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Methionine Restores the Venodilative Response to Nitroglycerin After The Development of Tolerance

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Depletion of sulfhydryl groups may contribute to nitroglycerin tolerance after long-term exposure. This study was performed to assess whether methionine, an amino acid capable of augmenting sulfhydryl availability, would restore the venodilative response to sublingual nitroglycerin once tolerance had developed. The venodilative response to organic nitrates was assessed with use of the equilibration technique of forearm plethysmography. Venous volume was measured before and after sublingual administration of 0.4 mg of nitroglycerin at baseline study and after 5 g of intravenous methionine. Retesting was performed 2 h after application of a 10 mg nitroglycerin patch and compared with the response after 74 h of nitroglycerin patch exposure before and after intravenous methionine.

Methionine alone had no intrinsic venodilative action. Although the venous volume at rest was unchanged after methionine administration, the response to sublingual nitroglycerin was potentiated compared with baseline values (37 ± 15% versus 32 ± 13%, $p < 0.02$). During nitroglycerin patch exposure, the response to sublingual nitroglycerin was significantly attenuated at 74 h compared with the response at 2 h of exposure (16 ± 10% versus 31 ± 13%, $p < 0.001$). The venodilative response to sublingual nitroglycerin was restored at 74 h after methionine administration (35 ± 14% versus 16 ± 10%, $p < 0.001$).

Thus, methionine potentiates the venodilative effect of sublingual nitroglycerin both immediately and in the setting of nitrate tolerance.

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Tolerance to both the hemodynamic and therapeutic effects of long-acting nitroglycerin after long-term use has been extensively documented (1–21). Numerous studies (7–16) have shown that continued exposure to transdermal nitroglycerin patches rapidly results in attenuation of both the hemodynamic and antianginal effects initially seen immediately after application. A leading theory to explain the mechanism of nitrate tolerance involves depletion of sulfhydryl groups, which combine with nitrates as an intermediate step leading to vasodilation (6,20,21). The direct and indirect effect of neurohumoral hormones, in part leading to plasma volume expansion, has also been demonstrated after long-term nitrate therapy (22,23) and may represent another active mechanism contributing to tolerance.

The concomitant administration of the sulfhydryl donor N-acetylcysteine has been shown (24–26) to potentiate the vasodilative effects of nitroglycerin during short- and long-term administration. Clinical use of N-acetylcysteine is limited, however, by gastrointestinal intolerance. In an attempt to circumvent this side effect, we have studied methionine, an essential amino acid capable of augmenting sulfhydryl availability. We (27) recently reported that methionine, like N-acetylcysteine, potentiates the immediate hemodynamic effects of intravenous nitroglycerin. This study was performed to assess whether methionine reverses the nitroglycerin tolerance observed after 74 h of continuous transdermal nitroglycerin exposure.

Methods

Study subjects. The study group comprised 14 volunteers (10 men and 4 women) ranging in age from 29 to 63 years. Eleven were normal subjects and three had a history of chronic coronary artery disease. No subject had been recently exposed to any nitrate preparation. One subject was taking a beta-adrenergic blocker, one was taking a calcium channel blocker and one was taking both a beta-blocker and calcium channel blocker at the time of study. Written informed consent for this study was obtained from all subjects, and the protocol was approved by the Committee on Human Investigation of the George Washington University Medical Center. Exclusion criteria included a heart rate at rest >120 beats/min, systolic blood pressure <100 mm Hg, significant valvular heart disease or pregnancy.

Evaluation of venodilative response to nitroglycerin. Forearm plethysmography is an established method for evaluating the venodilative response to pharmacologic

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agents (28–35). We adapted this in vivo technique for testing the venodilative response to nitroglycerin from the previous work of Zelis and Mason (33). The effect of nitroglycerin on venous tone was evaluated by measuring the change in venous volume after a sublingual dose of nitroglycerin. Venous volume was determined by the equilibrium technique of acute occlusion forearm plethysmography, employing a mercury in Silastic strain gauge. The signal was balanced with a plethysmograph (model 271, Parks Medical Electronics) and recorded on a single channel strip chart recorder. All subjects were studied at the same time of day while recumbent in a warm, quiet environment. The hand was isolated from the circulation by inflating a wrist cuff to suprasystolic pressure. A forearm occlusion cuff was inflated to 30 mm Hg above cuff zero. Cuff zero is that pressure above which forearm volume increases and approximates venous pressure. After inflation of the forearm cuff, venous volume increased for 3 to 4 min until a plateau was reached. This value was considered to be the baseline (prenitroglycerin) venous volume. Immediately after attaining equilibration, 0.4 mg of sublingual nitroglycerin was administered with continued forearm volume recording until a new plateau was achieved or for a maximum of 5 min. The change in forearm volume from the first to the second plateau was considered to be the venodilative effect of nitroglycerin. Results are expressed as venous volume at 30 mm Hg (VV[30]) in cc/100 cc arm ± SD and are a reflection of venous tone.

Study protocol. With use of forearm plethysmography the venodilative response (as reflected by a change in venous volume) to sublingual nitroglycerin was measured serially in each subject to reflect the hemodynamic effect of organic nitrates. On day 1 of the study, plethysmographically measured venous volume was recorded before and after administration of 0.4 mg of sublingual nitroglycerin, first at baseline study and then 30 min after administration of 5 g of intravenous methionine. A 10 mg nitroglycerin patch (Transderm-Nitro 10) was then applied and each subject returned for retesting before and after administration of sublingual nitroglycerin 2 h after patch application. These results were recorded as the response to sublingual nitroglycerin in the setting of short-term exposure to transdermal nitroglycerin.

The second phase of the study consisted of 3 days of continuous application of transdermal nitroglycerin, changing the patch every 24 h. Subjects were permitted to reduce the dosage of the transdermal nitroglycerin patch from 10 to 5 mg/24 h if headaches became intolerable. All subjects, however, once again applied a 10 mg patch on the final day of study so that all venous volume measurements (day 1 and day 4) were carried out while the subject was exposed to a 10 mg patch. Subjects returned after 74 h of continuous transdermal nitroglycerin exposure (2 h after application of the fourth patch) for repeat testing. The venodilative response to 0.4 mg of sublingual nitroglycerin was again measured while this final 10 mg patch was in place before and after administration of 5 g of intravenous methionine.

Heart rate was continuously monitored and blood pressure was measured every 3 min during the study sessions. Serial body weight and hematocrit data were obtained on day 1 and day 4 of the study as an indirect measure of plasma volume status.

Methionine preparation. The methionine infusion was prepared from pharmaceutical grade pure L-methionine to yield a 2.5% solution in 5% dextrose in water and was microfiltered by 0.22 μm millipore filtration. Methionine (5 g) was administered in 200 ml of 5% dextrose in water.

Statistical analysis. Group comparisons of baseline venous volume measurements and the nitroglycerin-induced change in venous volume were assessed by two-tailed, unpaired Student's *t* tests. Statistical comparisons of serial changes in venous volume, blood pressure, heart rate, weight and hematocrit were performed by a repeated measures analysis of variance F test to assess group and order effects. Results are expressed as the mean value ± SD. For all analyses, a p value ≤ 0.05 was considered significant.

Results

Study subjects. Fourteen volunteers entered the study and one withdrew during the initial phase because of intolerance to intravenous methionine (manifested as nausea). Two additional subjects withdrew during the long-term phase because of intolerable headache. Thus, results are based on 13 subjects for the baseline data and 11 subjects for the long-term phase.

Venodilative response at baseline and during acute transdermal nitroglycerin exposure. Venous volume measurements on day 1 of the study at baseline and after 5 g of intravenous methionine are presented in Table 1. At baseline, a 0.4 mg bolus of sublingual nitroglycerin induced a significant increase in venous volume (2.58 ± 0.9 to 3.37 ± 1.0 cc/100 cc arm, $p < 0.01$) with a mean change of 32%.

After the administration of 5 g of intravenous methionine, there was no significant change in the prenitroglycerin venous volume (2.58 versus 2.56 cc/100 cc arm); thus, methionine itself had no hemodynamic effect. After methionine, the change in venous volume after sublingual nitroglycerin (2.56 ± 0.8 to 3.45 ± 0.9 cc/100 cc arm) was significantly greater than at baseline (37 ± 15% versus 32 ± 13%, $p < 0.02$). This confirms our earlier hemodynamic finding (27) and demonstrates that methionine is capable of immediately potentiating the venodilative effect of sublingual nitroglycerin.

Venous volume measurements during short- and long-term exposure to transdermal nitroglycerin patches are presented in Table 2. Two hours after application of the first 10 mg nitroglycerin patch, there was a significant increase in venous volume after sublingual nitroglycerin (2.8 ± 1.0 to 3.6 ± 1.0 cc/100 cc arm, $p < 0.01$). This vasodilative response was similar to that seen at baseline study (32% versus 31%, p = NS).

Table 1. Venous Volume Measurements After Sublingual Nitroglycerin but Before Nitroglycerin Patch Application

Subject No.	Baseline			Post-Methionine		
	Pre-NTG VV	Post-NTG VV	% Change	Pre-NTG VV	Post-NTG VV	% Change
1	1.24	1.52	23	1.46	1.85	27
2	3.49	4.24	21	3.89	4.80	23
3	1.98	3.34	69	1.94	3.29	70
4	1.89	2.57	36	1.70	2.36	39
5	2.44	3.41	40	2.34	3.61	54
6	2.66	3.48	31	2.44	3.55	45
7	3.34	4.31	29	3.53	4.82	37
8	2.74	3.79	38	2.82	4.13	46
9	1.89	2.54	34	1.78	2.31	30
10	2.44	3.18	30	2.38	3.31	39
11	1.96	2.63	34	1.82	2.64	45
12	2.75	3.05	11	3.21	3.63	13
13	4.73	5.79	22	3.94	4.54	15
Mean	2.58	3.37	32.2	2.56	3.45	37.2*
± SD	0.86	1.00	13.2	0.82	0.93	15.2

*$p < 0.02$ versus value at baseline. NTG = 0.4 mg of sublingual nitroglycerin; Pre = before; Post = after; VV = venous volume measured plethysmographically in cc/100 cc arm.

Venodilative response after long-term transdermal nitroglycerin exposure. After 74 h of nitroglycerin patch exposure, although the induced change in venous volume by sublingual nitroglycerin remained significant (2.8 ± 1.0 to 3.2 ± 1.0 cc/100 cc arm, $p < 0.01$), this was significantly blunted compared with the response at 2 h of exposure (16 ± 10% versus 31 ± 13%, $p < 0.001$). Administration of 5 g of intravenous methionine restored responsiveness to sublingual nitroglycerin (2.67 ± 0.9 to 3.53 ± 1.0 cc/100 cc arm, $p < 0.01$), with a mean change of 35%. This was significantly augmented from the response at 74 h before methionine (35 ± 14% versus 16 ± 10%, $p < 0.001$). All subjects demonstrated attenuation of the venodilative response to sublingual nitroglycerin after 74 h of continuous transdermal nitroglycerin exposure, with a restoration of responsiveness after administration of methionine (Fig. 1).

Serial heart rate and blood pressure measurements (Tables 3 and 4). The increase in heart rate after sublingual nitroglycerin and methionine administration was augmented by comparison with baseline values on day 1 (20.4 ± 12.4% versus 12.0 ± 5.4%, $p < 0.02$). However, there was no significant influence by methionine on mean blood pressure changes after sublingual nitroglycerin (3.3 ± 4.1% versus 2.9 ± 3.2%) and no significant hypotension was seen.

As with venous volume, the change in heart rate after sublingual nitroglycerin after 74 h of transdermal nitroglycerin exposure was blunted compared with that seen after 2 h of nitroglycerin patch exposure (10.3 ± 5.1% versus 3.7 ± 6.3%,

Table 2. Venous Volume Measurements During Exposure to Nitroglycerin Patch

Subject No.	2 h TDNTG Exposure			74 h TDNTG Exposure			74 h TDNTG Post-Methionine		
	Pre-NTG VV	Post-NTG VV	% Change	Pre-NTG VV	Post-NTG VV	% Change	Pre-NTG VV	Post-NTG VV	% Change
3	2.15	3.44	60	2.31	3.23	40	2.26	3.82	69
4	1.98	2.64	33	1.66	1.87	13	1.74	2.36	36
5	2.34	3.21	37	1.83	2.33	27	2.00	2.97	49
6	2.51	3.73	49	3.12	3.50	12	2.58	3.55	38
7	4.33	5.08	17	4.58	5.05	10	4.75	5.95	25
8	3.35	4.35	30	3.15	3.47	10	2.88	3.74	30
9	1.75	2.23	27	2.32	2.67	15	2.12	2.62	24
10	2.32	2.84	22	2.57	2.93	14	2.40	3.13	30
11	1.69	2.23	32	1.70	2.09	23	1.59	2.18	37
12	4.52	5.26	16	3.51	3.67	5	3.20	3.81	19
13	3.91	4.55	16	4.09	4.51	10	3.86	4.74	23
Mean	2.80	3.60	30.8	2.80	3.21	16.2*	2.67	3.53	34.6
SD	1.00	1.04	13.3	1.00	1.00	10.0	0.91	1.05	13.6

*$p < 0.001$ versus value at 2 h and 74 h after methionine. TDNTG = 10 mg transdermal nitroglycerin patch; other abbreviations and units as in Table 1.

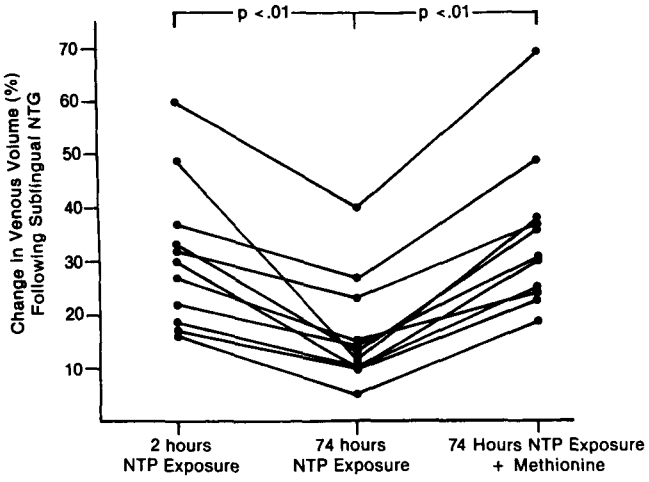

Figure 1. The change in venous volume (%) for each subject after 0.4 mg of sublingual nitroglycerin (NTG) 2 h after the application of the first 10 mg transdermal nitroglycerin patch (NTP), after 74 h of continuous transdermal nitroglycerin exposure and after 5 g of intravenous methionine and 74 h of continuous transdermal nitroglycerin exposure. Group mean values ± standard deviation are 31 ± 13% 2 h after nitroglycerin patch exposure and 16 ± 10% 74 h after nitroglycerin patch exposure and 35 ± 14% after 74 h of nitroglycerin patch exposure plus methionine.

$p < 0.01$). After methionine administration at 74 h of exposure, the change in heart rate after sublingual nitroglycerin was restored (15.5 ± 7.2% versus 3.7 ± 6.3%, $p < 0.01$) and was not significantly different from that seen at 2 h of exposure.

Blood pressure during the nitroglycerin patch portion of the study did not demonstrate any significant changes serially (Table 4).

Change in weight and hematocrit. Serial weight and hematocrit measurements were analyzed as an indirect measure of plasma volume changes. After 74 h of nitroglycerin patch exposure, there was a significant decrease in hematocrit (43.5 ± 3.6 to 40.9 ± 3.5%, $p < 0.01$) and an increase in weight (69.4 ± 15.9 to 69.8 ± 15.5 kg, $p < 0.01$) in the study group. Both changes suggest the possibility of plasma volume expansion.

Table 3. Heart Rate and Blood Pressure Response to Sublingual Nitroglycerin at Baseline

	Baseline	Post-Methionine	p Value
Heart rate (beats/min)			
Pre-NTG	57.1 ± 7.5	54.5 ± 8.6	NS
Post-NTG	63.8 ± 7.4	65.4 ± 10.4	
p value	<0.001	<0.001	
% change	12.0 ± 5.4	20.4 ± 12.4	<0.02
Blood pressure (mm Hg)			
Pre-NTG	78.1 ± 4.4	78.5 ± 4.3	NS
Post-NTG	75.6 ± 5.7	76.8 ± 4.3	
p value	<0.02	<0.05	
% change	3.3 ± 4.1	2.9 ± 3.2	NS

Abbreviations as in Table 1.

Table 4. Heart Rate and Blood Pressure Response to Sublingual Nitroglycerin During Transdermal Nitroglycerin Patch Exposure

	2 h TDNTG Exposure	74 h TDNTG Exposure	74 h TDNTG Post-methionine
Heart rate (beats/min)			
Pre-NTG	59.0 ± 7.4	59.7 ± 6.2	56.9 ± 8.0
Post-NTG	65.1 ± 8.8	62.2 ± 9.2	65.7 ± 10.6
p value	<0.001	<0.001	<0.001
% change	10.3 ± 5.1*	3.7 ± 6.3	15.5 ± 7.2*
Blood pressure (mm Hg)			
Pre-NTG	76.5 ± 4.8	78.2 ± 4.4	77.5 ± 6.5
Post-NTG	75.3 ± 5.1	77.6 ± 5.4	77.5 ± 6.5
p value	NS	NS	NS
% change	1.6 ± 3.3	0.7 ± 5.2	2.6 ± 4.0

*$p < 0.01$ vs 74 h transdermal nitroglycerin patch exposure. Abbreviations as in Tables 1 and 2.

Discussion

Background and mechanism of nitrate tolerance. Nitrates have been used to treat angina pectoris for >100 years (36); however, rapid tolerance to their pharmacologic effects was recognized soon after their introduction (1–3). Recently, there has been renewed interest in the concept of nitrate tolerance as basic research has more clearly elucidated the mechanism of action of nitroglycerin. Needleman et al. (37,38) first reported that sulfhydryl groups were required for the relaxation of smooth muscle and interaction with organic nitrates. Ignarro et al. (39) proposed that nitrates combine with sulfhydryl groups present in vascular smooth muscle to form S-nitrosothiols. These in turn activate guanylate cyclase, which stimulates the production of cyclic guanadine monophosphate (GMP), which is known to mediate smooth muscle relaxation. Depletion of the intracellular stores of sulfhydryl groups has been suggested as the mechanism of tolerance to organic nitrates.

Effect of sulfhydryl donation. Several investigators (24–26,40) have previously demonstrated that N-acetylcysteine, a source of cysteine rich in sulfhydryl groups, can potentiate the hemodynamic effects of nitroglycerin in vitro (25,40) and in vivo (24,26). In some cases, it has restored, at least in part, the hemodynamic effects of nitroglycerin after tolerance had developed (21,22,40,41). Others (42,43) have failed to show a beneficial interaction between N-acetylcysteine and organic nitrates. These apparently contradictory findings may be explained by a difference in kinetics between nitroglycerin and the isosorbide dinitrate used by Parker et al. (42) as well as by the marked 17-fold higher nitroglycerin dose used by Munzel et al. (43). In clinically applicable dosing regimens, N-acetylcysteine has repeatedly been shown to potentiate the response to nitroglycerin both immediately and in the tolerant state.

We recently reported (27) that methionine, an essential amino acid capable of augmenting sulfhydryl availability by means of its metabolic conversion to cysteine, can potentiate the immediate hemodynamic effects of intravenous nitro-

glycerin to the same degree as N-acetylcysteine. N-acetylcysteine is a noxious substance because of its sulfhydryl group and is often poorly tolerated when administered orally over a prolonged period. Methionine has been widely used to treat acute acetaminophen overdose and has demonstrated an efficacy equal to that of N-acetylcysteine, but its oral form is far better tolerated (44,45). There is the potential for some degree of methionine intolerance in a small number of patients as a result of partial cystathionine-synthetase deficiency without overt homocystinuria (46,47). Screening procedures to identify these patients has been previously described (47).

Results of the present study. We have demonstrated that intravenous methionine is capable of restoring the venodilative response to sublingual nitroglycerin after tolerance had developed. In addition, tolerance to the venodilative effect of nitroglycerin develops rapidly and can be documented by noninvasive forearm plethysmography.

Limitations. The degree to which intravenous methionine potentiated the venodilative effect of sublingual nitroglycerin at baseline study before long-term exposure to transdermal nitroglycerin was small but significant (from 32% to 37%, $p < 0.02$) One can speculate that a greater degree of potentiation was not seen because of a possible "ceiling" effect on the maximal amount of venodilation possible in the veins of the forearm. Perhaps a lower dose of sublingual nitroglycerin would have resulted in a greater initial potentiation after intravenous administration of methionine.

Overcoming nitrate tolerance. Tolerance to the venodilative effect of sublingual nitroglycerin was clearly demonstrated by this noninvasive technique, with every subject showing tolerance. Once tolerance had developed after 74 h of continuous transdermal nitroglycerin exposure, 5 g of intravenous methionine restored the venodilative response to sublingual nitroglycerin in every subject.

It is likely that the addition of methionine in subjects in the tolerant state did not truly reverse tolerance as much as it potentiated the effect of nitroglycerin after sulfhydryl depletion had occurred. This potentiation was of a much greater magnitude than that seen at baseline study and would likely be of benefit in a clinical setting. Whether the concomitant administration of any sulfhydryl donor, be it N-acetylcysteine or methionine, and nitroglycerin is capable of preventing the development of tolerance is unknown, and further study is required.

Plasma volume expansion. Rapid plasma volume expansion due to neurohumoral activation in patients with long-term exposure to nitrates has also been suggested as contributing to the development of nitrate tolerance (22,23). Packer et al. (22) and Bennett et al. (23) documented a decrease in hematocrit and an increase in weight during continuous nitroglycerin administration. Packer et al. (22) noted increased renin and norepinephrine concentrations in patients who developed tolerance to long-term nitroglycerin administration. Bennett et al. (23) further observed that although the hemodynamic effects of nitroglycerin became attenuated, there was sustained dilation of intraabdominal conduction vessels, suggesting that plasma volume expansion rather than neurohumoral vasoconstriction may be responsible for nitroglycerin tolerance. Our findings of a decrease in hematocrit and an increase in weight associated with the attenuated response to sublingual nitroglycerin add to the evidence that plasma volume expansion may occur after prolonged nitroglycerin exposure; however, its relative contribution to tolerance is unclear. Even in this setting of possible plasma volume expansion, sulfhydryl donation appears to have been sufficient to overcome any modifying influence that volume status renders on the vasodilative response to nitrates.

Conclusions. It is likely that both direct biochemical and indirect neurohumoral mechanisms have independent and additive effects in producing nitrate tolerance in the intact circulation. Patients with "complete tolerance" to organic nitrates still have a significant though blunted response to sublingual nitroglycerin. This observation suggests the presence of some residual sulfhydryl groups, permitting drug action. Furthermore, nitrate attenuation may be only partially reversed with sulfhydryl donors, indicating additional indirect effects of counterregulatory forces.

Evidence for attenuation of long-acting nitrate administration remains a clinical problem. Further clinical trials are needed, using therapies with sulfhydryl donors and neurohumoral blocking agents that may prevent attenuation and permit continuous nitrate administration.

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