increase (P=0.12). The spectral power and BRS estimates are given in Table 4.1. BRS LF gain decreased following the hemodynamic changes induced by NTG, as did the spontaneous BRS calculated in the time domain (Fig. 4.1F). There were no differences in BRS or power spectral density between the patients with a negative vs. positive test outcome in the selected periods. Although the BRS LF gain

results were lower compared to the time domain BRS estimates, methods correlated well (Pearson's R=0.79 with P<0.001).

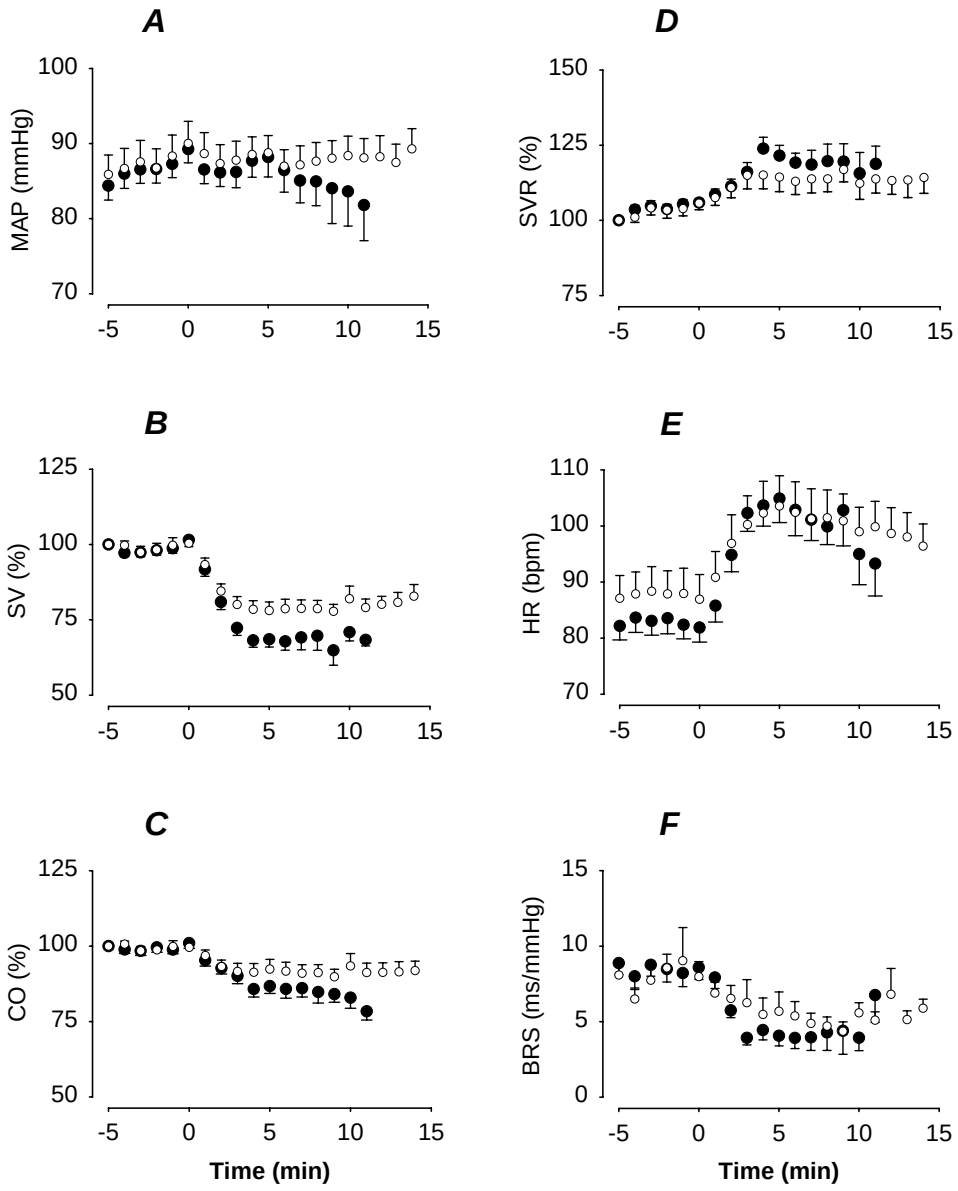

Figure 4.1
Hemodynamic response to nitroglycerine during the 60º-tilt test.
At 0 minutes NTG is administered. **A** Mean arterial pressure (MAP); **B** Stoke volume (SV); **C** Cardiac output (CO); **D** Systemic vascular resistance (SVR); **E** Heart rate (HR); **F** Baroreflex sensitivity (BRS). Circles denote minute averages and S.E.M. Empty circles, negative tilt test, solid circles, positive tilt test.

Computed trends after NTG administration

Cardiovascular trends in patients who experienced presyncope and those who did not are shown in Figure 4.1; the tilt-positive patients demonstrated a greater drop in SV, CO and arterial blood pressure. The HR in the period preceding NTG appears higher in the tilt-

negative group, this difference is not significant however (88±17 vs 83±12 bpm, P=0.3).

To avoid statistical testing of episodes where the number of tilt-positive patients was greatly reduced, trends were analyzed by calculation of the slope during the first 4 minutes after NTG administration (Fig 4.2). The tilt-positive patients had a steeper drop in SV compared to the tilt-negatives (P<0.05). The concomitant rise in HR was also steeper in the tilt-positives (P<0.05).

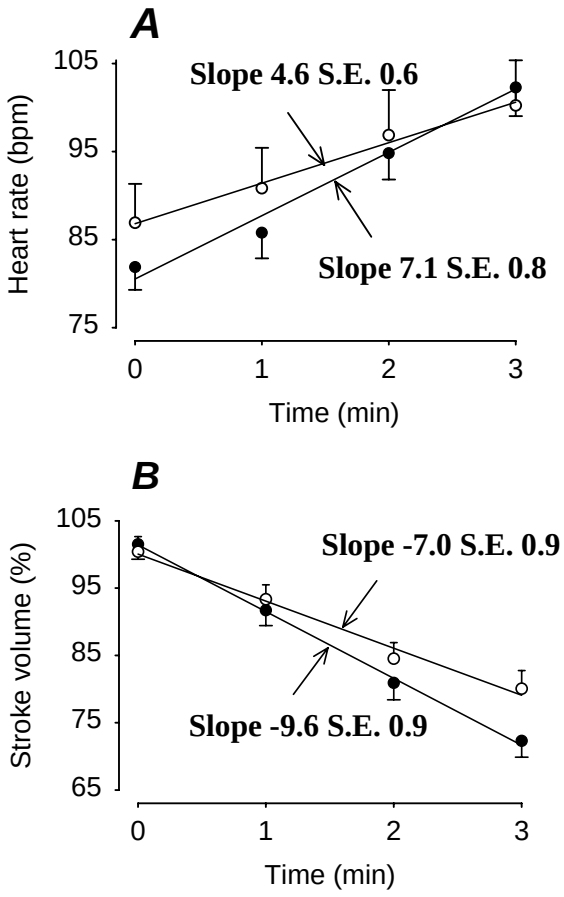

Figure 4.2
Computed trends of minute averages over 4 minutes immediately following NTG administration during 60º tilt
A Slope of heart rate; **B** Slope of stroke volume. Empty circles, negative tilt test; solid circles, positive tilt test. Bars indicate S.E.M.

Vasovagal episode

During the last 15 seconds prior to tilt back or counter-maneuver, the tilt-positives showed marked hypotension with a SAP of 81±11 mmHg and DAP of 54±10 mmHg. HR ranged from 45 to 111 bpm during this period, the mean HR was 78±23 bpm. Five of the 22 tilt positives had a HR>100 bpm while 7 had a HR<60 bpm. SV was 68±12 % of baseline, CO was 63±14 % and SVR was 126±44 %. SV and CO during the vasovagal episode were lower compared to that of tilt-negatives in their last minute of tilt, who had an average SV of 83±15 % (p=0.002) and CO of 92±12 % (P<0.001) (Fig. 4.1 B and C). During the near syncope, in 18 of the 22 tilt-positive patients SVR was increased compared to baseline. SVR and HR response were not related to age.

Cox regression model

Using the calculated slopes to model tilt test outcome (time to presyncope), Cox regression showed that rise in HR was related to the occurrence of a vasovagal response during tilt

testing (Figure 4.2A, Table 4.2). The drop in SV was also related to the test outcome; a steep drop in SV is associated with an increased hazard of a vasovagal response (Figure 4.2B, Table 4.2). Modeling both slopes of SV and HR together does not improve the model, as these variables are correlated (R=-0.54, P<0.01).

Table 4.2. Cox regression analysis for time to presyncope computed slopes

	Wald Chi-Square	Hazard Ratio (95% CI)	P-value
Model A: slope of HR (beats/min)	5.66	1.12 (1.02-1.22)	0.017
Model B: slope of SV (%/min)	7.91	0.86 (0.77-0.95)	0.005

HR, heart rate; SV, stroke volume; CI, confidence interval

Discussion

The present findings demonstrate that administration of NTG during routine tilt testing of otherwise healthy patients suspected of vasovagal syncope leads to a rapid drop in SV, a rise in SVR and HR, and initially a maintained arterial blood pressure in patients with a positive test outcome as well as those with a negative test outcome. This implies an adequate arterial resistance response to NTG-induced venous dilation and pooling, rendering impaired circulatory control due to NTG unlikely. The cardiovascular response to NTG was similar in vasovagal and non-vasovagal patients, but the response was more pronounced in tilt-positive patients; the reduction in SV after NTG administration was related to tilt test outcome. Despite an increase in HR, there was a reduction in cardiac output. NTG-induced vasovagal response therefore appears cardiac output mediated, and is not preceded by a decrease in SVR.

Effect of NTG on circulatory control

Baroreflex sensitivity was decreased in the period following NTG, which together with the rise in HR and SVR suggests sympathetic activation. Interestingly the rise in HR and SVR following NTG were not preceded by a reduction in arterial pressure. Possible explanations for this are firstly, that arterial baroreceptors respond to mechanical deformation and not pressure, and small reductions in effective blood volume are known to trigger baroreflex adjustments of arterial pressure [120]. Secondly, another pathway leading to an increase in SVR is via the cardiopulmonary reflex [136], which is sensitive to changes in venous pressure. Activation of the cardiopulmonary reflex is likely after NTG administration, which is known to result in venodilation and pooling of blood [90].

We found no difference in BRS between tilt-positives and tilt negatives prior to or following NTG administration. This seems at odds with a recent report by Samniah et al. of modification of BRS during VVS [106]. Their results are not comparable however as they

studied the BRS during tilt back immediately following full syncope, whereas we computed BRS in the lead-up to presyncope.

The present findings indicate an increase in SVR after NTG administration in all patients, and a sustained increase in SVR at the onset of presyncope. This seems at odds with previous reports of an early progressive decrease in SVR leading to syncope in healthy young subjects without use of NTG [37]. The supine recording is commonly used as control period for expressing SVR as percentage of baseline. In the present study however we explicitly omitted the supine recording as baseline and used the upright tilt recording prior to NTG administration as baseline, to avoid SV estimations during posture change [118]. After 20 minutes upright tilt with corresponding cardiovascular adjustments to orthostatic stress, NTG is administered. We have not analyzed the changes in SV, CO and SVR from the supine to the upright tilt position, and we therefore limit our conclusions to the effect of NTG administration during routine tilt testing in otherwise healthy, medication-free patients.

Central effects of NTG

Since the discovery that NO is not only a regulator of smooth muscle tone but also a neuromodulator within the central and peripheral nervous system [19; 49], it is likely that the cardiovascular actions of NO are not confined to its direct effects on blood vessels but include effects on the central and peripheral nervous system [133]. In humans the effect of NO-donors on cardiovascular autonomic control has been investigated using infusions of sodium nitroprusside (SNP), and the results suggested SNP had no effects on the cardiac/vagal limb of the baroreflex [65]. SNP is, however, hydrophilic, and the compound has difficulty crossing membranes. Nitroglycerine on the other hand is lipophilic and the compounds readily enter cells to form NO. Results of animal studies suggest that within the central nervous system there are sites that modulate the cardiovascular effects of NTG and the hypotensive effects of NTG may be modified by central noradrenergic activity controlling the circulation [88]. In the present study we demonstrate that baroreflex sensitivity, established using time and frequency domain methods, diminished following sublingual NTG. The increase in DAP and SVR however, together with the increase in HR and IBI LF spectral power, provide strong circumstantial evidence of increased sympathetic outflow [99]. We therefore consider sympathico-inhibition due to a central effect of NTG as used during routine clinical tilt testing unlikely.

Study limitations

The present results were obtained in patients with no cardiovascular or neurological diseases and no medication. The patient group has thus been selected, resulting in a group that is relatively younger and healthier than the total of patients referred for unexplained syncope. Included were only those patients who did not have a vasovagal episode before NTG, thus excluding the most outspoken cases. Vasovagal response was aborted before loss of consciousness set in, and we therefore limited our analysis and our conclusions to the prodromal phase and the onset of vasovagal response.

We used a new method of BRS computation (time domain cross-correlations) and an established method (frequency domain cross-spectral calculations). Although these methods correlated well, the frequency domain BRS gain was lower compared to the time domain BRS. Considering that both methods calculated the correlation between spontaneous variations in SAP and IBI, we would ideally expect identical results. However, in the frequency domain method we have made a frequency band selection (the LF band),

whereas the time domain method in principle includes all frequencies, which might explain the greater BRS estimates we found using the latter method.

Conclusions
Our study of otherwise healthy patients suspected of vasovagal syncope demonstrates a rapid decrease in stroke volume and an increase in systemic vascular resistance and heart rate following NTG administration during tilt testing. We found strong indications that sublingual NTG induces an increase in sympathetic outflow, resulting in initially maintained arterial pressure. The NTG-triggered syncopal episode is not preceded by a decrease in SVR, but appears cardiac output mediated. Our finding that the decrease in stroke volume after NTG administration is related to the time to presyncope supports this.

Acknowledgements

This study was supported by a grant of the Space Research Organization Netherlands (SRON), project MG-052, and a grant of the Netherlands Heart Foundation, project 99.181. We thank Dr D.L. Jardine and Dr J.J. van Lieshout for their review of the manuscript.