Development of Tolerance to Nitroglycerin in the Arterial and Venous Circulation of Dogs

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The purpose of this study was to define the effects of nitroglycerin on venous tone and to investigate the time course of nitroglycerin tolerance in the peripheral circulation. The changes in the arterial and venous circulation resulting from an intravenous infusion of nitroglycerin (5 μg/kg per min) after 5 minutes (acute infusion) were compared with those changes that occurred after 2 hours (chronic infusion) of the same infusion in six splenectomized, ganglion-blocked dogs. Hemodynamics, blood volume and venous and arterial compliance were measured during each infusion.

Nitroglycerin initially decreased mean arterial pressure from 81.5 ± 2.0 to 57.6 ± 2.7 mm Hg (p < 0.01). Central blood volume decreased from 21.1 ± 1.4 to 15.9 ± 1.1 ml/kg (p < 0.01), while total blood volume and unstressed vascular volume did not change. In the acute study, nitroglycerin increased venous compliance 33% from 1.75 ± 0.14 to 2.32 ± 0.16 ml/mm Hg per kg (p < 0.01) and arterial compliance 33% from 0.049 ± 0.007 to 0.065 ± 0.007 ml/mm Hg per kg (p < 0.01). At the end of the 2 hour infusion, arterial pressure increased and was now unchanged from control. Central blood volume had returned to baseline, 17.8 ± 0.9 ml/kg. Total blood volume and unstressed vascular volume remained unchanged. With the long-term infusion, both arterial and venous compliance decreased (p < 0.02) to 0.050 ± 0.006 and 1.50 ± 0.06 ml/mm Hg per kg, respectively, such that neither value was different from control. Nitroglycerin levels remained constant throughout.

Thus, acute administration of nitroglycerin causes arterial and venous dilation by increasing compliance in each vascular bed. The duration of this response is short-lived with the development of tolerance within 2 hours. (J Am Coll Cardiol 1987;10:1335-41)

Nitroglycerin has been used for almost 100 years for the treatment of angina pectoris. More recently, there has been interest in using nitroglycerin and other nitrate preparations to treat congestive heart failure. Although the therapeutic benefits of nitrates are apparent, there is evidence that long-term administration of these agents leads to tolerance (1-3). In clinical studies, tolerance to nitrates has been demonstrated by showing no difference in the anti-ischemic effects compared to placebo at specific time intervals with serial exercise testing or by showing no change in symptoms in patients with heart failure (2). Because the major effect of nitroglycerin is on preload, most investigators believe that tolerance to nitroglycerin occurs in the venous system. What specifically happens in the venous system is not known, in part because in clinical studies it is not possible to measure the true determinants of venous capacitance—mean circulatory filling pressure, venous compliance and unstressed vascular volume.

Changes in systemic venous tone can be measured in intact animals by measuring mean circulatory filling pressure, venous compliance and unstressed vascular volume (4-6). The use of an intact animal model has allowed us to simultaneously study the heart and the peripheral circulation. This is important because changes in one compartment of the cardiovascular system can affect changes elsewhere. For example, a change in volume in the venous system will affect the heart. In addition, the measurement of systemic venous tone eliminates the need to extrapolate changes observed in one type of vein to the entire venous system (7).

The purpose of this study, therefore, was to use an intact dog model to establish the precise changes in the peripheral circulation and the time course of nitroglycerin-induced changes in the arterial and venous circulation. Specifically,
we hypothesized that nitroglycerin increased arterial and venous compliance. These acute effects were altered by the chronic administration of nitroglycerin that resulted in normalization of compliance and therefore tolerance.

Methods

**Animal preparation.** Six mongrel dogs (18.2 ± 1.4 kg) underwent elective splenectomy through a midline abdominal incision under sterile conditions and general anesthesia with halothane and oxygen. The dogs were allowed to recover for 2 weeks and were then sedated with acepromazine (0.02 mg/kg), anesthetized with halothane and nitrous oxide and intubated. Using sterile technique, a 7F thermodilution catheter was inserted percutaneously in the right external jugular vein and advanced into the pulmonary artery. A 7F angiographic micromanometer-tipped catheter (Millar Instruments, Inc.) was inserted percutaneously through the left femoral artery to measure aortic pressure. A 14 gauge plastic catheter was then placed in the foreleg for intravenous access. All pressure measurements and experiments were performed with the animals placed on a warming blanket with their left side down. The dogs were extubated before the experiments. Further sedation was accomplished with diazepam (0.5 mg/kg) and hydromorphone (0.3 mg/kg) after recovery from general anesthesia.

**Baseline measurements.** After the placement of all catheters, ganglionic blockade was induced with hexamethonium chloride (30 mg/kg). Baseline heart rate and pulmonary artery, pulmonary artery wedge, right atrial and aortic pressures were then recorded. Cardiac output was measured in triplicate with the thermodilution technique using a minicomputer (Edwards, model 9510-A); averaged values were reported. The thermodilution technique has been verified in our laboratory using dye-dilution measurements of cardiac output (4,6). Systemic vascular resistance index was calculated as the difference between aortic and right atrial pressures divided by the cardiac index.

**Central blood volume, defined as the blood volume in the heart and lungs,** was determined by the Stewart-Hamilton principle using indocyanine green dye (4–6). Total blood volume was measured using the Evans Blue technique. A 0.45% (weight/volume) solution of dye in a total volume of 1 ml was injected into the circulation system through an external jugular vein sheath. Five minutes after injection, an arterial blood sample of 7 ml was withdrawn. After centrifugation, the dye concentration was determined spectrophotometrically. Total blood volume was calculated: Total blood volume = plasma volume ÷ 1 - hematocrit. This technique to measure blood volume is comparable to using radioisotope techniques (5). Mean circulatory filling pressure was measured after transient asystole (5 to 10 seconds) was produced by a bolus injection of acetylcholine (3 to 10 mg) into the superior vena cava as previously described by our laboratory (4-6). This pressure was calculated as: Mean circulatory filling pressure = VPP + (APP - VPP) × arterial compliance/venous compliance, where VPP = venous plateau pressure, and APP = arterial plateau pressure measured at 7 seconds after the start of asystole. An arterial to venous compliance ratio of 1/30 was assumed (8).

**Calculation of venous compliance.** Venous compliance was defined as the change in volume divided by the change in pressure of the total systemic venous bed. This compliance measurement was obtained by serial determinations of mean circulatory filling pressure after acute volume changes over a range of physiologic venous pressures as previously described (4,6). Mean circulatory filling pressure was measured at baseline and then after volume expansion of 5 ml/kg with body temperature dextran and then with a combination of dextran and blood to increase blood volume 10 ml/kg. After the 5 ml/kg volume load with dextran, 5 ml/kg of blood was withdrawn. and this plus another 5 ml/kg of dextran were used for 10 ml/kg volume load. These volume changes were performed manually by infusions within 30 seconds. This time period was chosen to minimize and reverse stress relaxation. There was at least a 10 minute recovery period between each measurement of mean circulatory filling pressure to allow hemodynamic variables to return to baseline. Venous compliance was defined as the reciprocal of the slope of the line obtained by plotting mean circulatory filling pressure versus blood volume. The unstressed vascular volume, or the volume in the blood vessels at zero transmural pressure, was obtained by extrapolating this pressure-volume relation to the zero pressure intercept.

**Calculation of arterial compliance.** The method for calculating arterial compliance has been previously reported from our laboratory (4,6). Arterial compliance was measured based on the simple windkessel model of the circulation (9). The equations used were obtained from an electrical analog of a single capacitor, one resistor circuit. This model assumes that arterial pressure initially decays monoexponentially as a function of time from an initial driving pressure with a rate constant k3. Instantaneous pressure was calculated as:

\[ P(t) = k_1 + k_2 e^{-t/T}, \]  

where \( k_1 \) = arterial pressure plateau (mm Hg), \( k_2 = \) the arterial driving pressure (mm Hg) and \( P(t) = \) instantaneous pressure (mm Hg).

Arterial compliance was then calculated as:

\[ T = CR, \]

where \( T = \) time constant, \( C = \) compliance (ml/mm Hg) and \( R = \) systemic vascular resistance (mm Hg.min-kg/ml).

According to equation 1, the time constant is inversely proportional to the slope of a plot of log pressure versus
Experimental protocol. Hemodynamic variables, including arterial and venous compliance, were measured under control conditions. On a separate day, control hemodynamic measurements were repeated, and an infusion of nitroglycerin (5 \( \mu \)g/kg per min) was begun. Five minutes after the start of an intravenous infusion, arterial and venous compliance was measured. On a subsequent day, control hemodynamic variables were again measured, and nitroglycerin (5 \( \mu \)g/kg per min) was infused for 2 hours, at which time arterial and venous compliance was measured. The dose of nitroglycerin had been chosen in pilot studies because it acutely resulted in a 25% decrease in the mean aortic pressure. The 2 hour time interval was chosen because, in pilot studies, the arterial pressure had increased to near control values by this time, despite the continuous nitroglycerin infusion. Total blood volume and central blood volume were measured whenever venous compliance was measured. Nitroglycerin blood levels (National Medical Services) were measured during the control period and at 5, 15, 30, 60 and 120 minutes into the study.

This protocol was approved by the animal research committees of the Tucson Veterans Administration Medical Center and the University of Arizona. Particular attention was given to the welfare of the animals, the adequacy of anesthesia and the method of instrumentation. During the measurement of mean circulatory filling pressure, cardiac electrical activity resumed spontaneously in all dogs; no seizures occurred, and there was no evidence of pain or suffering as defined in the Federal Animal Welfare Act and in accordance with the NIH Guide for care and use of laboratory animals.

Statistical analysis. Data are expressed as mean ± standard error of the mean. Statistical analysis was performed by repeated measures of analysis of variance to compare serial measurements to control. Venous compliance was determined by regression analysis using the method of least squares for each mean circulatory filling pressure-blood volume curve. The volume shifts in the arterial circulation were compared using a t test for paired values.

Results

Acute hemodynamic and circulatory effects of nitroglycerin after 5 minutes (Tables 1 and 2). At a dose of 5 \( \mu \)g/kg per min, nitroglycerin initially lowered mean aortic pressure from 81.5 ± 2.0 to 57.6 ± 2.7 mm Hg (p < 0.01). A representative recording of the rapid fall in arterial pressure is shown in Figure 1. There were no changes in any other hemodynamic variables, including heart rate, cardiac output, systemic vascular resistance and right atrial,

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<thead>
<tr>
<th>Table 1. Effects of Nitroglycerin (5 ( \mu )g/kg per min) on Hemodynamics in Six Splenectomized Dogs After Ganglionic Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mm Hg)</td>
</tr>
<tr>
<td>Pulmonary artery wedge pressure (mm Hg)</td>
</tr>
<tr>
<td>Cardiac index (ml/min per kg)</td>
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<tr>
<td>Systemic vascular resistance (mm Hg·min·kg/ml)</td>
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</tbody>
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Values are mean ± SE after ganglionic blockade with hexamethonium (30 mg/kg). *p < 0.01 compared with control value.

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<thead>
<tr>
<th>Table 2. Effects of Nitroglycerin (5 ( \mu )g/kg per min) on the Peripheral Circulation and Blood Volume in Six Splenectomized Dogs After Ganglionic Blockade</th>
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<tbody>
<tr>
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<tr>
<td>Mean circulatory filling pressure (mm Hg)</td>
</tr>
<tr>
<td>Venous compliance (ml/mm Hg per kg)</td>
</tr>
<tr>
<td>Unstressed vascular volume (ml/kg)</td>
</tr>
<tr>
<td>Arterial compliance (ml/mm Hg per kg)</td>
</tr>
<tr>
<td>Arterial volume shift (ml/kg)</td>
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<tr>
<td>Central blood volume (ml/kg)</td>
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<td>Total blood volume (ml/kg)</td>
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</tbody>
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Values are mean ± SE after ganglionic blockade with hexamethonium (30 mg/kg). *p < 0.02; †p < 0.02; \( \sharp p < 0.01 \), compared with control value; \$p < 0.05, compared with the 5 minute value.
pulmonary artery and pulmonary artery wedge pressures. Central blood volume decreased from 21.1 ± 1.4 to 15.9 ± 1.1 ml/kg (p < 0.01). Total blood volume and central blood volume were unchanged.

The major effects of nitroglycerin were on the peripheral circulation (Table 2). Figure 2 shows serial circulatory arrests, from which measurements of mean circulatory filling pressure and arterial and venous compliance were obtained. After 5 minutes there was no change in mean circulatory filling pressure, but venous compliance increased 33% (p < 0.01) and unstressed vascular volume did not change. The correlation coefficient for the control compliance curve was r = 0.998 (p < 0.001), and the correlation coefficient for the acute nitroglycerin compliance curve was r = 0.987 (p < 0.001). Arterial compliance also increased 33% (p < 0.01), and the volume of blood transferred out of the arterial circulation was 1.9 ± 0.4 ml/kg. The time constant of decay in the arterial system did not change (2.2 ± 0.1 versus 2.5 ± 0.1 seconds).

Hemodynamic and circulatory effects of nitroglycerin after 2 hours. (Tables 1 and 2). During the 2 hour infusion, the arterial pressure increased such that it was not different than control. Similar to the acute responses to nitroglycerin, there were no changes in any other hemodynamic variables and total blood volume compared with control. Central blood volume had returned to baseline. In the peripheral circulation, the effects that were seen after the acute infusion were reversed. Both the venous and arterial compliance decreased (p < 0.01) compared to their 5 minute values, and after the 2 hour infusion neither value was different from control (Table 2). The correlation coefficient for the chronic venous compliance curve was r = 0.997 (p < 0.001). The unstressed vascular volume remained unchanged. The volume of blood shifted out of the arterial circulation (1.1 ± 0.2 ml/kg) was less (p < 0.05) at 2 hours than at 5 minutes. The time constant of arterial pressure decay was unchanged (2.5 ± 0.2 seconds).

There were no differences in baseline hemodynamic values on the three study days. The nitroglycerin levels were undetectable during the control period and increased to 15.8 ± 1.5 ng/ml at 5 minutes, 17.0 ± 1.8 ng/ml at 15 minutes, 25.3 ± 7.9 ng/ml at 30 minutes, 28.0 ± 3.6 ng/ml at 60 minutes and 22.0 ± 9.3 ng/ml at 120 minutes.

Discussion

The results of this study show that within 5 minutes of starting an intravenous infusion of nitroglycerin, there was arterial and venous dilation. Arterial and venous compliance increased by 33%. This occurred without changes in total blood volume or unstressed vascular volume. With continuous infusion, tolerance had developed within 2 hours with
a return of arterial and venous compliance to baseline. The blood pooled in the venous circulation during the acute infusion was shifted back into the central and aortic compartments with chronic administration. These alterations in vascular tone occurred with no change in the dose and the serum level of nitroglycerin.

**Model to study and measure venous capacitance.**

The dog model and experimental techniques used in this study have been previously described from our laboratory (4,6) to measure the effects of pharmacologic agents on the peripheral circulation. This closed chest model has distinct advantages over previous investigations that used anesthetized, open chest preparations. There is less trauma, the pericardium is intact and the method evaluates the venous circulation as a whole. Splenectomy was performed to eliminate volume shifts that occur in dogs because the spleen is a large volume reservoir. Ganglion blockade was used to evaluate the direct effects of nitroglycerin and minimize active reflex compensation in the measurement of venous compliance.

In clinical studies, changes in central venous or pulmonary wedge pressure have been equated with changes in venous tone. These measurements of ventricular preload are affected by heart rate, chamber stiffness, afterload and contractility (10). Measurement of right or left atrial pressure, therefore, cannot be used to reflect the status of the venous capacitance system. Changes in total vascular capacitance also cannot be extrapolated from changes in one compartment, for example, in limb vessels (7). Because it is not possible to study the isolated venous system intact, we studied venous capacitance in the dog by measuring mean circulatory filling pressure, venous compliance and unstressed vascular volume. The mean circulatory filling pressure of a system was defined by Guyton et al. as “that pressure measured at all points in the circulatory system if the heart were stopped suddenly and blood redistributed instantly in such a manner that all pressures were equal” (11). It is assumed to be equivalent to the pressure distending the capacitance vessels.

**Mean circulatory filling pressure is determined by the compliance of the system and contained blood volume (10).** The vascular compliance of a system is defined as the change in vascular volume divided by a concomitant change in pressure. Because the venous system is 30 times more compliant than the arterial system, changes in whole body compliance are mainly reflected as a change in venous compliance. The compliance of the venous circulation can be obtained by measuring mean circulatory filling pressure after serial volume changes and constructing a pressure-volume curve. Because blood vessels possess nonlinear distensible properties when measurements are done outside physiologic filling pressures, it is essential to make these measurements within a narrow range of pressure and volume changes. A linear pressure-volume relation has been found to exist between mean circulatory filling pressures of 5 and 25 mmHg.
Hg (12). In addition, to minimize reflex changes during alterations in blood volume, the measurement of compliance should be performed after ganglion blockade. Finally, the unstressed vascular volume is defined as that volume of blood in the vascular system at zero transmural pressure. This is a virtual volume and can be obtained only by extrapolating a linear portion of the pressure-volume relation to zero transmural pressure. An increase or decrease in unstressed vascular volume will represent an increase or decrease in venous capacitance or may result from changes in stressed volume in the arterial and pulmonary circuit.

Effect of nitroglycerin. The baseline hemodynamic changes that occurred after acute nitroglycerin administration are consistent with earlier reports in dogs (13). Although nitroglycerin is a known venodilator, the exact mechanism by which this occurs has not been previously studied. Rothe (10) and Greenway et al. (14) have previously suggested that venodilation can occur with a change in venous compliance or unstressed vascular volume, or both. The present study suggests that the major effect of nitroglycerin is an increase in venous compliance. After a 5 minute infusion, mean aortic pressure decreased significantly. Because of the associated increase in aortic compliance, blood was shifted out of the large arteries. The increase in venous compliance with the acute infusion shifted volume from the arterial and central compartments into the systemic venous system. This results in a decrease in preload and eventually a decrease in stroke volume.

Clinical implications and comparison with previous studies. Tolerance to nitroglycerin, defined as a diminished response to the same dose, has been documented in various preparations and clinical studies. In rat aorta and guinea pig ileum stimulated with norepinephrine, preincubation with nitroglycerin for 1 hour decreased nitroglycerin’s ability to cause relaxation (15,16). Similar results were seen in strips of aorta removed from rats that had received a 6 hour infusion of nitroglycerin (17). In patients, nitrate tolerance has been seen after a 5 day treatment with isosorbide dinitrate and a 24 hour treatment with nitroglycerin patches (1–3,18,19). In these studies, the time course of the development of tolerance was not investigated. Because we examined the specific in vivo effects of nitroglycerin on venous tone, we were able to document the time course of these changes. The present study showed that tolerance developed early. Venous compliance returned to normal within 2 hours in the venous system and, similarly, changes in the arterial circulation were blunted. Because we were unable to continually monitor venous compliance, it is possible that these changes may occur even sooner than we documented.

The mechanisms producing tolerance are not completely understood but appear to be related to cyclic guanosine monophosphate (GMP) production. Nitroglycerin has been shown to increase smooth muscle levels of cyclic GMP before producing relaxation, and the concentration of cyclic GMP correlated with the degree of smooth muscle relaxation. In tissues showing tolerance, the rise in cyclic GMP production is blunted (15,16).

Potential limitations of this study. The dose of nitroglycerin employed in our study was larger than that most commonly used in clinical settings. This was done intentionally to maximize changes in vascular tone. To measure vascular compliance, it is necessary to block reflexes with hexamethonium. This will decrease baseline vascular tone, resulting in venodilation. The fact that nitroglycerin still resulted in venodilation supports our hypothesis. The qualitative changes and the time course of tolerance should not be dose dependent.

Care should be taken in extrapolating these data to patients with congestive heart failure. Previous work from our laboratory has shown that there is generalized venoconstriction in an animal model of chronic heart failure (20). Thus, in heart failure, the venodilatory effects on constricted veins may be more pronounced than reported in this study. However, the mechanism of the shift in the vascular volume-pressure relation should be similar.

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References