Long-term nitroglycerin treatment: effect on direct and endothelium-mediated large coronary artery dilation in conscious dogs

DUNCAN J. STEWART, M.D., JÜRGEN HOLTZ, M.D., AND EBERHARD BASSENGE, M.D.

ABSTRACT We examined the effect of nitroglycerin (GTN) tolerance on an important determinant of nitrate-antianginal action, large coronary artery dilation, in 11 chronically instrumented conscious dogs. In addition, endothelium-mediated coronary artery dilation was studied because this shares a common dilator pathway with the nitrates, i.e., activation of soluble guanylate cyclase. With long-term GTN (1.5 \(\mu g/kg/min\) iv for 5 days) the diameters of the left circumflex and anterior descending coronary arteries showed an initial increase of 8.2 \(\pm\) 0.3% and 10.8 \(\pm\) 0.9%, respectively, returning to control levels by the second to third day of treatment. On days 4 and 5, the dose-response relations for GTN-induced epicardial artery dilation were shifted (\(p < .01\)) to 17- to 20-fold higher doses. However, there was no attenuation of epicardial artery dilation induced by SIN-1 (\(n = 7\)), another activator of guanylate cyclase, or of endothelium-mediated dilation assessed both as flow-dependent dilation (\(n = 7\)) and as direct intra-arterial acetylcholine-induced dilation (\(n = 4\)). In addition, there was no clear tolerance to the peripheral vascular actions of GTN responsible for reflex tachycardia and increased coronary flow. We conclude that a moderate degree of nitrate tolerance to epicardial artery dilation does not affect the responsiveness to other exogenous or endogenous activators of guanylate cyclase. However, this tolerance to epicardial artery dilation, together with the maintenance of peripheral vascular actions that can induce reflex tachycardia, result in a potentially unfavorable balance of GTN effects.


IN PATIENTS with atherosclerotic heart disease, nitroglycerin (GTN) owes its well-recognized antianginal properties largely to the selective dilation of two principle vascular sections, large coronary arteries and peripheral venous capacitance vessels.1, 2 This occurs at dosages that have little overall arteriolar action.1-5 Therefore, blood flow to ischemic myocardium can be augmented by dilation of compliant epicardial coronary artery stenoses6 and/or intercoronary collateral vessels,1 whereas myocardial oxygen demand is reduced (and distribution of myocardial blood flow is improved) due predominantly to a reduction in preload.1 At the same time, the potentially deleterious effects of “coronary steal”7 and “oxygen wasting” reflex tachycardia1 are minimal.

During long-term nitrate exposure, attenuation of the antianginal efficacy of GTN is well documented.8-13 However, the mechanisms underlying this attenuation are controversial.6, 9, 14-17 Nitrate tolerance does not appear to develop uniformly in all sections of the vascular tree.8, 9, 14-17 Rapid development of “arterial” tolerance, using arterial pressure as an index of GTN effect, has previously been reported.9, 12, 17-19 However, with respect to hemodynamic and antianginal consequences, a clear distinction must be made between the different “arterial” actions of nitrates, i.e., large artery vs peripheral arteriolar dilation.1 Furthermore, a decrease in arterial pressure can result from either of two GTN actions: (1) arteriolar dilation and decreased peripheral vascular resistance (as is often assumed) or (2) venodilation and decreased venous return.1, 20, 21 In addition, this hypotensive action is subject to potent secondary reflex counterregulation.15, 20 With these reflexes inhibited, we have recently documented selective tolerance to GTN-induced venodilation, one of its important antianginal actions, without attenuation of its peripheral arteriolar effects.15

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Supported by the Deutsche Forschungsgemeinschaft, grant Ba 408/12-6. Dr. Stewart is a research fellow of the Medical Research Council of Canada.

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Received Aug. 8, 1986; revision accepted Dec. 18, 1986.
Therefore, the aim of this study was to assess whether GTN-induced dilation of epicardial coronary arteries, which constitutes a second important antianginal action, is also subject to tolerance under long-term GTN treatment. We now show that, with the same nitrate exposure as in the previous report, a specific tolerance develops to GTN’s action on large coronary arteries but, as before, not to its peripheral vascular effects, which can result in “oxygen wasting” reflex tachycardia.

Although it is now recognized that endothelium-dependent and nitrate-induced dilation might share a common mechanism of action, the effect of nitrate tolerance on endothelium-mediated dilation of epicardial arteries in vivo has not yet been evaluated. Endothelium-derived relaxing factor (EDRF) is a potent nonprostanoid endogenous vasodilator. A critical step in EDRF-induced dilation is the stimulation of soluble guanylate cyclase and the subsequent increase in smooth muscle cyclic GMP. This mechanism is shared with the nitrovasodilators and recent evidence in vitro implicates a depression of the activity of guanylate cyclase as mediating tolerance to nitrate actions, which develops under high-level exposure. To assess whether EDRF-induced dilation is also attenuated under conditions of GTN tolerance in vivo, flow-dependent dilation was studied as a physiologic marker of endothelium-mediated vasomotion and, in some animals, acetylcholine-induced dilation served as an example of receptor-mediated EDRF release.

Methods

Animals. Eleven mongrel dogs of either sex and weighing 24 to 31 kg were selected from a group of 16 animals and instrumented under pentobarbital anesthesia for the measurement of coronary flow and epicardial coronary artery diameters (figure 1). Criteria for the inclusion into this study were: a good recovery from the thoracotomy for implantation of instruments (3 weeks or more before entry), resting body temperature below 39.0°C, readiness to lie quietly on the experimental table for periods of 3 hr, proper functioning of the implanted instruments, and a minimal epicardial artery dilation of 100 μm in response to 2 μg/kg iv GTN in both branches of the left coronary artery, which were equipped with perivascular piezoelectric crystals for continuous diameter recording. Thus, those dogs were eliminated from the study in which postcoronary cicatriziation induced by the implanted instruments had severely impaired the dilation potential of the epicardial arteries. However, some perivascular accumulation of connective tissue was always found at postmortem inspection in the dogs included in this study. Therefore some impairment of epicardial artery dilation probably also existed even in the animals selected for study. Seven dogs equipped as shown in figure 1 were designated as group A; four dogs (group B) had, in addition to the equipment shown in figure 1, coronary catheters (0.8 mm od) implanted into the circumflex branch of the left coronary artery 2 cm proximal to the perivascular crystals. This catheter was inserted by the Herd-Barger technique. All dogs used in the study had a polyethylene catheter implanted in the pulmonary artery for long-term infusion of GTN. Before instrumentation of the dogs, a common carotid artery had been translocated into a cutaneous loop at the ventral surface of the neck.

Throughout the study, the dogs were kept on their standard diet containing 2 to 4 meq/kg sodium per day, with free access to tap water. Before inclusion into this study, none of the dogs had been used in other experiments. Care of the dogs was in accordance to the guidelines of the American Physiological Society.

Design of the long-term GTN treatment study. The dogs in groups A and B were evaluated on two separate days under control conditions, on each of 5 days under long-term GTN infusion, and during the withdrawal phase after infusion was stopped (see table 1). The animals were studied on each day between 8 and 12 A.M. while resting quietly on the experimental table. For long-term GTN exposure, a dose of 1.5 μg/kg/min GTN was infused continuously over 5 days via the pulmonary artery catheter, starting in the afternoon of day zero (see figure 2). Portable battery-operated electrolytic pumps (Sage instruments, model 216) were used and each animal received 0.5 ml/hr of 99% ethanol as solvent for the GTN throughout the treatment period. GTN solutions were always freshly prepared immediately before charging the 20 ml glass reservoir of the pump. One to 2 weeks after the end of the GTN application, three dogs of group A were used again to evaluate the effects of sham treatment (0.5 ml/hr ethanol without GTN) in an identical protocol.

Experimental protocols. When the dogs were lying on the experimental table, the carotid artery in the skin loop and a tibial vein were punctured with canulas, and baseline variables were measured after a resting period of 30 min. Thereafter the hemodynamic effects of cumulative infusions of GTN or SIN-1, the flow-diameter relation of the epicardial arteries, or the actions of intracoronary acetylcholine were analyzed as indicated in table 1. No experiment exceeded 3 hr.

For the assessment of large artery and hemodynamic effects of GTN infusions, three doses were applied intravenously during one experiment, 15 min at each concentration, with a 20 min interval between. Coronary flow was measured with a Gould SP 2202 flowmeter, coronary artery external diameters by ultrasonic transit time crystals, and arterial pressure by a Statham P 23 pressure transducer connected to the cannula in the carotid artery; heart rate was derived from an arterial pressure signal. Phasic and mean tracings of all variables were recorded on a Beckman dynograph and readings of variables were averaged over 30 sec. In the control and withdrawal phases, the GTN doses applied were 0.15, 0.5 and 1.5 μg/kg/min, whereas during long-term GTN treatment (continuous application of 1.5 μg/kg/min into the pulmonary catheter), the additional doses of 1.5, 5.0, and 15.0 μg/kg/min were infused, yielding total doses of 3.0, 6.5, and 16.5 μg/kg/min, respectively (see table 1). For assessment of the actions of SIN-1, doses of 0.1, 0.3, and 1.0 μg/kg/min were infused both in the control phase and during the phase of long-term GTN treatment in an identical protocol.

The flow-diameter relation of the circumflex branch of the left coronary artery was analyzed as a quantititative variable of the endothelium-mediated, flow dependent dilation. It was studied in the resting conscious dogs (group A) by manipulating left circumflex coronary flow with a pneumatic occlusion cuff implanted distal to the site of diameter measurement (figure 1). The approach has been described previously and is illustrated briefly by the examples shown in figure 1. For one flow-diameter relation, five to 10 values of mean coronary flow between 40% and 230% of control flow were studied as shown in figure 1, manipulations 1 and 2. These determinations were performed
FIGURE 1. Schema of long-term instrumentation (left) and analysis of flow-dependent epicardial artery dilation (right). Tracings from a typical experiment in a resting, conscious dog under control conditions. Abscissa depicts time on the experimental table. Ordinates (from top to bottom): mean arterial pressure (AP<sub>m</sub>); heart rate (HR); coronary diameter (CD) of the LAD and LC; coronary flow (CF); mean coronary flow (CF<sub>m</sub>), and mean coronary diameter (CD<sub>m</sub>) of the LC. Experimental manipulations (from left to right): (1) Mean coronary flow is reduced to 50% of control for 2 min by partially inflating the pneumatic cuff, resulting in a gradual reduction of LC diameter by 50 μm without any change in LAD diameter. (2) After occlusion of the LC by the cuff for 30 sec, the cuff is partially deflated, allowing mean coronary flow to rise to 56% above preocclusion control. Mean coronary flow is limited at this level by manually adjusting the cuff, until flow returns back to control. LC diameter falls slightly during the occlusion and then rises by 60 μm above preocclusion control. Several Δ-values of CF and CD obtained with manipulations 1 and 2 were used to obtain the flow-diameter relation. (3) Occlusion of the LC for 30 sec and the unrestrained postocclusion reactive hyperemia induce the "reactive dilation" of the LC described previously. Several manipulations 1 through 3, there is always a delay from the onset of the increase in flow to the onset of diameter changes. (4) GTN intravenously causes dilation of both epicardial branches, thereby demonstrating dilatory responsiveness of the control branch (LAD).

TABLE 1

<table>
<thead>
<tr>
<th>Protocol phases</th>
<th>Control</th>
<th>Long-term GTN</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -1</td>
<td>Day 0</td>
<td>Day 4</td>
<td>Day 5</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 4</td>
<td>Day 5</td>
<td>Day 9</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 4</td>
<td>Day 5</td>
<td>Day 9</td>
</tr>
<tr>
<td>Group A</td>
<td>GTN: 0.15–1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>GTN: 3.0–16.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>GTN: 0.15–1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n = 7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>SIN-1: 0.1–1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SIN-1: 0.1–1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SIN-1: 0.1–1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group B</td>
<td>GTN: 0.15–1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>GTN: 3.0–16.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(n = 4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ACh ic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ACh ic&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>In both groups, basal values of hemodynamic parameters were obtained following 30 min of rest on each day of the study protocol.

<sup>b</sup>Doses are given in μg/kg/min iv.

<sup>c</sup>During intracoronary infusion of acetylcholine (ACh, 0.2 to 0.3 μg/kg/min), mean coronary flow was kept constant mechanically (see Methods). For each dog, the same dose was applied in the two protocol phases.
Results

Baseline data. Baseline values for the seven group A dogs are presented in figure 2 for coronary artery diameters and hemodynamic variables. The fairly wide SEM for values of coronary artery diameter represents the variations in baseline diameter between different dogs (mean control left circumflex [LC] diameter 3.41 ± 0.20 mm, range 2.58 to 3.99; left anterior descending [LAD] diameter 2.67 ± 0.21 mm, range 2.08 to 3.40). On the other hand, the variation in diameters for each dog during the 2 days of the control period was very low (i.e., mean variation of LC diameter 0.01 ± 0.02 mm, range 0.00 to 0.05). On day 1 of long-term treatment with GTN 1.5 μg/kg/min, the diameters of both coronary arteries rose significantly (p < .001) to 3.69 ± 0.21 mm for the LC and 2.97 ± 0.24 mm for the LAD, representing an increase of 8.3 ± 0.4% and 11.2 ± 1.3%, respectively. Under continuous exposure to GTN, baseline diameters of both coronary arteries rapidly returned to control levels, indicating development of tolerance, and were not significantly different from the control values by the second (LAD) or third (LC) day of treatment. During long-term GTN treatment there were no significant changes in the hemodynamic variables measured, although there was a trend toward increased heart rate that persisted for much of the infusion period.

Short-term GTN and SIN-1 challenges. Coronary diameter, coronary flow and hemodynamic variables for the dogs of group A are presented in figure 3 during short-term challenge doses of GTN, in both the tolerant and control states. Note that the GTN challenge doses presented are greater than 10-fold higher in the tolerant than in the control state. Therefore the similar degree of large coronary dilation depicted actually reflects tolerance to large coronary dilation. However, at these doses of GTN that are equipotent in terms of epicardial artery dilation, the heart rate and coronary flow responses were substantially greater in the tolerant dogs. In figure 4, the same variables are shown during SIN-1 challenges. Dosages of SIN-1 were the same for both states, but the tolerant dogs received the ongoing infusion of 1.5 μg/kg/min GTN throughout the protocol. Large coronary artery dilation in response to SIN-1 was identical in both states, indicating an absence of cross-tolerance between GTN and SIN-1. However, as before, heart rate and coronary flow responses were augmented in the dogs undergoing long-term GTN infusion.

In figure 5, the responses to GTN and SIN-1 challenges are normalized in terms of percent change. It is now apparent that the increases in large coronary artery
RESPONSIVENESS TO SHORT-TERM GTN CHALLENGES

**FIGURE 3.** Responsiveness to short-term GTN challenges in the seven dogs of group A during the control phase (closed symbols) and on day 4 or 5 of long-term GTN treatment (tolerance, open symbols). Abbreviations as in figure 2. Note that under tolerance, the long-term GTN infusion of 1.5 μg/kg/min is supplemented by short-term infusions yielding the challenge doses of 3.0, 6.5, and 16.5 μg/kg/min, respectively. Asterisks indicate significance of differences from the last pre-challenge value: *p < .05; **p < .01.

The diameter in response to GTN and SIN-1 were highly uniform and reproducible in the control state. On days 4 and 5 of the long-term GTN infusion, the repeat GTN dose-response relation for coronary diameter was shifted to 18- (LC) and 22-fold (LAD) higher doses. However, no shift in SIN-1 responsiveness was detectable for coronary artery diameters. Although responses of heart rate and coronary flow to GTN or SIN-1 challenges did not reach significance under control conditions, larger, significant responses were observed to the challenge doses delivered during long-term GTN treatment (figure 5).

Three days after the withdrawal of long-term GTN (day 9 of protocol, see table 1), epicardial artery dilations in response to GTN challenge doses (0.15 to 1.5 μg/kg/min) did not differ significantly in maximal dilatation or ED₅₀ value from the GTN dilations in the control phase, indicating resolution of tolerance.

**ENDOTHELIUM-MEDIATED DILATION.** In group A, flow-dependent dilation was used as an indication of endothelium-mediated dilatory reactivity, as shown in figure 1. No attenuation of this reaction could be observed under tolerance (days 4 and 5 of long-term GTN treatment) using any of three approaches for quantification (table 2).

In group B, acetylcholine (0.2 to 0.3 μg/kg/min) was infused into the coronary artery, while mean coronary flow was maintained at the preinfusion level by partially inflating the pneumatic occluder. Under these conditions, the left circumflex diameter increased by 3.2 ± 1.0% (p < .05) in the control state. On day 4 or 5 of long-term GTN infusion, this dilation was similar, amounting to 3.4 ± 1.1%. Heart rate and mean arteri-
al pressure remained constant during the infusion under both states.

In group B, the maximal dilation on day 1 of long-term GTN treatment amounted to 8.1 ± 0.4% above control for the LC and to 10.1 ± 1.3% for the LAD. On days 4 and 5 of long-term treatment, diameters had returned to control levels, but the dilatory response of the epicardial arteries to GTN challenge was shifted to 15- and 17-fold higher doses in the two arteries, respectively.

By combining the data from groups A and B, we obtained a maximal dilation on day 1 of 8.2 ± 0.3% above control for the LC and of 10.8 ± 0.9% for the LAD, respectively. The log (ED$_{50}$ control/ED$_{50}$ tolerance) of the epicardial artery GTN response amounted to 1.23 ± 0.09 for the LC and to 1.30 ± 0.09 for the LAD, indicating a 17- and 20-fold shift, respectively.

**Alcohol sham treatment.** In the sham-treated dogs (n = 3) there was no change in baseline coronary diameter, coronary flow, or hemodynamic variables during the 5 day sham infusion of alcohol (0.5 ml/hr 99% ethanol). In addition, dose responses to GTN were completely comparable between the control state and on day 5 of sham treatment, yielding a log (ED$_{50}$ control/ED$_{50}$ sham) of 0.04 ± 0.06 (not significantly different from zero). Variables of flow-dependent dilation were not modified significantly (table 2).

**Discussion**

**GTN tolerance of epicardial arteries.** Dilation of large coronary arteries represents a selective and clinically relevant variable of nitrate action that has been studied over the short term both in patients and animals. However, much less is known concerning the responses in vivo of these vascular diameters to long-term nitrate treatment. Our results in conscious dogs equipped with ultrasonic vascular diameter gauges show that tolerance to GTN-induced epicardial artery dilation develops readily during a continuous par- enteral infusion of GTN. This tolerance was well established by the second to third day of treatment (figure 2).

A potential problem with the technique of long-term diameter registration with piezoelectric crystals must be considered briefly. To a greater or lesser extent, a degree of perivascular fibrosis develops in all animals so instrumented. However, despite this potential limitation to dilation, these vessels exhibited a similar degree of dilation in terms of change in cross-sectional area as that reported with angiography. Furthermore, care was taken in the study design to avoid potential errors relating to this scarification process. GTN dilation was always compared with another vasodilator (SIN-1) when dose responses were assessed. Furthermore, after withdrawal of long-term GTN, repeat GTN dose responses served as controls for nonspecific reductions of epicardial artery responsiveness with time.

**Partial GTN tolerance.** In this study, the tolerance to GTN observed under long-term treatment was only a partial tolerance. Although the dose response of epicardial arteries was shifted to 20-fold higher doses (figure 5), no clear tolerance could be demonstrated for heart rate and coronary blood flow responses. Tachycardia induced by GTN represents a secondary in-
crease in sympathetic tone because of the activation of baroreflexes, counteracting hypotensive influences.\textsuperscript{1, 14, 20, 21} The action of these reflexes can effectively mask the blood pressure changes\textsuperscript{15, 20} and are likely responsible for the small magnitude of arterial pressure declines observed in the present report. Although GTN-induced hypotension can be caused by venous as well as arteriolar dilation, a rapid development of venous tolerance has been documented in a recent report.\textsuperscript{38} In addition, we have shown that tolerance to the venous action of GTN develops more readily than for its effects on peripheral vascular resistance\textsuperscript{15} and discussed in detail the reasons for this apparent conflict with some earlier reports,\textsuperscript{9, 17} which include difficulties in choosing selective indexes of venous and arteriolar action as well as the effect of counterregulatory influences.\textsuperscript{14, 15} Thus, under long-term treatment, the increase in tachycardic response to GTN relative to large coronary dilation (figure 3) is likely secondary to reflex sympathetic activation induced by continued GTN action on the peripheral arteriole in the face of tolerance to its effects on epicardial arteries. The increase in coronary blood flow in the tolerant dogs receiving the higher doses of GTN (figure 3) parallels the heart rate response and to a large extent probably reflects increased myocardial oxygen consumption due to tachycardia, although an additional direct effect of GTN on coronary arterioles cannot be excluded.

The findings with SIN-1 likely reflect this same process. In the tolerant state, there was a greater increase in heart rate (and less so in coronary flow) at the same doses of SIN-1 (figure 4). This can be explained if one assumes a continued influence of the long-term 1.5 \(\mu g/kg/min\) GTN infusion on parameters of peripheral resistance, so that the additional doses of SIN-1 are additive with respect to these peripheral actions and result in a more intense reflex activation. Therefore these observations with two different vasodilators both point to a partial (and selective) GTN tolerance to a large coronary artery dilation, sparing its peripheral arteriolar actions.

**Endothelium-mediated dilation.** Despite documented attenuation of direct GTN-epicardial artery dilation during long-term exposure, we were unable to demonstrate any change in endothelium-mediated arterial dilation in vivo. Flow-dependent dilation is the dilation of an artery that occurs in response to an increase in blood flow, independent of local changes in pressure.\textsuperscript{31-33} It is an active process that has been demonstrated in vivo to be dependent on the presence of endothelium.\textsuperscript{33} Recent evidence suggests that this mechanically induced dilation is mediated by a nonprostanoid endothelium-derived factor that is likely identical to EDRF.\textsuperscript{39,41} By any of three variables of flow-dependent dilation, i.e., the change in artery diameter per change in coronary flow, the time delay to the onset of flow-dependent dilation, and the peak dilation in response to unrestrained postocclusion reactive hyperemia, we could not obtain evidence for an attenuation of the flow-dependent dilation under GTN tolerance in our dogs (table 2).

By its very nature, flow-dependent dilation is an indirect variable because the change in shear stress must be sensed by the endothelium and translated into a vasodilator signal.\textsuperscript{32, 33, 39, 40} Therefore, in some of the dogs (group B) we analyzed the receptor-mediated endothelial stimulation by acetylcholine. This represents

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**TABLE 2**

Flow-dependent dilation

<table>
<thead>
<tr>
<th>Protocol phases</th>
<th>Slope of flow-diameter relation (%Δdiameter/100%Δflow)</th>
<th>Postocclusion reactive hyperemia</th>
<th>Peak &quot;reactive dilation&quot; (%Δdiameter)</th>
<th>Calculated delay\textsuperscript{b} for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occlusion (sec)</td>
<td>Peak flow (%Δflow)</td>
<td>50%Δflow (sec)</td>
<td>400%Δflow (sec)</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>5.6±1.1</td>
<td>26±2</td>
<td>424±62</td>
<td>4.65±0.18</td>
</tr>
<tr>
<td>Long-term GTN (n = 7)</td>
<td>5.4±1.3</td>
<td>27±2</td>
<td>438±78</td>
<td>4.51±0.19</td>
</tr>
<tr>
<td>Repeat control (n = 3)</td>
<td>5.1±0.8</td>
<td>25±2</td>
<td>442±82</td>
<td>4.2±0.24</td>
</tr>
<tr>
<td>Sham treatment (n = 3)</td>
<td>5.2±0.8</td>
<td>25±2</td>
<td>429±83</td>
<td>4.1±0.24</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Calculated in each dog according to: diameter = intercept + slope · Δflow. Intercept was always within ±0.2% of zero; \(r^2\) varied between .81 and .99.

\textsuperscript{b}Calculated in each dog according to: delay = intercept + slope · log(Δflow); \(r^2\) varied between .58 and .98.
the “classic” pathway for inducing EDRF release and has been well characterized in a variety of models. Endothelium-mediated dilation by acetylcholine-induced EDRF release was also unchanged after development of GTN tolerance (table 2), thus substantiating the flow-dependent dilation findings.

The maintenance of endothelial dilator function in the face of tolerance to GTN has several important implications. First, in all probability the degree of GTN tolerance to epicardial artery dilation in this study is somewhat underestimated. As discussed above, with the higher doses of GTN that were required to achieve large vessel dilation in the tolerant animals, a substantial increase in coronary flow was observed, which might have contributed to the observed epicardial artery dilation by the mechanism of the flow-dependent dilation. Second, receptor-mediated EDRF release is thought to be important in reducing vasoconstriction under a variety of stimuli (i.e., ergonovine, thrombin, platelet-released serotonin, ADP, etc.). Moreover, considerable baseline EDRF release from native endothelium has been documented in the unstimulated state. Therefore substantial depression of this endothelial function would be expected to alter baseline or stimulated vasomotor tone and increase the tendency of the vessel wall toward vasoconstriction. The absence of such undesirable features in usual states of GTN tolerance is consistent with our present findings of a well-preserved endothelial dilatory function under long-term application of a clinically relevant dosage of GTN (i.e., 100 μg/min for the average adult).

However, under extreme conditions, such as in industrial exposure or in laboratory studies using high-dose prolonged treatment, nitrate-withdrawal complications have been reported. In such states there appears to be a marked increase in the tendency of the vessel wall toward vasoconstriction, both stimulated and spontaneous. Whether these changes are associated with a depression of endothelium-dependent dilation awaits future investigation with high-dose, “supraclinical,” long-term GTN treatment.

Cellular mechanism of GTN tolerance. Substantial experimental evidence supports the hypothesis that both EDRF and GTN produce vessel dilation by stimulation of soluble guanylate cyclase and increasing cyclic GMP (for review see ref. 26). Studies have demonstrated that GTN tolerance in vitro is associated with a decrease in smooth muscle cyclic GMP accumulation, which has recently been shown to be caused by a depression of guanylate cyclase activity. Therefore, under these conditions a decrease in endothelium-mediated dilation would be expected in the GTN-tolerant state. The lack of an attenuation of endothelium-mediated dilation seen in this study might be explained by one of several possibilities: (1) EDRF and GTN may activate different “pools” of (or different sites on) guanylate cyclase, and therefore induction of GTN tolerance leaves the activity of “EDRF-guanylate cyclase” unaltered. (2) Some attenuation in endothelium-mediated dilation was present but was overcome by supramaximal endothelial cell stimulation. This is unlikely since a full range of changes in flow were investigated, with small flow increases resulting in only threshold dilation. (3) GTN tolerance in this study was not due primarily to depression of guanylate cyclase activity but was related to another mechanism.

Of these explanations, the latter is the most likely. Considerable data have accumulated documenting a nitrate interaction with cytoplasmic sulfhydryl groups, which generates an active species necessary for dilation. This interaction likely occurs at two levels. Alteration in the redox state of tissue sulfhydryl donors under conditions of tolerance to nitrates has been proposed, and prevention and reversal of GTN tolerance with substances that replenish these groups (i.e., N-acetylcysteine) has been demonstrated. Such a mechanism of tolerance agrees well with the maintenance of SIN-1 potency in the face of tolerance to GTN observed in the present report (figures 4 and 5). SIN-1 is the active metabolite of molsidomine, an NO-containing vasodilator that, unlike GTN, does not require cytoplasmic interaction with reduced sulfhydryl groups to stimulate guanylate cyclase and produce smooth muscle dilation. This lack of cross-tolerance to SIN-1 points to a cellular alteration occurring before the shared pathway of guanylate cyclase stimulation accounting for nitrate tolerance, at least under the modest level of GTN exposure in vivo studied here.

Possible relevance to antianginal action of GTN. GTN improves the ratio of oxygen supply to demand in ischemic myocardium by virtue of its direct effects on the coronary vasculature, as well as indirectly, via effects in the peripheral circulation. Dilation of large coronary arteries and collateral vessels may increase perfusion of ischemic muscle distal to a stenosis, whereas systemic venodilation reduces ventricular diastolic pressure and improves the ratio of subendocardial to subepicardial blood flow. A reduction in ventricular filling pressure may also result in a reduction in ventricular volume, which, together with any lowering of vascular resistance and outflow impedance, reduces myocardial wall tension and oxygen consumption. Recently it has been shown that toler-
 ance develops rapidly to GTN effects on venous compliance.15,38 We now show that tolerance develops to GTN effects on large coronary arteries to a similar degree and with a similar time course as for its venous effects. Taken together, these two mechanisms likely contribute to the rapid attenuation of antianginal efficacy observed in clinical studies of long-term continuous nitrate therapy.10-13

However, our observations might have further implications to the long-term clinical use of parenteral GTN. Compared with the nontolerant state, the higher doses of GTN that are required to produce a comparable dilation of large coronary arteries (a determinant of antianginal effect) in the tolerant state now result in substantially more tachycardia and coronary flow changes (figures 3 and 5). Tachycardia itself will increase myocardial oxygen demand,1 reducing, or reversing, any potential net benefit derived from an associated lowering of wall tension caused by GTN’s effects on ventricular volumes and afterload. This, in conjunction with any possible direct coronary arterial dilation (i.e., “coronary steal”) at these higher doses, is potentially harmful in patients with ischemic heart disease.1 Therefore the spectrum of GTN action under tolerance is shifted to a less desirable (and possibly deleterious) balance of vascular effects. Whether a similar shift in the dilator spectrum of GTN occurs in patients with coronary artery disease under nitrate tolerance must be shown before its relevance to the treatment of angina can be assessed. Its demonstration, however, would substantially improve our current understanding of the clinical long-term use of nitrates.

In summary, continuous parenteral treatment with a modest dosage of GTN results in tolerance to GTN-induced large coronary artery dilation in vivo. There is no alteration in the potency of other stimuli that activate guanylate cyclase to bring about dilation (i.e., EDRF and SIN-1), suggesting that the mechanism of tolerance likely involves an earlier step in the cellular pathway of dilation. Thus potential dangers of depression of endothelium-mediated vasomotion in patients with ischemic heart disease would not appear to be a problem with clinically relevant states of GTN tolerance. However, the balance of vascular effects under tolerance is altered, reducing GTN’s beneficial properties in anginal syndromes (i.e., venous and large artery coronary dilation) and enhancing its potentially harmful influence on determinants of myocardial oxygen consumption (tachycardia) and possibly supply (coronary steal) should higher dosages be used with the intention of overcoming the tolerance to antianginal actions.

We are indebted to Helmut Siegel for excellent and careful technical assistance and to Christian Holtz for performing the statistical analysis. The nitroglycerin was a generous gift from Pohl-Boskamp, Hohenlockstedt.

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Circulation. 1987;75:847-856
doi: 10.1161/01.CIR.75.4.847

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/75/4/847

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