Effect of Nitroglycerin on Aortic Impedance, Diameter, and Pulse-Wave Velocity

Terry W. Latson, William C. Hunter, Norihisa Katoh, and Kiichi Sagawa

The beneficial effects of nitroglycerin on myocardial ischemia may be mediated in part by changes in ventricular loading. The ability of nitroglycerin to reduce ventricular preload by venodilation has been extensively documented. Although the effects of nitroglycerin on ventricular afterload have received much less attention, recent studies by O'Rourke et al and Yaginuma et al have emphasized that reduction in aortic impedance may also play an important role in nitroglycerin's therapeutic effect. Documented effects of nitroglycerin on ventricular afterload include: 1) decreased systolic pressures with less, if any, change in aortic diastolic pressure; 2) increased arterial compliance, as determined by both the rate of aortic diastolic pressure decay and pressure-diameter relations in peripheral arteries; and 3) decreased magnitude of arterial pulse-wave reflections.

This study used an acute canine preparation to further define the effects of nitroglycerin on systemic arterial properties. We measured the effects of intravenous nitroglycerin on aortic impedance, diameter, and pulse-wave velocity (PWV). The use of spectral analysis techniques, in conjunction with random cardiac pacing, allowed us to determine the impedance spectrum with a frequency resolution as small as 0.1 Hz. Our results indicate that nitroglycerin has significant effects on both the magnitude and timing of peripheral vascular reflections.

In Vivo Methods

Nine mongrel dogs (body weight 17–23 kg) were anesthetized with a combination of pentobarbital and fentanyl (initial dose: 12 mg/kg pentobarbital and 50 μg/kg fentanyl; subsequent infusion: 1.1 mg/kg/hr and 15 μg/kg/hr, respectively). Instrumentation in all dogs included a nonoccluding ultrasonic flow probe (model T-101, Transonic Systems, Ithaca, New York) in the ascending aorta, a Millar pressure catheter (Houston, Texas) in the ascending aorta, inserted through the right common carotid artery, and a central venous pressure catheter passed through the femoral vein. The Millar catheter was positioned at the same level as the aortic flow probe by palpating the catheter tip within the aorta. Additional instrumentation in seven dogs included a second Millar catheter in the femoral artery, inserted and attached with a nonoccluding purse-string suture, and a pair of ultrasonic crystals on the aortic arch (5-MHz crystals connected to a sonomicrometer; model 401, Schuessler, Cardiff by the Sea, California). These ultrasonic crystals, used to measure aortic diameter, were attached to opposing sides of the aortic adventitia with either fine sutures (tied to a flexible woven backing sheet) and/or a small amount of cyanoacrylate glue. Permanent aterioventricular block was produced in all dogs by injection of 40% formaldehyde into the proximal His bundle.

Calibration of the Transonic Systems flowmeter and probe was confirmed in vivo by comparisons with calibrated bypass pump flows in three separate dog experiments (r=0.98). The dynamic frequency characteristics of the flow system, as obtained from the manufacturer, could be approximated as a linear phase shift of 2.9°/Hz up to 30 Hz (±7%), with less than a 5% decrease in amplitude up to 15 Hz. The accuracy of ultrasonic blood flow measurements has also been documented in previous studies.

Measurements of aortic flow, aortic pressure, aortic diameter, and femoral pressure were made during random ventricular pacing at rates between 80 and 300 beats/min. Pacing spikes were triggered by an IBM
personal computer. To enhance the low frequency content of the derived data, we used a modification of the square-wave rate-modulation technique described by Ringo et al. Rather than switching between two predetermined heart rates, as described by these authors, the computer was programmed to switch between two cycles with different heart rate ranges. During each cycle, the next RR interval was randomly selected but was limited to intervals corresponding to rates within the allowable range for that cycle. Typically, the first pacing cycle included rates between 80 and 170 beats/min, while the second cycle included rates between 210 and 300 beats/min. Each cycle was active in turn for 5.12 seconds, corresponding to a modulation frequency of 0.0976 Hz (Figure 1). This frequency is the lowest for which spectral functions were calculated.

All data were collected and stored on the IBM personal computer, which was equipped with a Data-Translation DT-2801A analog to digital converter. For each experimental record, data were collected by burst sampling of all channels (less than 0.3 msec to scan all channels) every 10 msec for 82 seconds.

Control measurements were made after the dogs' mean blood pressure had been stable for at least 15 minutes. Immediately after these control measurements, intravenous nitroglycerin infusion was begun at the rate of 1 (µg/kg/min. To avoid the confounding effects of passive, pressure-induced changes in vascular properties, volume infusion (0-300 ml) was given when needed to maintain mean blood pressure within 5 mm Hg of control blood pressure. Nitroglycerin measurements reported here were made between 5 and 10 minutes after the start of drug infusion. Impedance spectra derived from repeated measurements during both control and nitroglycerin infusion periods exhibited very little variation (Figure 2).

Data Analysis

For calculation of vascular impedance, the data records of pressure and flow were divided into eight 10.24-second segments. The mean and trend of the data in each segment were determined with least-squares linear regression. These pressure and flow means were used to calculate the zero-frequency term of impedance, also known as the input resistance (IR) (thus, IR = mean pressure/mean flow averaged over all eight segments). The trends were subtracted from each segment, and these modified segments were subjected to a Hanning window. The Hanning window acts to reduce leakage by tapering the ends of each data segment. The resulting segments were transformed into the frequency domain with a real radix-2 Fast Fourier Transform (frequency interval of 0.0976 Hz).

Using standard notations, we can denote the Fourier transform of each flow segment by $X_k(f,T)$ and of each pressure segment by $Y_k(f,T)$. Here, $k$ corresponds to the sequential segments one through eight; $f$ represents frequency; and $T$ is the length of the segments (10.24 seconds). The required corrections for phase delay in the flowmeter (2.9°/Hz) and any distance between the flow and pressure transducers (0-2 cm) were applied to these transformed functions. The one-sided autospectral density functions ($G_{xx}$ and $G_{yy}$) and cross-spectral density function ($G_{xy}$) for flow and pressure were then estimated according to the following equations:

$$G_{xx} = \frac{(2/T)}{E[|X_k(f,T)|^2]}$$

$$G_{yy} = \frac{(2/T)}{E[|Y_k(f,T)|^2]}$$

$$G_{xy} = \frac{(2/T)}{E[X_k^*(f,T)Y_k(f,T)]}$$

where the expected value operator, $E$, denotes an averaging operation over the index $k$, and $X_k^*(f,T)$ is the complex conjugate of $X_k(f,T)$. The vascular impedance spectrum $Z(f)$ is now given by

$$Z(f) = \frac{G_{xy}}{G_{xx}}$$

The coherence [$C(f)$] of these $Z(f)$ values can be calculated from the equation

$$C(f) = \frac{G_{xy}}{|G_{xx}G_{yy}|}$$

where $C(f)$ has a value between 0 and 1 and is conceptually analogous to the linear correlation coefficient $r$. That is, a high value of $C(f)$ indicates that changes in the pressure harmonic at frequency $f$
correlated highly with changes in the flow harmonic at frequency f. Characteristic impedance \([Z_c]\) was calculated as the average value of \(|\mathcal{Z}(f)\)| between frequencies of 5 and 20 Hz. Only values of \(|\mathcal{Z}(f)\)| with a corresponding \(C(f)\) of greater than 0.8 were used in this calculation.

We determined two separate indexes that relate to the overall intensity of peripheral reflections. These computed indexes only measure the summated effect from multiple reflection sites rather than representing precise measurements of reflection amplitudes at individual sites. The reflection factor \(RF\) was calculated as the amplitude of impedance oscillations (i.e., the difference between the maximum and minimum impedance moduli for frequencies beginning at the initial impedance minimum) divided by the value of \(Z_c\). The frequency-dependent reflection coefficient \([RFC(f)]\) was derived from \(Z(f)\) and \(Z_c\) according to the following equation:

\[
RFC(f) = \frac{[Z(f) - Z_c]}{[Z(f) + Z_c]}
\]

Average pulse-wave velocity (PWV) along the aorta was calculated with the derived transfer function between simultaneously measured aortic and femoral pressures along with the measured distance \(D\) between these two recording sites; \(D\) was determined by passing the femoral catheter into the aorta up to the site of the aortic pressure transducer and then by measuring this insertion distance. This transfer function \(AP(f)\) was determined in a manner analogous to the determination of \(Z(f)\) above, except that \(X_c(f,T)\) would denote aortic pressure and \(Y_c(f,T)\) would denote femoral pressure. Two separate methods were used. The first method was to calculate the average slope of the phase of \(AP(f)\) between 0 and 20 Hz. PWV is then given by:

\[
PWV = \frac{(2\pi)D}{\text{slope}}
\]

where \(\pi\) is the numeric constant 3.14159. This method is equivalent to averaging the apparent phase velocity over the measured frequency range. By averaging these apparent phase velocities, the influence of reflections is reduced, and a more accurate estimate of true phase velocity is obtained.\(^{17}\) The second method was to transform the frequency domain function \([AP(f)]\) into a time domain function \([AP(t)]\) with an inverse Fourier transform. This method has previously been used to study the timing of vascular reflections in the time domain.\(^{18,19}\) \(AP(t)\) represents the impulse response of the transfer function between aortic and femoral pressures. Conceptually, it is the pressure that would be recorded at the femoral recording site when an impulse (very narrow pulse) of pressure was created at the aortic site at time zero (i.e., the peak of this impulse would occur at \(t = 0\)). The timing of the peak of \(AP(t)\) thus provides a measure of the time required for the pressure pulse to travel between the aortic and femoral sites. PWV is then given by the time to the peak of \(AP(t)\) divided by \(D\). The advantage of using \(AP(t)\) rather than the peaks of the original pressure tracings is that the peak of \(AP(t)\) should be less affected by peripheral vascular reflections. The calculated PWV, as determined by either of these methods, represents both a spatially averaged and a time-averaged (or pressure-averaged) value. Because of the nonuniform properties of the aorta (e.g., radius, wall thickness, compliance), PWV varies significantly along its length. Instantaneous PWV also varies with changes in instantaneous aortic pressure. However, the spatial pathway and range of pulse pressures over which averaging occurs were very similar for both control and nitroglycerin measurements.

The effective reflection distance (ERD) is a modeling parameter representing the distance to the major "effective" reflection site in the peripheral arterial system.\(^6\) ERD was calculated from the frequency of the first impedance minimum (\(F_m\)) and PWV according to the equation:

\[
ERD = PWV \times (1/4) \times (1/F_m)
\]

These calculations for ERD are based on a model of a single uniform tube with a closed end. Since these latter conditions are not present in the real arterial system, ERD should not be interpreted as a real physical distance.

The Windkessel Compliance \(Cw\) is another modeling parameter that was calculated from \(Z(f)\). \(Cw\) represents the compliance of a distensible reservoir that would be needed to model the low frequency impedance properties of the measured aortic impedance. Stated another way, \(Cw\) relates to the effective compliance of the total arterial system. The calculations for \(Cw\) are based on the impedance properties \(Zw(f)\) of a three-element Windkessel model composed of a series resistance \(Zc\) and a parallel capacitance-resistance combination \(Cw\) and parallel resistance. The logarithmic gain-frequency characteristics of such a first-order system can be used for parameter estimation.\(^{21}\) When plotted on log-log coordinates \((y = \log[Zw(f)])\), the value of \(Cw\) can be estimated from the point of intersection of two lines: the horizontal line \(y = \log[Z(0)]\) and a line with slope of \(-1\) passing through the midpoint of the initial descent of the \(\log[Zw(f)]\) curve. The \(y\) value of midpoint is equal to \(\frac{1}{2} (\log[Zw(0)] + \log[Zc])\), and the \(x\) value of midpoint is determined by the \(\log[Zw(f)]\) curve. The frequency coordinate of the point of intersection is an estimate of the "break frequency" \((BF)\), which can be used to determine \(Cw\) as follows:

\[
Cw = 1/[2\pi \times BF \times Z(0)]
\]

For the experimental data, \(Cw\) was calculated in an analogous fashion by first transforming \(Z(f)\) into log-log coordinates and then by determining midpoint, point of intersection, and \(BF\). Because the low frequency data points were not continuous, the \(x\) value of midpoint was extrapolated from a line determined by applying least-squares linear regression to the low frequency data points. The frequency interval chosen for this regression was 0.5-1.8 Hz; the \(r\) values of all lines exceeded 0.98. Although our parameter \(Cw\) is roughly analogous to compliance values determined...
from the decay pattern of diastolic pressure waveforms, it has the advantage of being determined from \( Z(f) \) values over a range of frequencies rather than from data at a single heart rate. Other difficulties involved in determining compliance values directly from the arterial pressure waveform have recently been reviewed.

The mean aortic diameter was calculated from all of the diameter data points from a given run. The theoretical resolution of the Schuessler ultrasonic dimension system used in these experiments is 0.1 mm.

**Results**

In every dog studied, nitroglycerin infusion produced a leftward shift in both impedance modulus and phase. Typical results from a single dog were previously shown in Figure 2, and averaged results for all dogs are shown in Figure 3. Despite individual differences in pulse-wave velocity and body length among dogs, this averaged-impedance spectrum still illustrates well the leftward shift in modulus and phase seen in the individual spectra. The average coherence \( C(f) \) of the individual \( Z(f) \) measurements was greater than 0.8 from 0 Hz up to 12 Hz.

**Discussion**

The major finding of this study is that nitroglycerin produced a consistent leftward shift in the impedance spectrum and that this shift occurred even in the absence of any change in mean blood pressure. Although a leftward shift in impedance has been described with vasodilators in the past, opinion has varied as to whether this shift was due to a passive change in vascular properties resulting from the uncontrolled decrease in mean blood pressure or due to some direct pharmacological effect of the drug.

Tabulated results from control and nitroglycerin (TNG) measurements. Values in parentheses are standard errors. BP, mean blood pressure; IR, input resistance; AOD, mean aortic diameter; Zc, characteristic impedance; Cw, Windkessel compliance; RF, reflection factor; PWV-P, pulse-wave velocity by phase-slope method; PWV-I, pulse-wave velocity by impulse-response method; ERD, effective reflection distance.

*\( p < 0.05 \); t \( p < 0.01 \) (paired Student's \( t \) test).
(measured along the aortic arch). Taken together, these latter measurements indicate that there was no significant change in proximal aortic compliance. This finding is in contrast to the observed increase in $C_w$, which may be considered as an index of the effective compliance of the total arterial system. Prior work by Simon et al., in which "arterial compliance" values were determined from the rate of diastolic pressure decay, has also documented an increase in effective arterial compliance with nitroglycerin. The greater increase in compliance reported by Simon et al (73% over control) may relate to the fact that their results were obtained in hyperensive patients in whom the response to nitroglycerin may differ compared with normotensive subjects. The increase in $C_w$ observed in our canine experiments can be considered as a manifestation of the leftward shift in impedance; that is, to model such an impedance shift requires a higher compliance value.

Based on their early studies in dogs, O'Rourke and Taylor1 suggested that the leftward shift in impedance seen with acetylcholine was due to changes in PWV. Pepine et al2 and Brin and Yin,3 in their studies with nitroprusside, speculated that other factors might also be involved in such vasodilator-induced impedance shifts. Actual measurements of PWV were not taken in any of these previous studies. Our measurements of PWV, along with calculations of ERD, indicate that the observed decrease in average PWV along the aorta was insufficient to explain the magnitude of impedance shift seen with nitroglycerin administration. In other words, a leftward shift in impedance implies that there has been a delay in the return of peripheral vascular reflections to the aortic root. This delay could result from a decrease in PWV and/or other factors such as an increase in the distance to the effective reflection site. If the measured decrease in average PWV along the aorta were sufficient to explain the magnitude of delay, then we would not have observed an increase in the calculated parameter ERD.

It must be emphasized that ERD is a modeling parameter and not an actual physical distance. The calculations for ERD assume a uniform tube with a single closed end (i.e., there is no change in PWV along the length of the tube; there is only one reflection site; and no phase delay occurs at this reflection site). These assumptions are not valid for the intact arterial system. The ERD calculation represents a distance to a hypothetical "effective" reflection site that results from the summated effects of the many different peripheral reflection sites within the arterial tree. The increase in ERD does imply, however, that nitroglycerin produced an alteration in the pattern of peripheral arterial reflections such that the return of these reflections to the aortic root was delayed to an extent greater than can be accounted for solely by the decrease in average PWV measured along the aorta.

Prior work by Taylor13 has shown that an increase in arterial cross-sectional area at peripheral bifurcations could theoretically produce this type of alteration in peripheral reflection patterns. The impedance changes calculated from Taylor's multibranched tubular model with this type of intervention (Figure 5) are very similar to the observed changes produced by nitroglycerin in the present experiments. As explained by Taylor, an increase in the total cross-sectional area at vascular bifurcations will reduce the intensity of reflections at these bifurcations such that summed reflections will originate more exclusively at vascular terminations. The "effective" site of reflections is thus moved farther from the origin. In support of this hypothesis, prior work by Simon et al has demonstrated the ability of nitroglycerin to dilate peripheral arteries.

Alternative explanations for this increase in ERD relate to some of the experimental and analytical limitations of this type of study. Average PWV was measured only along the aorta, which constitutes just one segment of the reflection pathway. Additional segments of the reflection pathway lie in more peripheral arteries. If nitroglycerin produces a relatively greater decrease in PWV along peripheral arteries than that measured along the aorta, this could also explain the increase in the calculated ERD. The analytical limitations of PWV calculations (see "Materials and Methods") must also be considered. As mentioned previously, the calculations of ERD are based on a model that assumes there is no phase delay at the reflection site. Most reflection sites within the vascular system exhibit complex reflection coefficients that do involve some phase delay. By altering the dynamic compliance of the peripheral arteries, nitroglycerin
might increase the phase delay seen at peripheral reflection sites. This increased phase delay would also alter the pattern of peripheral reflections in a manner that would result in an increase in the calculated ERD.

Stone and Dujardin and Dujardin et al have demonstrated that aortic smooth muscle tone can be influenced by autonomic reflexes, with resultant changes in pressure-diameter relations and \( z_c \). These reflex changes were studied under the conditions of volume expansion and hemorrhage. The applicability of their work to the present study is uncertain. Under the design of our study, volume status was not a controlled variable. Both the venodilatation produced by nitroglycerin as well as the supplemental volume administered to maintain mean blood pressure potentially could have altered volume status between control and nitroglycerin measurements. However, the fact that our experimental results were similar in all dogs despite a varied range of administered volumes (0–300 ml) would suggest that such volume changes did not play a major role in our findings.

We cannot exclude the possibility that our pacing protocol may have had some influence on autonomic reflexes. The wide range of heart rates used did produce significant alterations in pulse pressure as well as small cyclical alterations in mean pressure. The maximum cycle time for these alterations, due to the two different heart rate ranges used, was relatively short (5.12 seconds). Both the fact that our \( C_f \) measurements were relatively high and that repeated measurements initiated at random during the pacing process were always in good agreement suggest that possible phasic reflexes had little influence on our results. The pacing protocol may have produced some level of “tonic” reflex changes, although such changes would have been present during both the control and nitroglycerin measurements.

O'Rourke et al and Yaginuma et al have also emphasized the role of vascular reflections in explaining the effects of nitroglycerin on vascular impedance. Based on their findings in humans, they hypothesized that the “beneficial effects of nitroglycerin resulting from altered LV load in adult humans are predominantly due to reduction in peripheral wave reflection.” This disparity in results, however, may relate to differences in experimental conditions. Their study was performed in awake humans after the administration of sublingual nitroglycerin. Our study was performed in anesthetized dogs during an intravenous infusion of nitroglycerin. Furthermore, in the study by Yaginuma et al, the mean blood pressure was allowed to fall, and no supplemental volume was administered. The fall in mean pressure and the change in relative volume status (due to venodilatation) could have produced changes in aortic smooth muscle tone that would not have been produced under the conditions of our study. Because a delay in the return of vascular reflections can produce a beneficial effect in terms of ventricular-vascular coupling, this disparity in experimental results deserves further study.

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References


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