Altered spectrum of nitroglycerin action in long-term treatment: nitroglycerin-specific venous tolerance with maintenance of arterial vasodepressor potency

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ABSTRACT The study of venodilator tolerance to nitroglycerin has been complicated by reflex compensation and by problems in analyzing venous tone in the presence of multiple determinants of venous pressure. We assessed venous tone as total effective vascular compliance (TEVC) under autonomic blockade in six dogs, in the nontolerant state, and during a 5 day infusion of nitroglycerin (1.5 μg/kg/min). Under long-term treatment, baseline TEVC was unaffected and the nitroglycerin dose-response relationship for TEVC was shifted to greater than 10-fold higher doses, whereas baseline mean arterial pressure (MAP) was lowered by 17 ± 3 mm Hg without any shift in nitroglycerin responsiveness. This lowering of MAP was observed only after autonomic blockade. In six additional dogs instrumented with aortic flow probes, nitroglycerin (1.5 μg/kg/min) induced a 15 ± 1% decline in peripheral vascular resistance (PVR) under autonomic blockade, but with reflexes intact these dogs showed no change in PVR and a 21 ± 10% increase in norepinephrine release rate. We conclude that modest long-term exposure to nitroglycerin results in tolerance to its venodilating effects, whereas arteriolar action is maintained. This tolerance-induced shift in action from venous toward arteriolar dilation is normally masked by compensatory reflexes.


THE HEMODYNAMIC EFFECTS of nitroglycerin are complex, and even with short-term administration the primary vasodilator action of nitroglycerin is subject to powerful counterregulatory reflex influences.1-3 Prolonged nitrate exposure results in a decrease of at least some of its therapeutic efficacy,6-15 although considerable controversy exists concerning the nature of this tolerance. It has been suggested that the action of regulatory mechanisms may result in an apparent "tolerance."5,7,16,17 However, more frequently nitroglycerin tolerance is considered to result from an alteration in the target tissue itself,18-21 with failure to relax arteries and/or veins. Although many studies have shown a partial tolerance, with a differential susceptibility of the venous and arterial systems to the development of nitrate tolerance in vivo,6,9,10,22 there is still no general agreement concerning the specific response of either system to long-term nitroglycerin. Some groups report a preferential venous vs arterial tolerance,9,10,23 and others demonstrate an opposite result,7,8 even questioning whether tolerance to venodilation occurs at all.8

The importance of choosing a selective index of drug effect in studying nitroglycerin tolerance has been stressed,15,22 as well as the need for consideration of compensatory mechanisms, especially in relation to the effects of nitroglycerin on arterial pressure.22 Therefore, this study was designed specifically to investigate the venodilator actions of nitroglycerin in vivo both before and during long-term exposure to the drug by measuring its effect on total vascular compliance. Autonomic blockade was used to assess the contribution of neurogenic mechanisms to the hemodynamic profile of nitroglycerin under tolerance.

In addition, we investigated the mechanism of nitrate tolerance in vivo by examining for possible cross-
tolerance with SIN-1, the NO-containing active metabolite of molsidomine. Because studies in vitro have shown continued activity of SIN-1 in the face of tolerance to nitroglycerin, we tested whether this selectivity could be demonstrated in vivo. Furthermore, since a deficit of cellular S-H groups has been proposed as a mechanism underlying nitroglycerin tolerance, the use of SIN-1, which has no such requisite interaction, might provide additional clues toward an understanding of this state.

In dogs under sustained parenteral nitrate treatment, we document specific tolerance to the venodilator effects of nitroglycerin but no demonstrable tolerance to its action on arterial pressure when autonomic reflexes were inhibited.

Methods

Animals. Six mongrel dogs of either sex, weighing 19 to 36 kg (mean 24 ± 3), were studied both in the control state and during long-term infusion of nitroglycerin in a crossover protocol. Two weeks before initiation into the series, polyethylene catheters for long-term drug infusion were implanted into the external jugular vein percutaneously, with animals under pentobarbital anesthesia, and tunneled subcutaneously to the back. At each subsequent evaluation, the dogs were again anesthetized and catheterized as described below. Intervals of at least 2 weeks were allowed between consecutive experiments in the same dog. The dogs were kept on a standard diet containing 2 to 4 meq/kg Na+ per day with free access to tap water. During the experimental period the dogs maintained their body weight and remained in vigorous condition. In a separate experimental series (validation experiments), an additional seven dogs were prepared for long-term study with electromagnetic aortic flow probes (Gould/Statham) implanted around the ascending aorta and aortic catheters. Instruments were implanted by means of a left thoracotomy, with animals under pentobarbital anesthesia. At least 10 days were allowed for full recovery before the first experimental evaluation. All studies were performed in accordance with the guidelines of the American Physiological Society.

Design of the long-term nitroglycerin treatment study. The six dogs in the long-term treatment series were evaluated both under control conditions and under long-term infusion of nitroglycerin (table 1). Three of these six animals were evaluated first under control conditions (i.e., without prior long-term exposure to nitroglycerin), and the second experiment, 14 days later, was performed during long-term nitroglycerin treatment.

In the other three dogs, the first experiment was performed during long-term nitroglycerin exposure, while the second experiment (again 14 days later) was the control evaluation. For long-term nitroglycerin exposure, a dose of 1.5 μg/kg/min nitroglycerin was infused continuously over a 4 day period via the external jugular catheter with a battery-operated electrolytic pump (Sage Instruments, model 216). This delivered nitroglycerin dissolved in a constant volume of ethanol so that each animal received 0.5 ml of 99% alcohol per hour. On the fifth day of treatment, the dogs were evaluated as described below ("Measurements"). Ten days after completion of this crossover protocol, two of the six dogs received 0.5 ml of 99% ethanol (without nitroglycerin) per hour for 4 days (sham treatment) and were reevaluated for the third time on the fifth day of ethanol sham treatment.

Experimental protocol. For each evaluation the dogs were anesthetized with 27 ± 1 mg/kg plus 2.5 ± 0.5 mg/kg/hr pentobarbital intravenously and breathed spontaneously through an endotracheal tube. Repeated measurement of arterial blood gases and pH were performed and values were kept within the physiologic range (PCO2 35 to 41 mm Hg, Po2 78 to 90 mm Hg, pH 7.36 to 7.44) by adjusting the infusion rate of anesthetic and/or infusing 8.4% sodium bicarbonate intravenously as necessary. Body temperature was kept at 37.0° to 38.5° C by means of a heating pad. Two peripheral intravenous lines were inserted for separate infusion sites. The femoral artery was punctured percutaneously and a catheter was inserted for the changing of blood volume and arterial blood sampling. A second catheter was placed in the right atrium via the external jugular vein for recording of central venous pressure (CVP). The position of both catheters was verified fluoroscopically. Reflexes were minimized by ganglionic blockade (hexamethonium 10 mg/kg and 10 mg/kg/hr, methylatropine 0.5 mg/kg) and β-blockade (2 mg/kg nadolol). Heparin was administered intravenously (500 U/kg initial and 250 U/kg/hr maintenance). A 4 ml/kg volume of dextran (Macrodex) was exchanged for the same volume of blood, which was stored in a water bath at 37° C. Saline and dextran were infused continuously, both at a rate of 2.5 ml/kg/hr after an initial infusion of 10 ml/kg over an equilibration period of 45 min. The total duration of the experimental protocol was 180 to 200 min. After completion of the evaluation, all infusions were stopped, protamine was given, the short-term catheters were removed, and the dogs were allowed to recover.

Measurements. Arterial and venous pressures were measured with Statham P23 pressure transducers and recorded continuously on a Watenabe linear recorder. Effective compliance of the total vascular bed (TEVC) was measured by infusion and withdrawal of whole blood in a defined cycle, with careful registration of the induced changes in CVP, according to a modification of the technique of Gauer’s group.26, 27 The stored blood (4 ml/kg) was reinfused into the abdominal aorta at a rate of 2 ml/kg/min. After a 1 min pause, the same volume of blood was withdrawn at the same rate, and 1 min thereafter an identical withdrawal was repeated. This was followed again by reinfusion in the same manner, the total cycle lasting 11 min. From the CVP tracings recorded during such cycles, 12 readings at 1 min intervals were obtained (integrating CVP over several respiratory cycles for each measurement) (figure 1). For each cycle of volume changes a linear regression was calculated, relating the observed venous pressure values to the induced changes in blood volume. The effective compliance of the vascular bed can be calculated as the inverse of the slope of this regression line (ml/mm Hg/kg).

TABLE 1

Long-term nitroglycerin (GTN) treatment series (n = 6)

<table>
<thead>
<tr>
<th>Nontolerant state</th>
<th>Tolerant state (4 days GTN)</th>
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</thead>
<tbody>
<tr>
<td>No ongoing GTN</td>
<td>Ongoing GTN 1.5 μg/kg/min</td>
</tr>
<tr>
<td>Hemodynamic variables</td>
<td>Hemodynamic variables</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>Anesthesia</td>
</tr>
<tr>
<td>Anesthesia + AB</td>
<td>Anesthesia + AB</td>
</tr>
<tr>
<td>Anesthesia + AB + NE</td>
<td>Anesthesia + AB + NE</td>
</tr>
<tr>
<td>GTN dose responses</td>
<td>GTN dose responses</td>
</tr>
<tr>
<td>Anesthesia + AB + NE</td>
<td>Anesthesia + AB + NE</td>
</tr>
</tbody>
</table>

AB = autonomic blockade; NE = norepinephrine.
above. Arterial and venous pressures were recorded continually. To produce a defined baseline state of vascular tone from which the venodilator responses to nitroglycerin and SIN-1 could be quantified, norepinephrine was administered in a continuous infusion of 0.15 μg/kg/min throughout the determination of the dose-response relationships. Hemodynamic and compliance measurements were repeated after establishment of a steady state. The dose response to nitroglycerin was then determined by intravenous infusion of increasing doses (i.e., 0.15, 0.5, 1.5 μg/kg/min in the nontolerant state). After 5 min at each dose, the cycle of volume changes was repeated. The nitroglycerin infusion was discontinued between each dose level. The unopposed norepinephrine steady state (which will be referred to as "baseline" in the rest of the text) was allowed to recur and the compliance determination was repeated. After the last such baseline measurement, increasing doses of SIN-1 (0.1, 0.3, 1.0 μg/kg/min) were infused through a separate intravenous line. Because of the longer half-life of SIN-1 compared with that of nitroglycerin, 10 min intervals were allowed for steady-state conditions to be achieved before the cycle of volume changes was repeated at each dose level, and the dosage was increased in a stepwise manner without discontinuation of SIN-1. The experimental protocol is summarized in figure 2.

With the dogs under long-term nitroglycerin exposure, the dose-response relationships were determined as described above, except that nitroglycerin was continuously infused, throughout the evaluation, at the level of long-term exposure (1.5 μg/kg/min) and higher nitroglycerin doses were required to establish a dose-response relationship (2.0, 3.0, 6.5, and 16.5 μg/kg/min). In the sham-treated dogs, the alcohol infusion was continued throughout the experiment, but in all other respects the dogs were handled in a manner identical to that for the animals in the nontolerant state. In both these groups, SIN-1 was administered as described above.

Validation experiments. In all seven of the dogs with aortic flow probes, the hemodynamic effects of a short-term nitroglycerin challenge (1.5 μg/kg/min) were measured in the conscious state (table 1). In addition, the rate of norepinephrine release into plasma was assessed as an indicator of integrated sympathetic activity. This was done by infusing a subthreshold dosage of 1-H-norepinephrine (0.02 μCi/kg/min = 0.15 ng/kg/min) intravenously and repeatedly measuring the ratio of titrated to cold norepinephrine in arterial plasma samples (for details see ref. 28). An initial 1 hr rest period was followed by a 20 min infusion of 1.5 μg/kg/min nitroglycerin. Plasma levels of catecholamines were determined by a radioenzymatic technique.20 The rate of endogenous norepinephrine (NE) release into plasma was calculated as:

\[
\frac{({}^3\text{H})\text{ NE infusion rate - steady-state plasma NE}}{\text{steady state plasma} \cdot ({}^3\text{H})\text{ NE}}
\]

In one of these dogs, a cable leak caused the aortic flow probe to malfunction. In the remaining six animals, the hemodynamic effects of nitroglycerin (0.5 and 1.5 μg/kg/min for 15 min) were measured during anesthesia, autonomic blockade, and norepinephrine infusion as described above for the long-term treatment series (table 2). During assessment of TEVC in these animals, sinus arrhythmia and cardiac cycles were temporarily inhibited by placing a cold probe over the carotid sinus. A control infusion of saline at the same rate as that of the test infusion was used to avoid a change in the cardiac cycle on the heart rate monitor. The control infusion was started 2 min before the test infusion was commenced. The pressure responses to 15 min of saline were measured during anesthesia, autonomic blockade, and norepinephrine infusion as described above for the long-term treatment series (table 2). During assessment of TEVC in these animals, sinus arrhythmia and cardiac cycles were temporarily inhibited by placing a cold probe over the carotid sinus. A control infusion of saline at the same rate as that of the test infusion was used to avoid a change in the cardiac cycle on the heart rate monitor. The control infusion was started 2 min before the test infusion was commenced. The pressure responses to 15 min of saline were measured during anesthesia, autonomic blockade, and norepinephrine infusion as described above for the long-term treatment series (table 2). During assessment of TEVC in these

![FIGURE 2](http://circ.ahajournals.org/lookup/fig/2)

FIGURE 2. Values of TEVC for the six animals of the long-term nitroglycerin (GTN) treatment series, in the nontolerant state, are shown throughout the experimental protocol. The infusions of ganglionic blockade and norepinephrine are indicated above by horizontal bars. The shaded columns represent short-term nitroglycerin or SIN-1 challenges, with the dosages (μg/kg/min) as shown. The two higher doses of nitroglycerin and SIN result in significant increases in TEVC (*p < .05; **p < .01) compared with the preceding baseline values.

**TABLE 2**

<table>
<thead>
<tr>
<th>Validation series in dogs in the nontolerant state (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conscious</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>No AB (reflexes intact)</td>
</tr>
<tr>
<td>Hemodynamic variables under short-term GTN</td>
</tr>
<tr>
<td>Catecholamine release rate</td>
</tr>
</tbody>
</table>

Abbreviations as in table 1.

*Data available in only six dogs (see text).
experiments, the percent change in cardiac output per ml/kg change in blood volume was assessed. Aortic flow was registered continuously (Statham SP 2202 flowmeter) and stroke volume was obtained by planimetry of aortic flow tracings recorded at 250 mm/sec paper speed on a Gould ES 1000 electrostatic recorder.

**Calculations and drugs used.** Statistical analysis was done by analysis of variance for multiple comparisons within the same group followed by t test with Bonferroni's correction for repeated comparisons as necessary. All values are expressed as mean ± SEM. Peripheral vascular resistance was calculated as mean arterial pressure (MAP) divided by mean aortic flow.

The following drugs and intravenous solutions were used (dosages refer to the bases of the salts): pentobarbital sodium (Ceva, Bad Segeberg), hexamethonium bromide and methyldopa (Merck, Darmstadt), nadolol (Von Heyden, Regensburg), heparin sodium (Hoffmann-La Roche, Grenzach-Wyhlen), l-norepinephrine HCl ( Hoechst, Frankfurt), l-(7-H(N))-norepinephrine, 23 Ci/mmol (N.E.N., Dreieich), nitroglycerin (Pohl-Boskamp, Höhenlockstedt), SIN-1 (Casella, Frankfurt), protamine HCl (Hoffmann-La Roche, Grenzach-Wyhlen), sodium bicarbonate 8.4% (Delta-Pharma, Pfungsten), dextran 60 (Macrodex, Schiwa, Glandorf).

**Results**

**Basal characteristics.** In the untreated dogs (nontolerant state) under ganglionic blockade, CVP was low (1.14 ± 0.2 mm Hg) and TEVC was high (3.31 ± 0.2 ml/kg/min Hg) compared with values from conscious dogs. In these same dogs in the tolerant state, the values of CVP and TEVC after ganglionic blockade were identical to the corresponding values for the nontolerant state (figure 3), even though they were receiving the ongoing infusion of 1.5 μg/kg/min nitroglycerin. However, MAP during ganglionic blockade was significantly lower in the dogs under long-term nitroglycerin exposure (tolerant state) than that in dogs in the nontolerant state.

The norepinephrine infusion (0.15 μg/kg/min) elevated CVP and lowered TEVC to values close to those found in the normal conscious dog, in both the tolerant and nontolerant states (figure 3). The baseline values repeated between each short-term nitroglycerin challenge remained essentially at these levels and were not significantly different between the two states (figure 3). Norepinephrine augmented MAP to 164 ± 8 mm Hg in the nontolerant state and to 147 ± 5 mm Hg in the tolerant state. In both states there was a parallel decline in these baseline values obtained between the nitroglycerin challenge infusions, but the baseline MAP in the nontolerant state was always significantly higher (by 17 ± 3 mm Hg) than that in the tolerant state (figure 3). The volume of fluid infused during the protocol was identical in both states (44 ± 8 and 45 ± 5 ml/kg for the nontolerant and tolerant states, respectively).

**Short-term nitroglycerin and SIN-1 challenges.** The response in TEVC of the dogs in the nontolerant state to increasing doses of nitroglycerin and SIN-1 is portrayed in figure 2. A dose-dependent increase in compliance was seen for both nitroglycerin and SIN-1, with a similar maximal value that approached the effective compliance of the animals after anesthesia and ganglionic blockade before norepinephrine (presumably a very low state of intrinsic venous tone). This was accompanied by a dose-dependent decrease in CVP from a baseline of 3.4 ± 0.8 to 1.9 ± 0.9 mm Hg under high-dose (i.e., 1.5 μg/kg/min) nitroglycerin infusion. In the tolerant state, however, the response of TEVC to nitroglycerin challenge infusions was shifted to greater than 10-fold higher doses (figure 4), although the maximum values remained similar in the two states. A similar 10-fold attenuation of response was seen in CVP, which decreased from a baseline value of 3.8 ± 0.6 mm Hg in the tolerant state to 2.1 ± 0.3 mm Hg under the 16.5 μg/kg/min nitroglycerin infusion. However, the increase in effective compliance observed with SIN-1 infusion was unchanged by

![Figure 3](image-url)
The actions of nitroglycerin on effective compliance and MAP are contrasted in figure 5, in both the nontolerant and tolerant states. From this comparison two features become obvious: (1) the baseline values of TEVC are identical for both states, but the baseline MAP is significantly lower in the tolerant state, and (2) the nitroglycerin dose-response relationship for TEVC is shifted by 10-fold in the tolerant state, but no such shift is detectable for the MAP response. Since the dogs in the tolerant state were receiving the ongoing infusion of 1.5 μg/kg/min nitroglycerin, these two observations suggest that tolerance had developed to the venous effects of nitroglycerin but not to the effects on arterial pressure.

This maintenance of the arterial vasodepressor potency of nitroglycerin in the face of tolerance to its venous effects could be observed only after institution of ganglionic blockade, as shown in figure 6. Before administration of hexamethonium, MAP was similar under both long-term nitroglycerin treatment and in the untreated state. The situation was dramatically different after hexamethonium, with the MAP in the chronically treated (i.e., tolerant) animals becoming significantly lowered.

**Alcohol sham treatment.** In the sham-treated dogs, there was no shift in either the response to nitroglycerin or to SIN-1, with TEVC values of 2.07, 2.31, and 4.29 ml/mm Hg/kg for 0.15, 0.5, and 1.5 μg/kg/min nitroglycerin and 2.17, 2.41, and 2.73 ml/mm Hg/kg for 0.1, 0.3, and 1.0 μg/kg/min SIN-1, respectively. In two of the dogs with aortic flow probes, the hemodynamic effects of short-term infusions of incremental concentrations of alcohol (equivalent to 0.5 to 5.0 ml/hr of the 99% solution) were studied. There was no discernible action of alcohol on any of the hemodynamic variables even at doses up to 10-fold higher than those encountered in the long-term infusion of nitroglycerin.

**Validation experiments.** In the six dogs in the nontolerant state with functional aortic flow probes, the effects of nitroglycerin were studied during anesthesia, autonomic blockade, and norepinephrine infusion as described above. Nitroglycerin caused a dose-dependent decline in MAP and a fall in peripheral resistance...
of 15 ± 1% (figure 7). TEVC was augmented in a dose-dependent manner by nitroglycerin in these dogs (data not shown). The variation in cardiac output induced by the blood volume changes necessary for the measurement of TEVC was rather low. With animals under baseline conditions (autonomic blockade and norepinephrine infusion) we observed a change in cardiac output of 0.5 ± 0.2% for each milliliter per kilogram change in blood volume. During infusion of nitroglycerin (0.5 and 1.5 μg/kg/min), the change in cardiac output was 0.3 ± 0.4% and 0.7 ± 0.4%, respectively. However, during autonomic blockade, before norepinephrine infusion, this value was significantly higher (2.5 ± 0.4% per ml/kg).

The effects of hexamethonium on MAP in the dogs under long-term nitroglycerin treatment (figure 6) implicate the autonomic nervous system in buffering the arteriolar effects of nitroglycerin. Therefore, variables of sympathetic nervous system activation were studied in the conscious dogs in the nontolerant state (reflexes intact) prepared for measurement of peripheral resistance (implanted flow probes) during infusion of nitroglycerin. The release rate of norepinephrine into plasma increased by 21 ± 10% during the 20 min infusion (figure 8). The plasma levels of norepinephrine and epinephrine increased similarly, whereas the clearance of norepinephrine (89 ± 3 ml/kg/min before nitroglycerin) was not changed (85 ± 8 ml/kg/min under nitroglycerin). Surprisingly, heart rate in the conscious dogs did not increase during infusion of nitroglycerin (figure 8). Peripheral resistance (n = 6; 0.80 ± 0.07 mm Hg·kg·min/ml) and cardiac output (n = 6; 129 ± 9 ml/kg/min) did not change significantly (0.80 ± 0.08 mm Hg·kg·min/ml and 126 ± 10 ml/kg/min, respectively) with nitroglycerin in these conscious dogs.

Discussion

We have shown that a 4 day exposure to a modest dosage of nitroglycerin results in tolerance to its effects on CVP and TEVC.

Critique of the model. Total vascular compliance reflects, for the most part, the compliance of the venous system, since the pulmonary circuit in dogs accounts for as little as 10% and the arterial system accounts for only one-thirtieth of the systemic circulation. Therefore the majority of the compliance of the vascular system resides in the veins.

Two significant limitations of the method of TEVC determination should be considered. First is the inability to control for changes in blood flow distribution between various beds during the application of the various drugs under study. This problem is shared by all methods that measure compliance in the entire vascular system. Any changes in the relative perfusion to
The second limitation is the inability to control for changes in cardiac output during the experimental manipulations. This limitation is more specific to the present model since, by virtue of its relatively noninvasive nature, it leaves the vascular circuit intact. Because cardiac output is not maintained constant, the compliance measured can be considered only an "effective" compliance. Under our conditions of norepinephrine stimulation, in which the dose-response relationships were established, there was remarkably little variation of cardiac output during the measurement of effective compliance (only 0.5% change per ml/kg). This approaches fulfillment of the prerequisite for a "true" compliance determination.

Therefore the increases in TEVC measured in response to nitroglycerin and SIN-1 reflect predominantly their effects on the venous system, and the tolerance with respect to changes in effective compliance seen after long-term nitroglycerin exposure reflects an attenuation of the venodilator effects of nitroglycerin.

"True" vs "pseudo" tolerance. The argument that this venodilator tolerance represents a tolerance at the cellular "effector" site, rather than a regulated "pseudo" tolerance, is supported by two lines of evidence in our data. First, during the measurement of venous tone, neurogenic vascular control was markedly attenuated by ganglionic blockade with hexamethonium and methylatropine. Second, the ability of SIN-1 to augment effective vascular compliance was unaltered by induction of nitroglycerin tolerance showing continued responsiveness of the low-pressure system to the venodilator effects of this NO-containing vasodilator. Thus this nitroglycerin tolerance could not have resulted from a nonspecific attenuation of dilator responsiveness of the venous system due to possible alterations of volume or electrolyte balance. The lack of cross-tolerance with SIN-1 is in agreement with observations in vitro which suggest that although nitroglycerin and SIN-1 share a common underlying cellular mechanism for their vasodilating effects (i.e., stimulation of guanylate cyclase and increased cyclic GMP levels), they exhibit different requirements with respect to earlier steps in the pathway, in particular the somewhat controversial requisite nitrate interaction with cytoplasmic cysteine.

Before autonomic blockade, the arterial pressure in the tolerant state (under ongoing nitroglycerin infusion) was not lower than that of the same dogs in the nontolerant state (no ongoing nitroglycerin), apparently indicating tolerance of arterial pressure to nitroglycerin (figure 6). This dose of nitroglycerin (1.5 μg/kg/min) is below that usually described as the threshold.

FIGURE 8. Sympathetic activation induced by nitroglycerin (GTN) infusion (1.5 μg/kg/min) in seven conscious dogs prepared with aortic flow probes. Asterisks indicate significance of differences of values under nitroglycerin compared with the preceding baseline value (Fig 8. *p .05 < p < .1, **p < .05, ***p < .01). NE-RR = norepinephrine release rate; PCC = plasma catecholamine levels; HR = heart rate.

a vascular bed with "slow venous return" (i.e., a large time constant for venous return), away from a bed with "fast venous return" (small time constant), can be misinterpreted as an active change in the capacitance (and hence the compliance) of the venous system due to venodilation. Although redistribution of blood flow toward the splanchnic bed (i.e., "slow venous return") could have contributed to our observed increases in TEVC under nitroglycerin and SIN-1 treatment, this is unlikely for the following reasons: (1) Studies on the effect of nitrates on regional distribution of blood flow have not shown a preferential dilation of the splanchnic bed. When analyzed in terms of the two-compartment model of Caldini et al., an increase in venous compliance of 35% (i.e., similar to that found in the present report) was documented without any change in blood flow distribution to the two compartments. (2) In our model, no increase in mesenteric flow, measured by electromagnetic flow probes, was seen in response to these doses of nitroglycerin under identical experimental conditions (Stewart et al., unpublished observations).
for arteriolar effects\textsuperscript{37, 38} and did not result in a lowering of peripheral resistance in our conscious dogs in the nontolerant state (see Results: Validation experiments). However, at the same time, the rate of norepinephrine release was significantly increased in these conscious dogs (figure 8), indicating a compensatory increase in sympathetic activity. When anesthesia and autonomic blockade were applied in the same animals without tolerance, this dosage of nitroglycerin resulted in a highly significant decrease in peripheral vascular resistance and a substantial decline in MAP (figure 7). Similarly, in the dogs under long-term nitroglycerin exposure (i.e., the tolerant state), autonomic blockade unmasked a continued hypotensive action of the ongoing nitroglycerin infusion (figure 6), demonstrating that the apparent tolerance to nitroglycerin’s arterial pressure effects was, in all likelihood, only a pseudo tolerance. Even though cardiac output was not directly measured in the chronically treated group, it seems reasonable to attribute the arterial pressure response to the persistence of the same action of nitroglycerin on the arteriole (and hence vascular resistance) described with short-term exposure. This is especially true in view of the blunting of nitroglycerin’s venous effects (i.e., on preload), so that the cardiac output of the animals under tolerance should be greater, not less, than that of animals in the nontolerant state.

Therefore, during a 5 day course of intravenous nitroglycerin, we observe a true nitroglycerin-specific tolerance to venodilation, but only a pseudo tolerance to nitroglycerin effects on peripheral vascular resistance, mediated by a discrete action of neurogenic compensatory mechanisms.

Venous vs arterial tolerance. In a plethysmographic study, Zelis and Mason\textsuperscript{10} showed the development of tolerance to the venous effects of nitroglycerin in healthy subjects on long-term isosorbide dinitrate treatment but observed a maintenance of its effects on resistance vessels in this localized bed. Since this observation, the relative susceptibility of the arteriolar and venous beds to nitrate tolerance has been a matter of dispute. Results in patients with congestive heart failure in particular have been interpreted as suggesting arteriolar rather than venous tolerance on sustained nitrate exposure.\textsuperscript{8} The rapid attenuation of nitrate effects on arterial pressure, which has been described in many clinical studies,\textsuperscript{6–8} has strengthened this impression.

However, few studies have measured the venodilator effects of nitroglycerin directly. Recently Manyari et al.\textsuperscript{15} reported that long-term nitrate treatment markedly attenuates the effects of nitroglycerin on vascular capacitance. Our results confirm that tolerance to nitroglycerin venodilation develops readily. Furthermore, we have shown that venous tolerance is not altered by attenuation of compensatory reflexes. However, after minimization of these influences, a preserved action of nitroglycerin on resistance vessels becomes apparent.

In this context it should be stressed that the arteriolar events during long-term nitroglycerin exposure are complex. At least three mechanisms may contribute to the blunting of nitroglycerin hypotensive effects during long-term nitrate treatment observed clinically:

1. Development of venous tolerance per se. The drop in arterial pressure caused by nitrate administration is often associated with a decrease in cardiac output and hence no change in peripheral resistance.\textsuperscript{3, 37, 39} Therefore, to a large extent, this hypotensive response reflects a decrease in venous return due to venodilation, with any possible arteriolar actions effectively masked by sympathetic reflexes.\textsuperscript{3, 37} Thus, if nitroglycerin-induced venodilation is attenuated (i.e., by tolerance), regardless of its direct arteriolar effects there will be an attenuation of the nitroglycerin hypotensive action.

2. Sympathetic reflexes. These have been shown to completely mask direct arteriolar effects of nitroglycerin during short-term treatment.\textsuperscript{1–3} and our results point to a similar mechanism operating during long-term exposure. In clinical studies of nitrate tolerance, the extent to which this may contribute must be assessed by indirect methods.\textsuperscript{3} Heart rate alone is probably an insensitive index of sympathetic activity as evidenced by the lack of a change in this variable in our conscious dogs even when the measured rate of norepinephrine release was significantly increased (figure 8).

3. The development of “true” tolerance to nitroglycerin arteriolar actions. Although this was not achieved in the present study, it is likely that a higher degree or a longer interval of exposure would result in tolerance at the level of the resistance vessels themselves. What is important is that the time course and/or threshold for development of tolerance to nitrates differs between the compliance and the resistance beds. In our study, during a relatively brief, continuous intravenous infusion (comparable to a course of intravenous nitroglycerin in an intensive care unit), venous tolerance predominated.

Relevance to therapeutic actions of nitroglycerin. Recent studies have documented development of tolerance to the antianginal effects of nitrates with long-term treatment.\textsuperscript{5, 7, 11, 12} The mechanisms by which nitrates relieve myocardial ischemia are complex.\textsuperscript{40} However, in
chronic stable angina, the venous effects of nitroglycerin are considered to be of “paramount importance.”  Therefore the tolerance to nitroglycerin-induced venodilation observed in our study agrees well with these clinical observations and with the observations of others.  In another study we have observed tolerance to large coronary artery dilation in awake dogs, with the same nitroglycerin exposure and similar time course as for venodilation (Stewart et al., unpublished observations), and such a mechanism might contribute to antianginal tolerance.

Studies on long-term use of nitrates in patients with congestive heart failure have generally shown a sustained therapeutic benefit. In particular, Leier et al. have suggested that a persistent lowering of pulmonary wedge pressure, in patients with congestive heart failure on long-term nitrate treatment, reflects the absence of tolerance to venodilation, whereas the loss of nitrate action on arterial pressure and peripheral resistance indicates an arteriolar tolerance. This is in apparent conflict with our observations. However, this lowering of pulmonary wedge pressure was seen 5 to 6 hr after the last dose of isosorbide dinitrate and was not further lowered 1 hr after an additional dose. Franciosa and Cohn demonstrated a similar decrease in pulmonary arterial wedge pressure in their long-term treatment group more than 8 hr after the last dose of isosorbide dinitrate, which, as they pointed out, exceeds the known duration of action of a single dose. Under long-term nitrate treatment, its duration of action may even be shorter (i.e., less than 3 hr). They argue that this decrease in pulmonary arterial wedge pressure “need not necessarily reflect a venodilatory action,”  but rather “decreased end-systolic and end-diastolic volumes due to decreased ventricular out-flow resistance.” Experience with short-term vasodilator treatment in patients with congestive heart failure suggests that only modest decreases in left ventricular end-diastolic pressures can be achieved over the short term from predominantly arteriolar dilators such as hydralazine. But over the long term, improved ejection characteristics may result, through a variety of indirect mechanisms (i.e., increased renal perfusion, improved diuretic responsiveness), in further decreases in preload. This might explain the decreases in left heart filling pressures, seen in these studies, persisting past the expected duration of nitrate action. As discussed above, the lack of a decrease in arterial pressure or peripheral vascular resistance at rest should not necessarily be taken as evidence of arteriolar tolerance. We have shown that compensatory mechanisms strongly regulate these variables during long-term treatment. In fact, the increased exercise capacity observed in both studies may support the concept of continued arteriolar nitrate action.

We have described two processes occurring in healthy dogs under long-term treatment with nitroglycerin: true tolerance to nitroglycerin venodilation but continued arteriolar responsiveness offset by discrete neurogenic regulation. The extent to which these same mechanisms operate in patients under long-term nitrate treatment is not known. Experience with long-term nitrates in patients with chronic stable angina pectoris indirectly supports our venous findings, but while a recent clinical study directly confirms important nitroglycerin venous tolerance in patients. A continued action of nitroglycerin on resistance vessels, although normally masked by counterregulatory influences, might be manifested indirectly, contributing, for instance, to the improved hemodynamics and better exercise capacity observed in patients with heart failure on long-term nitrate therapy. Therefore, such a shift in the spectrum of nitroglycerin action from a predominant venodilator toward arteriolar action under conditions of nitroglycerin tolerance might have important clinical implications and warrants attention in future clinical trials.

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