Regulation of Shear Stress in the Canine Coronary Microcirculation

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Background—Physical forces, such as pressure and flow, are well known to affect vascular function in the coronary circulation. Increases in shear stress produce vasodilation in coronary arterioles in vitro, and constant-flow preparations suggest a role for shear stress–induced vasodilation during adjustments to metabolic demand in vivo. Hypothetically, the regulation of shear stress can be viewed as a negative feedback control scheme (increased velocity → increased shear → vasodilation → decreased velocity → shear normalized). Therefore, we hypothesized that shear stress would be at least partially regulated during conditions of elevated flow.

Methods and Results—We used fluorescence microangiography to measure microvascular diameters and velocities in the coronary circulation in vivo and used these variables to calculate shear stress. Measurements were obtained under basal conditions, during maximal coronary blood flow, and after inhibition of NO synthase. Basal shear stress in the coronary circulation averaged 10 dyn/cm² in small arteries and 19 dyn/cm² in arterioles. Regulation of shear stress was observed in small arteries during adenosine-induced increases in coronary blood flow, but arterioles showed minimal regulation. NO synthase blockade had no effect on basal shear stress but completely abolished its regulation in small arteries during vasodilation.

Conclusions—Our data provide the first quantitative estimates of microvascular shear stress in the coronary circulation. Moreover, our results suggest that shear stress in small coronary arteries is regulated by NO release from the endothelium. (Circulation. 1999;100:1555-1561.)

Key Words: stress ▪ circulation ▪ nitric oxide synthase

Factors that determine coronary vascular resistance remain one of the cardinal issues in understanding the regulation of blood flow to the heart. Although cardiac metabolism is clearly a major determinant of coronary blood flow, physical factors, such as changes in blood flow and intraluminal pressure, also contribute to the regulation of vascular resistance via shear stress and myogenic mechanisms, respectively. Of these 2 responses, shear stress has more potent effects on coronary vasomotor tone. In 1984, Hintze and Vatner observed that if flow through an epicardial artery was held constant during reactive hyperemia, vasodilation of that artery was lost. In vitro studies of coronary microvessels reveal a powerful vasodilatory response when luminal flow is increased. These observations provide evidence that flow-mediated dilation is present in large arteries in vivo and in coronary arterioles in vitro. However, it is not known whether flow-mediated vasodilation occurs in the coronary microcirculation in vivo. We emphasize this point because in vitro flow-induced coronary microvascular dilation peaks at very low shear stresses (4 dyn/cm²): thus, at the higher shear stresses that probably occur in vivo, this mechanism may already be saturated.

Flow-mediated dilation is induced by changes in shear stress. An increase in microvascular flow velocity produces increased shear stress when viscosity and diameter are constant. The increased shear stress stimulates the release of endothelium-dependent dilators, thereby increasing diameter and lowering shear stress. If shear stress is a regulated variable in the coronary microcirculation, the increase in diameter would hypothetically lower shear stress to a normalized value. Inhibition of endothelium-dependent dilators therefore would impair regulation of shear stress and lead to higher levels of intravascular shear during increases in blood flow, ie, shear stress would not be regulated at its baseline value.

The objective of the present study was to test the hypothesis that shear stress is a regulated variable in the canine coronary microcirculation and that this regulation is mediated by nitric oxide (NO). We measured shear stress in coronary microvessels (ranging from arterioles to small arteries) in vivo during basal conditions and maximal coronary blood flow with adenosine and during inhibition of NO synthase under both conditions. Our results indicate that NO regulates microvascular shear stress in the coronary circulation in vivo during elevations in blood flow. We also found that shear stress is effectively regulated, ie, remains constant in small coronary arteries, but not arterioles, during large changes in coronary blood flow.
Methods
Coronary Microvascular Preparation
Forty-eight adult mongrel dogs (5 to 15 kg) were anesthetized with sodium pentobarbital (Nembutal, 30 mg/kg IV). A core body temperature of 37°C was maintained by a homeothermic blanket. The right femoral artery and vein were cannulated for measurements of aortic pressure and arterial blood gases and the administration of drugs, respectively. A 5F fluid-filled catheter was advanced into the left ventricle from the left carotid artery for the measurement of left ventricular pressure. The first derivative of the left ventricular pressure trace (LV dP/dt) was obtained by an on-line differentiator. Hemodynamic data were acquired continuously by use of ACODAS data acquisition software from DATAQ. A tracheotomy was performed, and ventilation was provided by high-frequency jet ventilation. With the maximum LV dP/dt as a timing reference, a solenoid connected to a pressure source (100% O2) at 6 to 12 psi was triggered to open for 20 to 35 ms at the same time in each cardiac cycle. The small tidal volume minimizes respiratory movement, which occurs at the same frequency as the heart beat. Arterial pH and blood gases were monitored frequently and maintained within the following ranges by adjustment of the tracheal catheter or by administration of sodium bicarbonate: PCO2 25 to 40 mm Hg, PO2 100 to 200 mm Hg, pH 7.35 to 7.45. All animals received propranolol 1 mg/kg to reduce heart rate and minimize tissue motion.

To visualize the epicardial surface, the heart was exposed by a left thoracotomy at the fifth intercostal space and stabilized in a partial pericardial cradle. A large coronary artery was exposed and a 24-gauge cannula inserted to allow the measurement of coronary artery pressure and the administration of microspheres, intracoronary drugs, and fluorochromes. After an area of easily visible epicardial microvessels was identified, four 22-gauge pins were passed horizontally through the left ventricle to minimize vertical cardiac motion. Neither maneuver appears to compromise coronary tone, because resting blood flow and vasodilator reserve were unaffected in each case.

Measurement of Coronary Microvascular Diameter
To measure coronary microvascular diameter, the cardiac surface was illuminated by a stroboscope (Chadwick-Helmuth, 100-W xenon arc) that was triggered by the maximum LV dP/dt signal to flash once for 20 to 30 μs at the same point during each cardiac cycle. The strobe trigger signal was monitored in relation to left ventricular pressure for a precise determination of the position of the strobe in the cardiac cycle. The combined use of low-tidal-volume jet ventilation and brief epicardial illumination, both synchronized to the cardiac cycle, causes the surface coronary microvessels to appear virtually motionless when viewed through an intravital microscope (Leitz Ploemopak, Wild Leitz). The microscope objectives used were the Leitz EF4 (×4, numerical aperture 0.22) and the Leitz L10 (×10, numerical aperture 0.22).

To illuminate the inner diameters of the microvessels, 50- to 100-μL aliquots of a 25-mg/mL solution of FITC-dextran in 0.9% saline (MW 500 000) were injected through the coronary cannula. A Leitz H2 excitation-barrier filter was used to activate the fluorescein and receive the emitted light. Each injection causes arterial and venous vessels to fluoresce sequentially for 5 to 10 seconds. The anatomic landmarks of a particular vessel were identified, and 5 to 8 images were obtained over a period of <1 minute with a Cohu silicon-intensified tube video camera (Cohu Intensified CCD Camera). The images were digitized directly from the camera by a frame digitizer (Scion Image, National Institutes of Health) and were transferred to a Macintosh computer (Apple Computer Inc) for diameter measurements with appropriate software (Image 2.18, National Institutes of Health Research Services Branch). Diameters were measured by aligning cursors at the vessel edges, the measurements in pixels being converted to micrometers by use of a conversion factor determined with a micrometer grid. Typically, microvascular measurements over each image acquisition period vary by ±3% from the average value. Vessels in which the control microvascular diameters after interventions vary from the previous baseline by >10% were excluded from analysis.

Measurement of Coronary Microvascular Velocity
A procedure similar to that designed by Nellis et al6 was used to measure flow velocities in the epicardial coronary microcirculation in vivo. The strobe was triple-flashed during a single video frame (33 ms for 1 complete video image); thus, 3 images, separated in time (8 ms between flashes), are captured on the same video frame (Figure 1). The fluorescent particles appear 3 times in each image, and the velocity can be calculated as the quotient of the distance moved by the microspheres and the time interval between strobe flashes. Fluorescent microspheres (10 μm, Fluoresbrite YG plain microspheres, Polyscience, Inc) suspended in a mixture of dilute fluorescein solution and 0.9% saline were injected into the coronary artery,
Measurement of Coronary Blood Flow
To measure coronary blood flow, a segment of an epicardial coronary artery proximal to the insertion of the catheter was dissected free of surrounding adventitia, and a 2.5B Transonics flow probe was placed around the vessel. The probe was covered in an ultrasonic conduction jelly, and flow data were monitored on a Transonics T206 Flowmeter (Transonics Inc) and recorded on a Macintosh IIcx with LabVIEW 2 Software (National Instruments).

Calculation of Shear Stress
Shear stress was calculated from the measurements of microvascular flow velocities, blood viscosity, and the radius of the microvessel from the formula $\tau = (4v \eta)/r$, where $t$ is shear stress, $v$ is microvascular flow velocity, $\eta$ is blood viscosity, and $r$ is the radius of the blood vessel. Viscosity was estimated from an arterial blood hemocrit and shear rate by use of the relationship described by Brooks et al.? According to this relationship, when shear rates exceed 50 to 100 s$^{-1}$, variations in the hematocrit observed in these studies have negligible effects on viscosity. Because shear rates in this study exceed this level even in the basal state, we assumed that variations between the systemic and microvascular hematocrits would have no significant effect on shear stress calculations.

Experimental Protocols
Shear stress experiments were divided into 4 separate protocols. In each experiment, the preparation was allowed to stabilize 15 to 30 minutes, and basal diameters of each vessel were obtained. Animals were then introduced into 1 of the following protocols.

Basal Shear Stress
After stabilization and diameter acquisition, velocity measurements were obtained.

Shear Stress During Maximal Vasodilation
Adenosine 25 $\mu$g · kg$^{-1}$ · min$^{-1}$ was infused into the coronary artery for 10 minutes. Microvascular diameters and velocities were obtained during adenosine administration.

Basal Shear Stress After Inhibition of NO
N$^\text{G}$-Monomethyl-L-arginine (L-NMMA, 300 $\mu$g · kg$^{-1}$ · min$^{-1}$) was infused into the coronary artery for 20 minutes. Microvascular diameters and velocities were obtained after L-NMMA administration.

Shear Stress During Maximal Vasodilation After Inhibition of NO
L-NMMA was infused into the coronary artery for 20 minutes. Adenosine was then infused into the coronary artery for 10 minutes. Microvascular diameters and velocities were obtained during adenosine administration after L-NMMA.

Regulation of Shear Stress
The goal of this study was to determine the degree to which shear stress is regulated. In an unregulated system, such as a rigid tube, shear stress would increase proportionately to flow velocity. Therefore, a hypothetical unregulated shear stress would be basal shear stress increasing proportionately to velocity, ie, if adenosine doubled microvascular velocity, then the unregulated shear stress would also be doubled. The difference between the experimental measurement and the calculated unregulated value was the estimated regulation of shear stress. To calculate shear stress (SS) regulation, the following equation was used: $\text{SS regulation} = (\text{observed ADO SS} - \text{observed basal SS})/(\text{unregulated high-flow SS} - \text{observed basal SS}).$ If the observed shear stress during vasodilation were equal to the unregulated, shear stress regulation would be zero. If shear stress with adenosine were unchanged from basal, shear stress regulation would be 1, or perfect regulation. To account for the possible heterogeneity of shear stress regulation in the coronary circulation, we calculated shear stress regulation in small arteries (>160 $\mu$m in diameter) and arterioles (<160 $\mu$m).

Drugs
All drugs were dissolved in 0.9% saline solution. Propranolol, adenosine, and buffer components were purchased from Sigma Chemical Co. L-NMMA was purchased from RBI.

Statistics
All statistical analyses were performed on StatView software for the Macintosh (Abacus Concepts). Differences in shear stress, vascular diameter, and microvascular velocity were evaluated by ANOVA with Fisher’s protected least significant difference as the post hoc multiple comparison test. Regression lines were obtained by best-fit algorithms using StatView. Data are presented as mean±SEM. Significance was accepted at $P<0.05$ in all experiments.

Results
Preliminary Studies
We determined that the point in the cardiac cycle at which maximal velocity occurred was mid-diastole. In a comparison of midsystole (25% of the cardiac cycle from the onset of positive LV dP/dt) and mid-diastole (75% of the cardiac cycle), it was determined that blood velocity during diastole was 46% greater than that observed during systole (5 versus 7 mm/s, systole versus diastole, $n=3$). All subsequent measurements were taken during peak diastole.

We also optimized the individual interventions used in these studies, specifically, the effects of adenosine and L-NMMA on total coronary blood flow. Basal coronary blood flow was 12±3 mL/min (n=4), and intracoronary infusion of adenosine (25 $\mu$g · kg$^{-1}$ · min$^{-1}$) increased coronary blood flow 5.3-fold to 62±10 mL/min. The blood concentration of adenosine was estimated at 16 $\mu$mol/L (infusion concentration·infusion rate/coronary blood flow), which is far in excess of the ED$_{max}$ of 4 to 5 $\mu$mol/L reported previously.8 We are therefore confident that the intervention described produced maximal coronary vasodilation.

L-NMMA was used to inhibit NO at a dose that impaired both acetylcholine-mediated dilation (fluorescence microangiography) and increase in blood flow by >60%. L-NMMA (100 $\mu$g · kg$^{-1}$ · min$^{-1}$ or $\approx$300 $\mu$mol/L) had no effect on either resting (12±3 [control] versus 12±3 [L-NMMA] mL/min) or vasodilated (adenosine) (62±9 [control] versus 63±9 [L-NMMA] mL/min) coronary blood flow. Intracoronary infusion of adenosine or L-NMMA did not produce significant changes in aortic blood pressure or heart rate.

Finally, we determined the number of 10-$\mu$m microspheres that could be infused without perturbing coronary vascular hemodynamics. These experiments determined that $\approx4\times10^6$ spheres could be infused without effect on basal or maximal (adenosine-induced) coronary blood flow (±10%), heart rate, or aortic pressure. On the basis of these studies, no more than $4\times10^6$ microspheres were infused into the coronary artery.

Microvascular Velocity and Shear Stress:
Basal Conditions
The relationship between microvascular diameter and velocity is shown in Figure 2A. Velocity averaged 7±1 mm/s. The relationship between shear stress and diameter is shown in Figure 2B. Shear stress was heterogeneously distributed, with
arterioles (<160 μm) showing significantly higher shear stress than small arteries (>160 μm) (19 ± 2 [arterioles] versus 10 ± 2 [small arteries] dyn/cm², P < 0.005). The correlation between shear stress and diameter was best described by a second-order polynomial equation (P < 0.05).

Microvascular Velocity and Shear Stress Measurements: Maximal Coronary Vasodilation
The effect of maximal vasodilation caused by adenosine on the relationship between diameter and velocity is shown in Figure 3A. Microvascular velocity during adenosine infusion increased 114% to 15 ± 1 mm/s from baseline conditions (7 ± 1 mm/s, P = 0.0001). The effect of adenosine on shear stress is shown in Figure 3B. The relationship between shear stress and microvascular diameter was best fit by a second-order polynomial equation (P < 0.05). During vasodilation, shear stress was markedly increased in coronary arterioles (37 ± 5 dyn/cm²) compared with basal values (19 ± 2 dyn/cm², P < 0.05). In small arteries, shear stress was only modestly increased during elevated coronary blood flow, to 13 ± 3 dyn/cm², a value not significantly different from basal shear stress in these vessels.

Microvascular Velocity and Shear Stress Measurements During Inhibition of NO Synthase
The effect of NO synthase inhibition by L-NMMA on the relationship between diameter and velocity is shown in Figure 4A. Microvascular velocity after L-NMMA was not statistically different from control (7 ± 1 [basal] versus 10 ± 1 [L-NMMA] mm/s, P = 0.06). The effect of L-NMMA on shear stress is shown in Figure 4B. Shear stress was also not significantly altered by L-NMMA in either small arterioles (19 ± 2 [basal] versus 25 ± 6 [L-NMMA] dyn/cm²) or small arteries (10 ± 1 [basal] versus 10 ± 2 [L-NMMA] dyn/cm²). The relationship between shear stress and microvascular diameter was best fit by a second-order polynomial equation (P = 0.001).

The effect of L-NMMA during near-maximal coronary vasodilation on the relationship between diameter and velocity is shown in Figure 5A. Microvascular velocity during adenosine infusion (21 ± 1 mm/s) was higher on average by 133% than L-NMMA alone (10 ± 1 mm/s, P < 0.001) and 50% greater than adenosine without NO synthase blockade (15 ± 1 mm/s, P < 0.01). The effect of adenosine on shear stress is shown in Figure 5B. In arterioles, shear stress during adenosine dilatation after L-NMMA was 54 ± 5 mm/s, significantly greater than with L-NMMA alone (25 ± 6 mm/s, P < 0.001). Shear stress was also 45% greater than with adenosine alone (37 ± 5 [L-NMMA] versus 54 ± 5 [L-NMMA + adenosine] dyn/cm², P < 0.05). In small arteries, shear stress was on average 280% greater than with L-NMMA alone (10 ± 2 [L-NMMA] versus 28 ± 4
Regulation of Shear Stress

The degree of shear stress regulation across the coronary microcirculation is shown in Figure 6. Shear stress regulation was analyzed in small arteries (>160 μm) and arterioles (<160 μm). Small arteries demonstrated pronounced shear stress regulation, with shear stress increased only 30% in response to a 114% increase in velocity. Arterioles demonstrated negligible shear stress regulation, increasing shear stress by 94% when velocity increased by 114%. After L-NMMA, shear stress regulation in small arteries was virtually abolished, with shear stress increasing parallel to velocity. Shear stress regulation in arterioles was unaffected by L-NMMA.

Discussion

These data provide the first estimates of shear stresses in the canine coronary microvasculature in vivo and the first measurements of microvascular velocity during conditions of elevated blood flow. We also report the effects of inhibition of NO synthase on these variables. In small arteries, shear stress was increased 30% above basal during maximal coronary vasodilation, when velocity increased 114%. When NO was inhibited, shear stress in small arteries increased proportionately to velocity, implying that shear stress is regulated in these vessels by the production of NO. Arterioles, conversely, appeared to regulate shear stress only minimally. This finding suggests that despite the demonstrated ability of these vessels to regulate shear stress in vitro,3 other factors are primarily responsible for coronary arteriolar tone in vivo.

Critique of the Methodology

The experiments for measuring microvascular velocity have a number of caveats. First, only the epicardial circulation was visualized in these experiments. As has been discussed previously, the epicardial circulation may have features different from those of the endocardial circulation, although such differences are distinguished largely by magnitude rather than direction.9 A second concern would be that the velocity measurements of spheres moving too close to the vessel wall would be compounded by adhesion of the sphere...
Shear Stress in the Coronary Microcirculation

We have made several observations regarding shear stress in the coronary microvasculature in vivo. First, shear stress is heterogeneously distributed; arterioles <160 μm have increased levels of shear stress relative to small coronary arteries. Second, the levels of shear stress present in the microvasculature at rest are greater than has been reported to cause maximal arteriolar dilation in vitro. Finally, our evidence suggests that shear stress is at least partially regulated in the coronary microvasculature.

The observation that shear stress is elevated in microvascular vessels has been reported previously in other vascular beds. In 1978, Lipowsky and coworkers reported arteriolar shear stresses in the mesenteric bed averaging 47 dyn/cm² in vessels <100 μm in diameter. A similar range of velocity-diameter relationship in the cat mesentery and the rabbit omentum was reported earlier, and although shear stress was not calculated, it was probably similar to values reported in the Lipowsky study. In the present study, shear stress ranged 15 to 23 dyn/cm² at rest in vessels ranging from 100 to 150 μm in diameter. Although the range of diameters measured is not perfectly overlapping with previous reports, the relationship between shear stress and diameter described by our data predicts a similar range of shear stress values in arterioles of the caliber of those in these previous studies. This observation suggests that the relationship between diameter and shear stress may be similar across different vascular beds.

The high levels of shear stress observed in these studies present a puzzle about the vasoactive sensitivity of these vessels in vitro. Kuo et al reported that values of shear stress at 2 to 4 dyn/cm² produced maximal vasoconstriction in isolated coronary arterioles. This value is ≈10% of the values obtained in these in vivo studies. One explanation that accounts for both results could be the presence of blood in vivo. Because hemoglobin scavenges NO, it may cause a rightward shift in the relationship between NO and shear stress. Studies of isolated vessels perfused with blood are needed to address the role of interactions with hemoglobin and other blood products in modulating vascular responses to shear stress.

To determine whether shear stress was a regulated variable in the coronary microcirculation, we increased coronary flow velocity maximally with adenosine. We selected adenosine because it acts primarily at the level of the distal microvasculature. This allowed examination of downstream dilation of vessels without the confounding effects of direct dilation. If shear stress were a regulated variable in coronary microvessels (100 to 300 μm in diameter), it would remain constant as flow velocity increased. Alternatively, a proportionate increase in shear stress parallel to the increase in flow would argue against any regulation. We observed heterogeneous control of shear stress in coronary microvessels of different sizes during adenosine-induced vasodilation. In small coronary arteries (>160 μm), there was a pronounced degree of regulation; shear stress increased only 30% relative to a 114% increase in velocity (73% regulation). In contrast, arterioles showed poor regulation; shear stress increased 94% to a 114% increase in velocity (17% regulation). Taken together, these observations suggest that regulation of shear stress is heterogeneous in the coronary circulation and is more important in small coronary arteries than downstream arterioles.
Effect of NO Synthase Inhibition on Microvascular Shear Stress

To determine whether the observed modulation of shear stress was mediated by NO, L-NMMA was infused into the coronary circulation to antagonize NO synthase. Several observations were made. First, L-NMMA had no effect on either basal diameter or shear stress. Second, shear stress was considerably increased during adenosine infusions after L-NMMA relative to adenosine alone.

The effects of NO inhibition on resting hemodynamics are controversial. Some studies report reductions in blood flow or diameter in some preparations, but no effect in others. This variation may be accounted for by variations in dose, duration, and agent used. In the present experiments, we observed no effect of L-NMMA on resting diameter or shear stress. There are 2 potential caveats to this observation. The first is that we inhibited NO alone. Jones et al have observed microvascular constriction of small coronary arteries during administration of Nω-nitro-L-arginine methyl ester in conjunction with indomethacin. Previous studies have suggested a role for prostanoids in the response to changing shear. Because we observed no effect using a preparation very similar to that of Jones et al, we conclude that interactions between NO and prostanoids may account for the disparate results. The second important aspect is that these studies are performed in the coronary circulation, as opposed to the systemic circulation. Koller et al have observed pronounced effects of NO inhibition at rest in the cremaster preparation. Because the skeletal and other systemic circulations are under high basal tone at rest, it is possible that their sensitivity to shear might be greater than the coronary circulation, in which flow is relatively quite high. Finally, it should be stated that the effect of NO inhibition on resting coronary blood flow that could produce the 10% to 20% decrease reported in some studies might be due to very small changes in diameter, especially if those changes are distributed throughout various sizes of coronary arteries. Such changes may be beyond the resolution of our measurements. We therefore must exercise caution in any conclusion that NO does not modulate basal shear stress. However, we can maintain that the basal role of NO is small in the coronary microcirculation.

In contrast to the basal state, shear stress increased dramatically after NO inhibition when blood flow was elevated. In small coronary arteries, in which shear stress is normally well regulated, NO inhibition virtually abolished this regulation, ie, shear stress increased proportionally to velocity. In arterioles, shear stress was poorly regulated and showed little response to L-NMMA. We are compelled to mention the possibility that in vivo, coronary arterioles may regulate shear stress but at lower flow rates than produced by maximal dilation. However, we can state with conviction that small coronary arteries regulate shear stress much more effectively than coronary arterioles. Taken together, these observations demonstrate that in small coronary arteries, in which shear stress is well regulated during large changes in coronary blood flow, NO plays a central role in that control.

In conclusion, these studies provide new observations on the regulation of shear stress in the coronary microvasculature. In addition to the first estimation of coronary microvascular shear stress in vivo, we observed active regulation of this variable in the small coronary arteries by NO. We conclude that NO plays an important role at specific sites in the coronary circulation in regulating shear stress.

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