Long-Term Nitroglycerin Treatment Is Associated With Supersensitivity to Vasoconstrictors in Men With Stable Coronary Artery Disease: Prevention by Concomitant Treatment With Captopril

THOMAS HEITZER, MD, HANJÖRG JUST, MD, CARSTEN BROCKHOFF, MD,*
THOMAS MEINERTZ, MD,* MANFRED OLSCHEWSKI, THOMAS MÜNZEL, MD*

Freiburg and Hamburg, Germany

Objectives. We examined whether long-term nitroglycerin (NTG) treatment leads to an increase in sensitivity to vasoconstrictors. To assess a potential role of the renin-angiotensin system in mediating this phenomenon, we treated patients concomitantly with the angiotensin-converting enzyme (ACE) inhibitor captopril.

Background. The anti-ischemic efficacy of organic nitrates is rapidly blunted by the development of nitrate tolerance. The underlying mechanisms are most likely multifactorial and may involve increased vasoconstrictor responsiveness.

Methods. Forearm blood flow and vascular resistance were determined by using strain gauge plethysmography. The short-term responses to intraarterial angiotensin II (1, 3, 9 and 27 ng/min) and phenylephrine (an alpha-adrenergic agonist drug, 0.03, 0.1, 0.3 and 1 μg/min) were studied in 40 male patients with stable coronary artery disease. These patients were randomized into four groups receiving 48 h of treatment with NTG (0.5 μg/kg body weight per min) or placebo with or without the ACE inhibitor captopril (25 mg three times daily).

Results. In patients treated with NTG alone, the maximal reductions in forearm blood flow in response to angiotensin II and phenylephrine were markedly greater ($-64 \pm 3\%$ and $-53 \pm 4\%$, respectively) than those in patients receiving placebo ($-41 \pm 2\%$ and $-42 \pm 2\%$, respectively). Captopril treatment completely prevented the NTG-induced hypersensitivity to angiotensin II and phenylephrine ($-33 \pm 3\%$ and $-35 \pm 3\%$, respectively) but had no significant effect on blood flow responses in patients without NTG treatment ($-34 \pm 2\%$ and $-37 \pm 3\%$, respectively).

Conclusions. We conclude that continuous administration of NTG is associated with an increased sensitivity to phenylephrine and angiotensin II that is prevented by concomitant treatment with captopril. The prevention of NTG-induced hypersensitivity to vasoconstrictors by ACE inhibition indicates an involvement of the renin-angiotensin system in mediating this phenomenon.

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Nitroglycerin (NTG) remains one of the most useful drugs in the short-term management of acute ischemic coronary syndromes (for review see [1]). However, the long-term hemodynamic and anti-ischemic efficacy of organic nitrates is rapidly attenuated by the development of nitrate tolerance (2,3). The mechanisms involved in the attenuation of the vasodilator effects of NTG are probably multifactorial and may include neurohormonal counterregulatory mechanisms, a desensitization of the target enzyme guanylyl cyclase or a decreased NTG biotransformation (liberation of nitric oxide [NO]) of the organic nitrate (4,5). Much less attention has been brought to a potential role of vasoconstrictors in contributing to nitrate tolerance. Previous experimental studies (6,7) have shown that long-term treatment with high dose NTG is associated with an increase in sensitivity to alpha-adrenergic stimuli such as phenylephrine and epinephrine. Because part of these phenomena were blocked by using alpha$_1$-adrenoreceptor antagonists, it was hypothesized (6) that long-term treatment with NTG causes a specific sensitization of these receptors. Recently, however, we (8) demonstrated in an animal model of nitrate tolerance that 3 days of continuous treatment with NTG was associated with enhanced vasoconstrictions not only to norepinephrine but also to serotonin, angiotensin II and potassium chloride, suggesting that this phenomenon is not restricted to a specific upregulation of alpha-adrenoreceptors. The present study was therefore performed to determine, whether long-term NTG treatment induces increased sensitivity to various neurohormonal stimuli in patients with stable coronary artery disease.

To address a potential involvement of the renin-angiotensin system in mediating this phenomenon, we treated patients concomitantly with NTG and the angiotensin-converting enzyme (ACE) inhibitor captopril.

Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
ANOVA	=	analysis of variance
cGMP	=	cyclic guanosine monophosphate
i.v.	=	intravenous
NO	=	nitric oxide
NTG	=	nitroglycerin
PKC	=	protein kinase C
t.i.d.	=	three times daily

Figure 1. Schematic diagram of the protocol design. After initial hemodynamic evaluation, patients were allocated to receive 48 h of pretreatment with captopril or no captopril. Thereafter (0-h time point), patients with or without captopril therapy were randomized in a single blinded fashion to receive either i.v. infusion of NTG or placebo (NaCl 0.9%) for 48 h. After 48 h of treatment with NTG or placebo (48-h time point), forearm studies with intraarterial infusion of angiotensin II and phenylephrine were performed.

Methods

Study patients. Forty male patients (mean age 57 ± 4 years [range 39 to 72]) with stable coronary artery disease were studied (Table 1). All patients included in this study were admitted to the hospital for diagnostic cardiac catheterization. Subjects with unstable angina, recent myocardial infarction (within 6 weeks), valvular heart disease, ejection fraction $<35\%$, insulin-dependent diabetes mellitus, arterial hypertension $>160/90$ mm Hg, arterial hypotension (systolic pressure <110 mm Hg) or a history of nitrate intolerance were excluded. Cardiovascular medications (beta-adrenergic blocking agents, calcium channel antagonists, long-acting nitrates) were withheld for ≥ 48 h before participation in the study. All participants gave written informed consent, and the study protocol was approved by the Ethical Committee of the University of Freiburg.

Study protocol. A schematic diagram (Fig. 1) summarizes the study protocol. Subjects were initially screened by medical history and physical examination. Measurements of blood pressure, heart rate and rest forearm blood flow were obtained (-48 h time point). Patients were then recruited in randomized fashion to receive either ACE inhibitor treatment with captopril (25 mg three times daily [t.i.d.]) or no captopril. In patients allocated to receive ACE inhibitor treatment, the final captopril dose (25 mg t.i.d.) was titrated over a 2-day period. On day 1 all patients receiving captopril received 12.5 mg t.i.d.; on day 2 they received 25 mg t.i.d., a concentration previously shown (9) to prevent the development of nitrate tolerance in healthy volunteers. After this 2-day period, all patients were reevaluated in examinations that included measurement of blood pressure, heart rate and baseline forearm blood flow (0 h time point in the protocol, Fig. 1, Table 2). Thereafter, they were randomized in a single blinded fashion to receive either
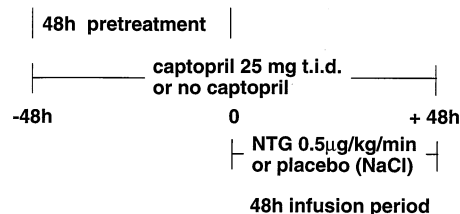
treatment with placebo infusion (NaCl 0.9%) or intravenous (i.v.) treatment with NTG. The chosen NTG concentration was 0.5 μg/kg per min and the infusion period was 48 h. Long-term NTG infusion was continued throughout the protocol. All patients were examined for possible adverse reactions and for recording of supine and standing blood pressure. No patient exhibited symptomatic postural hypotension or other significant side effects. The chosen NTG concentration (0.5 μg/kg body weight per min) for long-term infusion has been shown (10,11) to induce tolerance in both the systemic and coronary circulations within 24 to 48 h. Hemodynamic measurements were made in every patient between 11 and 12 AM after 48 h of continuous NTG or placebo infusion and 4 h after oral intake of the ACE inhibitor.

Hemodynamic measurements. Forearm flow was determined as recently described (12,13). These studies were performed in a quiet, temperature-controlled room. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was inserted into the brachial artery of the nondominant arm for infusion of drugs. This arm was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed on the widest part of the forearm. The strain gauge was connected to an electronically calibrated plethysmograph. A wrist cuff was inflated to suprasystolic pressures 1 min before and during each measurement to exclude the hand circulation. A cuff placed on the upper arm was inflated to 40 mm Hg to occlude venous outflow from the limb. Flow measurements were recorded for 5 s every 10 s and

Table 1. Patient Characteristics*

	Placebo (n = 10)	Placebo + Cap (n = 10)	48 h of NTG (n = 10)	48 h of NTG + Cap (n = 10)
Age (yr)	58.6 ± 4.9	59.4 ± 6.3	58.6 ± 4.9	60.4 ± 5.8
Male/female	10/0	10/0	10/0	10/0
Forearm length (cm)	27 ± 1	27.2 ± 1.1	27 ± 1	26.9 ± 0.9
Forearm volume (liters)	1.02 ± 0.2	1.1 ± 0.12	1.09 ± 0.17	1.06 ± 0.14

*p = NS for all comparisons. Data presented are mean value $\pm$ SEM or number of patients. Cap = captopril; NTG = nitroglycerin.

Table 2. Effects of 48 Hours of Treatment With NTG or Placebo on Systemic and Forearm Hemodynamic Variables in Patients With or Without Captopril Treatment*

	Placebo (n = 10)	Placebo + Cap (n = 10)	48 h of NTG (n = 10)	48 h of NTG + Cap (n = 10)
Mean arterial pressure (mm Hg)				
0 h	92 ± 3	92 ± 3	92 ± 3	94 ± 3
48 h	93 ± 4	91 ± 2	93 ± 3	89 ± 2
Heart rate (beats/min)				
0 h	67 ± 7	67 ± 11	65 ± 6	69 ± 10
48 h	69 ± 6	66 ± 8	71 ± 9	75 ± 7
Forearm blood flow (ml/min per 100 ml of tissue)				
0 h	3.0 ± 0.2	3.0 ± 0.3	2.9 ± 0.3	2.9 ± 0.2
48 h	3.1 ± 0.1	2.9 ± 0.2	2.9 ± 0.4	3.0 ± 0.2
Forearm vascular resistance (U)				
0 h	30.6 ± 1.6	31.2 ± 1.9	31.0 ± 1.1	30.5 ± 1.2
48 h	31.1 ± 0.9	29.9 ± 1.2	29.7 ± 1.6	29.9 ± 1.1

*p = NS for all comparisons. Data presented are mean value ± SEM. Abbreviations as in Table 1.

expressed as ml/min per 100 ml of tissue; the mean flow of seven consecutive readings was used for analyses.

All subjects rested ≥30 min after catheter placement to establish a stable baseline. Basal measurements were then obtained during intrarterial infusion of 0.9% saline solution at a rate of 1.66 ml/min. To assess alpha$_1$-adrenoceptor responsiveness, phenylephrine was administered at increasing doses of 0.03, 0.1, 0.3 and 1 μg/min. Angiotensin II was given at doses of 1, 3, 9 and 27 ng/min. Each dose was infused at a rate of 1.66 ml/min for 5 min, and forearm flow was measured during the last 2 min of each infusion. A 30-min rest period was allowed and basal measurements were repeated between the infusion of the two drugs. Throughout the infusion of vasoconstrictor agents, the long-term infusion of either NaCl 0.9% or NTG was continued.

Systolic, diastolic and mean arterial pressures and heart rate were determined at the contralateral arm with a Dinamap (845 oscillometric) blood pressure recorder. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow. The volume of the forearm, excluding hand and wrist, was measured by water displacement.

Statistical analysis. All values are reported as mean value ± SEM. Group comparisons with respect to baseline characteristics were done by using one-way analysis of variance (ANOVA). The influence of NTG and captopril treatment on the responses to angiotensin II and phenylephrine was assessed in a 2 × 2 factorial design and analyzed by three-way ANOVA for repeated measures considering interaction between the two treatments. If an interactive effect of NTG and captopril reached statistical significance, the nature of this interaction was further investigated by applying a two-way ANOVA for repeated measures to all four treatment groups (placebo, placebo plus captopril, NTG and NTG plus captopril) without consideration of the factorial design. Statistical significance was accepted at a level of alpha = 5%.

Results

Patient characteristics. In all patients studied, there was no significant difference between the four treatment groups in terms of age, blood pressure, heart rate, forearm length or forearm volume (Tables 1 and 2).

Effects of captopril on systemic and forearm hemodynamic variables in patients treated with and without captopril. Changes in systemic hemodynamics and forearm hemodynamic variables in response to long-term NTG or placebo treatment are summarized in Table 2. There were no significant differences with respect to systemic or forearm hemodynamic variables between measurements (before NTG or placebo treatment).

Effects of captopril on vasoconstrictor responses to angiotensin II and phenylephrine. Intraarterial infusion of angiotensin II and phenylephrine caused a dose-dependent decrease in forearm arterioles. The reductions in forearm blood flow in response to angiotensin II and phenylephrine were markedly greater in patients treated with NTG alone ($-64 \pm 3\%$ and $-53 \pm 4\%$, respectively) than in patients receiving placebo ($-41 \pm 2\%$ and $-42 \pm 2\%$, respectively) (Fig. 2 and 3). Concomitant treatment with the ACE inhibitor captopril completely prevented this hypersensitivity in the NTG-treated group ($-33 \pm 3\%$ vs. $-25 \pm 35\%$, respectively). Captopril therapy alone had no significant effect on vascular responses to either angiotensin II or phenylephrine in the placebo group ($-34 \pm 2\%$ and $-37 \pm 3\%$, respectively).

Changes in vascular resistance mirrored in a reciprocal fashion the responses of forearm flow in all four groups (Fig. 2 and 3).

Discussion

To our knowledge, this study provides the first evidence that long-term treatment with NTG induces hypersensitivity to
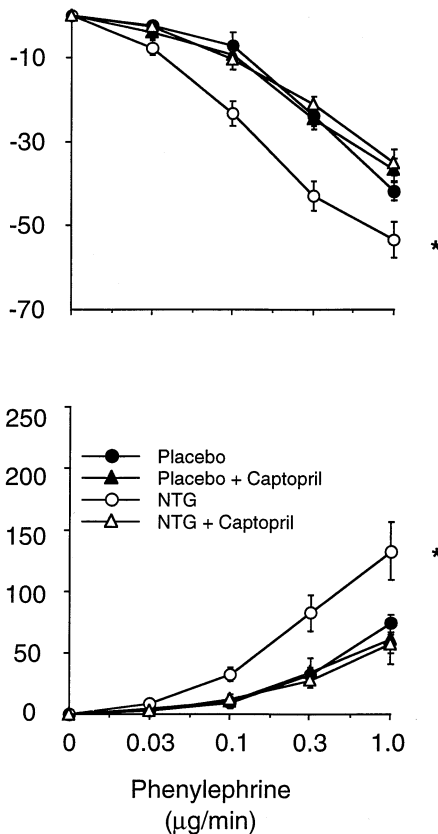
Figure 2. Mean ± SEM responses of forearm blood flow **(top panel)** and vascular resistance to intraarterial phenylephrine **(bottom panel)** in patients after 48 h of placebo infusion **(solid circles),** after 48 h of placebo infusion plus ACE inhibitor (captopril) treatment **(solid triangles),** after 48 h of NTG infusion **(open circles)** and after 48 h of NTG infusion plus ACE inhibitor treatment **(open triangles).** Continuous treatment with NTG for 48 h markedly increased the sensitivity to phenylephrine, a phenomenon that was prevented by concomitant treatment with captopril. The **asterisk** indicates a significant difference between the dose-response relation (two-way ANOVA) in patients treated with NTG alone and that in the other three groups.

Figure 3. Mean ± SEM responses of forearm blood flow **(top)** and vascular resistance **(bottom)** to intraarterial angiotensin II in patients after 48 h of placebo infusion **(solid circles),** after 48 h of placebo infusion plus ACE inhibitor (captopril) treatment **(solid triangles),** after 48 h of NTG infusion **(open circles)** and after 48 h of NTG infusion plus ACE inhibitor treatment **(open triangles).** Continuous treatment with NTG for 48 h markedly increased the sensitivity to angiotensin II, a phenomenon that was prevented by concomitant treatment with captopril. The **asterisk** indicates a significant difference between the dose-response relation (two-way ANOVA) in patients treated with NTG and that in the other three groups.

vasoconstrictors in forearm resistance vessels of patients with stable coronary artery disease. This hypersensitivity was completely prevented by concomitant treatment with the angiotensin I-converting enzyme inhibitor captopril, suggesting that this phenomenon is mediated by angiotensin II released either systemically or locally in response to nitrate therapy. It is likely that the observed increase in sensitivity of the vasculature to neurohormonal agents contributes to nitrate tolerance by counteracting vasodilator effects. Further, this enhanced vasoconstriction may contribute to rebound vasoconstriction and ischemia, which are frequently encountered on abrupt discontinuation of nitrate therapy (14,15).

Increased vasoconstrictor sensitivity in response to long-term NTG-treatment. Previous clinical (3,10,16) and experimental studies (17,18) have primarily focused on the concept that nitrate tolerance is associated with decreased vasodilation in response to the drug. Much less attention has been devoted to the possible role of vasoconstrictors in nitrate tolerance.
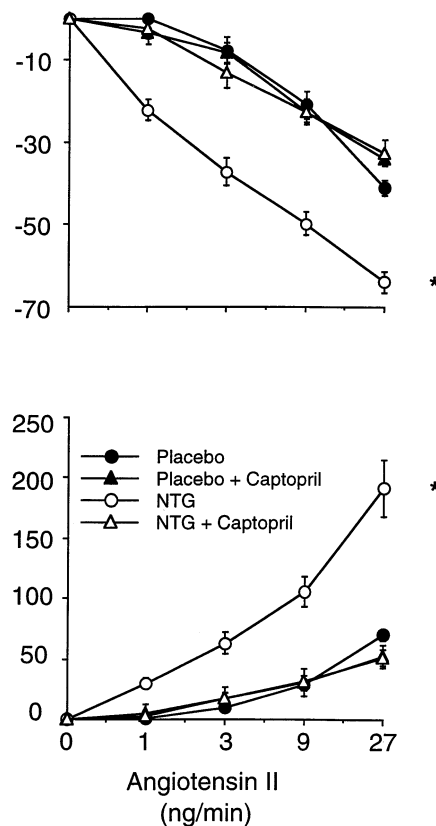
Previous experimental studies (6,7) have demonstrated that long-term treatment with high dose NTG was associated with an increase in sensitivity to alpha-adrenergic stimuli such as epinephrine and phenylephrine. By treating rats for 4 to 7 days with NTG (200 mg/kg subcutaneously), Molina et al. (7) observed that tolerance to NTG was associated with a marked cross tolerance to endothelium-dependent and -independent nitrovasodilators and with an increase in sensitivity to norepinephrine. They also observed that norepinephrine had no effect on cyclic guanosine monophosphate (cGMP) levels in control vessels, whereas in NTG-tolerant aorta norepinephrine significantly decreased cGMP levels in tolerant aorta. Therefore, they concluded that cGMP may play an important physiologic negative feedback signal in hormone- or autacoid-induced smooth muscle contraction and that the observed increase in sensitivity to norepinephrine may be explained by a desensitization of the guanylyl cyclase and also by the cGMP-lowering effects of norepinephrine in the setting of nitrate

tolerance (7). Rydell and Axelsson (6) reported that long-term treatment of mice with NTG caused a marked increase in epinephrine toxicity. Because epinephrine toxicity induced by NTG treatment was inhibited by the alpha$_1$-receptor antagonist prazosin, they (6) postulated that this phenomenon is a rather alpha$_1$-receptor–specific process that may contribute to withdrawal symptoms and sudden death in industrial workers exposed to NTG. With the present studies, however, we can extend these experimental observations by demonstrating that reductions in forearm blood flow in response to intraarterial infusions were markedly enhanced in response not only to the alpha-receptor agonist phenylephrine but also to angiotensin II in patients pretreated with NTG for 48 h. Therefore, the increase in sensitivity to two different vasoconstrictors suggests that this phenomenon is not specific to any one agonist but may more likely involve an intracellular signaling process shared by the different receptors activated by these agonists.

The observation of enhanced vasoconstriction to various agonists is in line with recent experimental studies (8) demonstrating that in the setting of nitrate tolerance in rabbit aorta, the sensitivity of the vasculature is increased to vasoconstrictors including phenylephrine, serotonin, angiotensin II and KCl. In vitro, increased vasoconstriction to phenylephrine and angiotensin II could be blocked by preincubating tolerant vessels with protein kinase C (PKC) inhibitors such as calphostin C and staurosporin, suggesting an involvement of PKC (an important second messenger for smooth muscle contraction [19]) in mediating this phenomenon. Our concept of a PKC-induced vasoconstrictor hypersensitivity in nitrate tolerance was recently confirmed by the observation that in vivo treatment of rats with the PKC antagonist N-benzoyl staurosporin completely prevented the increase in sensitivity to catecholamines and a thromboxane agonist (20).

What mechanisms are responsible for the activation of PKC in response to long-term NTG treatment? NTG therapy in a concentration of 0.3 to 0.5 μg/kg per min is associated with a transient activation of the circulating renin-angiotensin system and increases in circulating vasopressin levels (21,22). Angiotensin II and vasopressin activate PKC (19) and increase the autocrine (local) production of endothelin-1 in vascular tissue (23,24), a phenomenon that was recently demonstrated (8) to occur also in vivo in response to NTG treatment. Autocrine-produced endothelin-1 within vascular smooth muscle cells may serve as a "priming" stimulus for PKC, which in turn mediates the hypersensitivity to angiotensin II and phenylephrine (8). The hypothesis of an involvement of angiotensin II or endothelin, or both, in mediating NTG-induced hypersensitivity to vasoconstrictors is further supported by preliminary experimental observations demonstrating that concomitant treatment with the angiotensin II receptor blocker losartan (AT-1 receptor subtype) significantly inhibits tolerance, the development of a hypersensitivity to vasoconstrictors (25) and in parallel the expression of endothelin within the vascular media (Giaid, Montreal, personal communication).

In agreement with this concept, we observed in the present study that concomitant treatment with the angiotensin I-converting enzyme inhibitor captopril prevented the development of an NTG-induced hypersensitivity to vasoconstrictors. The ACE is identical with kininase II, an enzyme involved in the breakdown of the endothelium-dependent vasodilator bradykinin (26). Thus, high local concentrations of bradykinin in response to ACE inhibition may stimulate release of NO and prostacyclin by the endothelium, thereby promoting additional vasodilation (27). It is tempting to speculate that this could have contributed to the beneficial effect of ACE inhibition on hypercontractile responses of the vasculature. However, this possibility is considered less likely, because captopril therapy in itself had no significant effect on the forearm blood flow responses to intraarterial angiotensin II and phenylephrine in patients treated with placebo. It is therefore conceivable that angiotensin II- or endothelin-1–mediated cellular events account at least in part for the observed hypersensitivity to vasoconstrictors in response to long-term NTG treatment.

Pathophysiologic and therapeutic implications. By summarizing results obtained from vessels from nitrate-tolerant animals it is interesting to note that tolerance is associated with increased endothelial superoxide production (28), increased sensitivity to vasoconstrictors secondary to activation of PKC (8,20) and increased autocrine production of endothelin-1 within the vascular media (8). The present study extends these observations from animal studies by demonstrating that long-term NTG treatment induces hypersensitivity to vasoconstrictors in patients with stable coronary artery disease. This phenomenon occurs within 48 h of long-term i.v. NTG therapy in concentrations (25 mg/12 h) that are commonly used to treat patients with unstable angina or myocardial infarction. Thus, it is conceivable that in addition to venous tolerance (which occurs within 24 h of NTG treatment (29), enhanced propensity of arterioles for vasoconstriction to circulating neurohormones may contribute to the early attenuation of the vasodilator effects of NTG (11) and also to the rebound phenomena encountered after cessation of long-term NTG therapy (15). There is experimental evidence (25,31) that tolerance and enhanced vasoconstriction in the setting of nitrate tolerance might be beneficially influenced by the use of hydralazine (30), high dose AT-1 receptor blockade or high dose ACE inhibition, which might be at least in part attributed to the efficacy of these drugs to inhibit angiotensin II–induced superoxide production or PKC activation in endothelial and smooth muscle cells.

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