Nitric Oxide Mediates Flow-Dependent Epicardial Coronary Vasodilation to Changes in Pulse Frequency but Not Mean Flow in Conscious Dogs

John M. Canty, Jr, MD; Jeffrey S. Schwartz, MD

**Background** Although epicardial coronary arteries dilate in response to changes in flow, the mechanisms responsible for this and the mechanical stimuli that are sensed by the endothelium are not completely defined. We performed the present study to determine the importance of nitric oxide in eliciting epicardial dilation to sustained changes in mean flow and pulse frequency in the coronary circulation of conscious dogs.

**Methods and Results** Dogs were chronically instrumented with a circumflex coronary occluder, piezoelectric crystals to measure epicardial diameter, and a coronary artery catheter placed distal to the crystals for intracoronary drug infusion. Studies were conducted in dogs in the conscious state. We inhibited nitric oxide production by administering the arginine analog N'-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg IV), which attenuated the epicardial artery diameter changes to left atrial infusions of acetylcholine (10 µg/min) from 140±23 (±SEM) to 46±20 µm (P<.05). Epicardial dilation to sustained increases in mean coronary flow was examined by infusing adenosine into the distal coronary artery at a constant heart rate. Intracoronary adenosine increased mean flow to the same extent (180±21 versus 177±24 mL/min after L-NAME, P=NS), but inhibiting nitric oxide production had no effect on flow-mediated epicardial dilation, with coronary diameter increasing by 264±36 µm under control conditions and 294±67 µm after L-NAME (P=NS). In contrast, when pulse frequency was increased by pacing to a rate of 200 beats per minute, mean coronary flow increased to a similar level (78±9 versus 75±9 mL/min after L-NAME), but the epicardial diameter change to pacing was attenuated from 170±29 µm under control conditions to 54±23 µm after L-NAME (P<.01).

**Conclusions** These results demonstrate that in vivo, nitric oxide production is primarily responsible for eliciting epicardial coronary vasodilation to endothelium-dependent agonists and changes in coronary flow pulse frequency. The failure of L-NAME to affect epicardial vasodilation during sustained increases in mean flow when pulse frequency is held constant suggests that additional mechanisms are involved in flow-mediated vasodilation of epicardial coronary arteries. (*Circulation.* 1994;89:375-384.)

**Key Words** • nitric oxide • flow • vasodilation • endothelium

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were addressed. Does nitric oxide mediate coronary vasodilation to sustained increases in mean coronary flow when heart rate is constant? And to what extent is nitric oxide production responsible for regulating large coronary artery diameter in response to pacing-induced changes in pulse frequency in vivo?

**Methods**

Studies were conducted in chronically instrumented dogs. All experimental protocols were performed in accordance with institutional guidelines. A total of nine mongrel dogs (30±1 kg) were studied.

**Experimental Preparation**

Animals were fasted overnight and premedicated with Innovar-Vet (0.4 mg/mL fentanyl and 20 mg/mL droperidol, 1 to 3 mL IM). We induced anesthesia with sodium thiamylal (20 mg/kg IV), after which the dogs were intubated and mechanically ventilated. A surgical plane of anesthesia was maintained throughout the procedure with a nitrous oxide (=60%), oxygen (=40%), and halothane (=1% to 2%) mixture. Under aseptic conditions, we performed a thoracotomy in the fourth left intercostal space and suspended the heart in a pericardial cradle. Tygon catheters were placed into the descending aorta and left atrium. Pacing leads were sewn onto the right ventricular outflow tract. A micromanometer was placed into the left ventricle. We dissected a long segment (4 to 5 cm) of the proximal circumflex artery free and instrumented it with a hydraulic occluder and an ultrasonic flow probe (model 3R or 4R, Transonics, Ithaca, NY). Two miniature piezoelectric crystals (2-mm diameter) glued to Dacron patches were used to measure coronary artery diameter as previously described. The crystals were attached to the adventitia of the left circumflex artery distal to the hydraulic occluder. Care was taken to place them so that they were orthogonal to the long axis of the vessel and measured the major diameter. A small Teflon angiograph (connected to polyethylene tubing) was placed into the circumflex artery for drug infusion. The catheter was placed so that its tip was at least 3 mm distal to the ultrasonic crystals. A Teflon angiograph was also placed into the pulmonary artery for systemic drug infusion. At the conclusion of instrumentation, the chest was closed, and the pneumothorax was evacuated. The catheters and wires were exteriorized through the chest wall and placed in a jacket that the animal was trained to wear. Animals were given prophylactic antibiotics (300 mg streptomycin and 300 000 U procaine penicillin IM) for 3 to 5 days postoperatively. Postoperative analgesia (2 mg butorphanol IM PRN) was administered until the animals were free of subjective signs of discomfort. Fluid-filled catheters were flushed with saline at regular intervals and filled with heparin (1000 U/mL). Enteric-coated aspirin was begun on the third to fifth postoperative day (325 mg PO QD). The animals were allowed to recover for at least 2 weeks before experiments were conducted in the unanesthetized state.

**Experimental Protocols**

On the day of the study, the animals were sedated with Innovar-Vet (1 to 3 mL IM). They were placed in a sling and allowed to adjust to the laboratory for 60 minutes before beginning measurements. A demand pacemaker was set at the control heart rate of each animal to prevent reductions in heart rate during the various experimental interventions.

**Effect of L-NAME on Epicardial Artery Dilation to Acetylcholine**

In seven of the nine animals, we examined the effects of L-NAME on acetylcholine-induced vasodilation of the epicardial coronary artery. Acetylcholine (10 μg/mL saline) was infused into the left atrium at continuous rates of 5, 10, and 15 μg/min. These systemic infusion rates were chosen because they produced significant agonist-induced vasodilation of the epicardial coronary artery while having minimal effects on systemic hemodynamics and coronary flow.

**Effect of L-NAME on Epicardial Dilation to Sustained Changes in Mean Flow and Flow Pulse Frequency**

After determining baseline variables in the resting state, we assessed reactive dilation of the epicardial coronary artery to a 30-second occlusion (Fig 1, top). Once coronary flow and diameter had returned to resting values, adenosine (0.5 mg/mL of saline) was infused through the distal coronary artery at 0.5 mg/min to produce sustained coronary vasodilation (Fig 1, bottom). Coronary and systemic hemodynamics were sampled after diameter reached the steadystate (3 to 5 minutes after the infusion was started). After the adenosine infusion was stopped and coronary hemodynamics were allowed to return to baseline, animals were paced to produce a heart rate of about 200 beats per minute, and the hemodynamic measurements repeated after flow and diameter reached a new steady state. On completing the control series of interventions, we administered 10 mg/kg L-NAME IV to inhibit nitric oxide production. We have previously shown that this dose of L-NAME attenuates the coronary resistance vessel vasodilation to intracoronary acetylcholine. After 30 minutes, the previous series of interventions were repeated. At the end of each study, nitroglycerin (50 μg/mL saline) was infused into the pulmonary artery at 50 μg/min to assess maximal dilation of the epicardial artery. Two animals were excluded from the present analysis because their arteries were not reactive as defined by a <3% increase in epicardial diameter to nitroglycerin. Pacing-induced diameter changes could not be obtained in one animal due to nonfunctioning pacing electrodes. The effects of L-NAME on the coronary flow response to pacing in seven of the nine animals has been presented as part of a study that examined the role of nitric oxide in coronary resistance vessel control during autoregulation.

**Epicardial Diameter During Pulmonary Artery Adenosine Infusion and Intracoronary Adenosine With Restricted Flow**

In five of the animals, we determined the effects of pulmonary artery infusion of adenosine on coronary flow and diameter to exclude the possibility that adenosine infused into the distal circumflex artery recirculated and reached significant concentrations in the systemic arterial circulation. Adenosine (0.5 mg/min) was infused into the pulmonary artery, and measurements of coronary flow and diameter were compared with the preinfusion control values. In four of the animals, we were able to examine the effects of intracoronary adenosine infusion when coronary flow was restricted to values that were within 10% of the mean resting flow levels to exclude the
CONTROL

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Reactive Hyperemia

ADO

Adenosine .5 mg/min i.c. infusion
Effects of N\textsuperscript{\textdagger}\texthyphen\textregistered\textdash Nitro\texthyphen\textdagger\texthyphen\textregistered\textl\texthyphen\textregistered\texthyphen\textl\texthyphen\text registered Arginine Methyl Ester on Hemodynamics and Coronary Artery Diameter at Rest

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N\textsuperscript{\textdagger}\texthyphen\textregistered\textl\texthyphen\texthyphen\textregistered\texthyphen\textl\texthyphen\text registered Arginine Methyl Ester</th>
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<tbody>
<tr>
<td>Aortic pressure, mm Hg</td>
<td>100±3</td>
<td>126±3\textsuperscript{*}</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>85±4</td>
<td>86±5</td>
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<tr>
<td>Coronary flow, mL/min</td>
<td>42±4</td>
<td>41±3</td>
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<tr>
<td>Coronary diameter, μm</td>
<td>3454±369</td>
<td>3351±361\textsuperscript{*}</td>
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bpm indicates beats per minute. Values are given as mean±SEM. \textsuperscript{*}P<.01 vs control.

possibility of direct actions due to reflux or propagation of the distal effects of adenosine on the coronary diameter crystals.

Data Analysis

All data were recorded on a Gould 2800W recorder. Catheters were connected to Statham P23DB transducers with the zero reference level taken at the midchest to correspond with the estimated position of the left atrium. Coronary diameter was measured using a Triton 120 sonomicrometer. Mean coronary flow was recorded throughout each experiment. Hemodynamic data were digitized at a sampling rate of 200 Hz with a Data Translation DT2801A analog-to-digital convertor (Marlboro, Mass), which was interfaced to an IBM PC/AT computer. All steady-state hemodynamic data represent the average of a 15-second sampling interval that comprised at least 15 cardiac cycles. We determined both peak reactive hyperemic flow and the percentage of flow repayment to flow debt by integrating the area under the reactive hyperemic flow curve from the chart recordings as described by Coffman and Gregg,\textsuperscript{16} where blood flow debt (mL) is control flow rate (mL/min)×xocclusion time (min); excess flow during reactive hyperemia is total volume during reactive hyperemia (mL)−control flow rate (mL/min)×reactive hyperemia duration (min); repayment of flow debt (%) is (excess flow during reactive hyperemia [mL]/flow debt [mL])×100. Diameter measurements were expressed as either absolute values or as the change in diameter relative to the appropriate resting diameter under control conditions or after L-NAME. They were also normalized to the maximum nitroglycerin response using the following equation:

$$\%\text{Dilation} = \frac{(D - D_{\text{Rest}})}{D_{\text{Re}} - D_{\text{Rest}}} \times 100$$

where D is steady-state diameter during an intervention under control conditions or after L-NAME, D\textsubscript{Re} is resting diameter under control conditions or after L-NAME, and D\textsubscript{Rest} is maximum diameter during nitroglycerin infusion after L-NAME.

Data are presented as the mean±SEM. The constancy of systemic hemodynamics during interventions was assessed by a repeated-measures ANOVA. Significant differences between parameters before and after L-NAME were assessed using paired t tests. When comparing repeated measurements, we used the Bonferroni correction for performing multiple comparisons. The P<.05 level was considered significant.

Results

All animals were in good health at the time of study. Room air arterial blood gases were pH 7.37±0.01 (±SEM); PCO\textsubscript{2}, 34±1 mm Hg; and PO\textsubscript{2}, 88±1 mm Hg. Hematocrit averaged 36±2%. Resting hemodynamics under control conditions and after L-NAME are summarized in the Table. Measurements of coronary diameter at baseline, after L-NAME, and after nitroglycerin (in the presence of L-NAME) are illustrated in Fig 2.

Inhibiting nitric oxide production with L-NAME increased mean aortic pressure. Although heart rate (which was paced) and coronary flow remained unchanged, L-NAME constricted the epicardial coronary artery by 103±80 μm. Intravenous infusion of nitroglycerin dilated the epicardial artery by 536±88 μm (7.2±3.3% of the resting value obtained after L-NAME).

Effects of L-NAME on Acetylcholine-

Induced Dilation

Fig 3 demonstrates the effects of blocking nitric oxide on receptor-mediated epicardial dilation to systemic administration of acetylcholine. Under control conditions, left atrial infusion of acetylcholine at doses between 5 and 15 μg/min caused progressive dilation of the epicardial coronary artery that reached 53±9% of the maximum response found during nitroglycerin infusion. Heart rate, aortic pressure, and coronary flow did not change significantly with increasing doses of acetylcholine (repeated-measures ANOVA). Because coronary flow was not increased, the epicardial dilation represented the direct effects of acetylcholine on the endothelium as opposed to flow-dependent changes. After administration of L-NAME, the epicardial artery dilation to systemic acetylcholine was attenuated at all infusion rates (P<.001, ANOVA) and reached a maximum dilatation of 15±6% of the nitroglycerin response at the highest infusion rate (P<.05 versus control response). Thus, in the dose we used, L-NAME attenuated the vasodilation of epicardial conduit arteries to agonist-induced nitric oxide production.

Effect of L-NAME on Reactive Versus

Sustained Vasodilation

Fig 4 summarizes the effects of L-NAME on reactive hyperemia and reactive epicardial coronary dilation in all of the animals studied. Although L-NAME reduced peak reactive hyperemic flow by a small amount (19±6%, P<.01, L-NAME versus control), it had a much more pronounced effect on the percentage of flow repayment to flow debt, which decreased to 55±7% of the control response (P<.01, L-NAME versus control). The reduction in the volume of the reactive hyperemic response produced by inhibiting nitric oxide production was associated with an attenuation in the peak reactive epicardial arterial dilation to 47±11% of control values (P<.01).

In contrast to the attenuation of reactive dilation by L-NAME, the epicardial diameter change to sustained increases in flow produced by infusing adenosine into the distal coronary artery was not affected (Fig 5). L-NAME did not affect heart rate (94±6 versus 90±6 beats per minute, P=NS), resting coronary flow (42±4 versus 41±3 mL/min after L-NAME, P=NS), or the increase in mean flow during intracoronary adenosine infusion into the distal circumflex artery (180±21 versus 177±24 mL/min after L-NAME, P=NS). Similarly, changes in epicardial coronary diameter (Fig 5) to sustained adenosine infusion under control conditions (284±36 μm) were similar to those obtained after inhibiting nitric oxide production (294±67 μm, P=NS). There were no significant effects of intracoronary adenosine on arterial pressure.
Coronary Flow and Diameter During Pulmonary Artery Adenosine Infusion and Intracoronary Adenosine With Restricted Flow

To exclude the possibility that recirculation of adenosine infused into the distal coronary artery could have had direct effects on coronary flow and epicardial coronary artery diameter, we infused adenosine into the pulmonary artery in five animals. During pulmonary artery infusion of adenosine (0.5 mg/min), both coronary flow (45±6 mL/min under control conditions versus 48±7 mL/min during adenosine, P=NS) and epicardial coronary diameter (3110±390 μm under control conditions versus 3120±390 μm after adenosine, P=NS) were similar. Thus, the rapid metabolism of adenosine by both the pulmonary endothelium and red blood cells reduced arterial concentrations below those that could affect coronary flow and epicardial diameter directly.

Diameter changes during distal vasodilation with restricted coronary flow were evaluated in four animals. Resting flow averaged 41±5 mL/min and 41±6 mL/min with restricted coronary flow during distal infusion of adenosine (P=NS). Under these circumstances, epicardial diameter was not significantly different from that during intracoronary adenosine infusion (4260±516 μm under control conditions versus 4205±516 μm after adenosine, P=NS).

Effects of L-NAME on Pacing-Induced Increases in Flow and Coronary Artery Diameter

The effects of sustained pacing-induced increases in flow on epicardial coronary diameter are shown in Fig. 6. There was no effect of L-NAME on resting heart rate (87±4 versus 89±5 beats per minute after L-NAME, P=NS) or coronary flow (42±4 versus 41±4 mL/min after L-NAME, P=NS). During pacing, heart rate (196±5 versus 193±5 beats per minute after L-NAME) and coronary flow (78±7 versus 75±9 mL/min after L-NAME) increased to the same extent. Pacing-induced increases in coronary flow were modest in relation to maximal flows recruited by intracoronary adenosine inf-
fusion and peak ischemic vasodilation during reactive hyperemia. Nevertheless, the change in coronary artery diameter we found to a twofold increase in flow produced by pacing under control conditions approached that found when flow was increased fourfold by intracoronary adenosine (170±27 μm during pacing versus 264±36 μm during adenosine). After L-NAME, the diameter change to a similar increase in heart rate and flow was markedly attenuated, although not completely abolished (54±22 μm, P < .01 versus control, Fig 6).

Discussion

The major new finding of our study is that the dilation of epicardial coronary conduit arteries to sustained increases in mean flow at a constant heart rate is not mediated by endothelial nitric oxide production. Nevertheless, inhibiting nitric oxide attenuated the epicardial coronary dilation to pacing-induced increases in flow. The latter finding suggests that nitric oxide production plays an important role in modulating epicardial conduit artery tone in response to changes in coronary flow pulse frequency.

Methodological Limitations

The interpretation of our results is dependent on demonstrating that we inhibited nitric oxide production. We chose the L-arginine analog L-NAME because previous studies have shown it to be a potent inhibitor of nitric oxide synthase. Inhibition of receptor-mediated nitric oxide production was confirmed by demonstrating that the dose of L-NAME that was used inhibited acetylcholine-induced dilation in the large epicardial artery. In concert with findings in a variety of other preparations, however, L-NAME did not completely abolish the epicardial vasodilation to acetylcholine. This suggests that additional mechanisms, such as endothelium-dependent hyperpolarization of vascular smooth muscle, may also be involved in eliciting relaxation to this endothelium-dependent agonist.

In a previous study, we demonstrated that this dose of L-NAME attenuated the vasodilation of resistance vessels during intracoronary infusion of acetylcholine at concentrations that were on the plateau of the normal dose-response curve (estimated plasma concentrations, \(10^{-5}\) mol/L). In the latter study, the lowest doses of acetylcholine administered into the coronary artery (0.1
effects of adenosine

μg/min corresponding to an estimated coronary acetylcholine plasma concentration of 0.0033 μg/mL, or approximately 2×10⁻⁸ mol/L did not cause significant coronary vasodilation. This is twice as high as the maximum estimated plasma concentration during left atrial infusion that we used in the present study (range, 0.0005 to 0.0015 μg/mL assuming a resting cardiac output of 3 L/min). Thus, we were able to dissociate effects of acetylcholine that were directly related to agonist stimulation from those that were secondary to flow. Although this suggests a differential sensitivity of epicardial conduit arteries to the effects of acetylcholine, the dissociation between diameter changes and flow could also have been due to other factors. First, a reduction in the acetylcholine concentration to which the resistance vessels were subjected could have occurred through metabolism by plasma acetylcholinesterase. Alternatively, autoregulatory resistance adjustments in portions of the microcirculation less responsive to the dilator actions of low concentrations of acetylcholine could have kept flow constant.

Although it is clear that adenosine can dilate the epicardial coronary artery secondary to flow-mediated vasodilation, its direct effects are controversial. Hintze and Vatner ¹⁸ showed that the epicardial dilation to systemic infusion of adenosine was attenuated but not completely abolished by restricting coronary flow. They found that intravenous adenosine increased cross-sectional area of the epicardial artery by 28%. When they restricted flow, this was reduced to 12% but not completely abolished, suggesting a small direct effect of adenosine on the epicardial coronary artery. In contrast, Holtz et al ¹⁹ found that epicardial dilation to adenosine was abolished when flow was restricted to resting levels. We circumvented the potential for direct vasodilatory effects of adenosine by infusing it directly into the coronary artery through an intracoronary catheter that was inserted several centimeters distal to the coronary diameter crystals. When epicardial flow during intracoronary adenosine was restricted to values similar to those at rest, we found no changes in epicardial diameter, like the study of Holtz et al.¹⁹ In addition, epicardial dilation always occurred 10 to 15 seconds after coronary flow increased (Fig 1, bottom), which was the same time delay we found for the epicardial dilation during reactive hyperemia (Fig 1, top). Finally, we also demonstrated that adenosine infused at this rate was metabolized before recirculating to the systemic circulation because pulmonary artery infusion of adenosine did not increase coronary flow or dilate the epicardial coronary artery. These considerations substantiate our conclusion that the epicardial vasodilation we observed was mediated by changes in flow and not secondary to direct effects of adenosine on the epicardial coronary artery.

Contrasting Effects of L-NAME on Epicardial Dilation to Reactive Hyperemia Versus Sustained Increases in Flow

Reactive epicardial coronary dilation after a 30-second coronary occlusion was attenuated by L-NAME to a degree that was similar to the responses previously reported in conscious dogs with L-NMMA by Chu et al.¹⁰ In contrast to their study, however, we found that both peak reactive hyperemic flow and the percentage of flow repayment to flow debt were significantly reduced. When flow was increased by infusing adenosine into the distal coronary circulation, the epicardial dilation to a sustained increase in mean flow was not affected. These findings suggest that the dilation to changes in mean coronary flow was elicited by mechanisms that were not dependent on nitric oxide production. They support the notion that the attenuation of reactive epicardial dilation by L-NAME was most likely secondary to changes in the volume or duration of the reactive hyperemic flow response. Although our interpretation differs from that of Chu et al, their inability to detect significant effects of L-NMMA on reactive hyperemia may have been related to the statistical power of their data, which was limited by a small sample size (n=4). In this regard, it is important to point out that although they did not quantify the percentage of flow repayment to flow debt, they did observe a trend for...
L-NMMA to reduce peak hyperemic flow. We, as well as others, have consistently found that l-arginine analogs attenuate the coronary reactive hyperemic response.15,20,21

In contrast to the lack of effect of L-NAME on flow-mediated vasodilation in epicardial conduit arteries in our study, Kuo et al9 have shown that flow-mediated vasodilation in isolated subepicardial porcine arteries (≈80 μm resting diameter) is abolished by inhibiting nitric oxide production with L-NMMA. Flow-mediated vasodilation produced substantially larger changes in the diameter of resistance arterioles (≈30% of resting diameter) than we found in epicardial conduit arteries (≈5% to 10% of resting diameter). In addition, like the more rapid time course of the coronary reactive hyperemic flow response, flow-mediated changes in arteriolar caliber appeared to reach steady state much more rapidly (10 to 30 seconds, see Fig 4, Reference 8) than flow-mediated vasodilation of epicardial conduit arteries, which peaked after 60 seconds and lasted 5 to 10 minutes (Fig 1, top). A number of studies have demonstrated differential responses of coronary conduit and resistance vessels to pharmacological stimuli such as thrombin, vasopressin, and nitrates.22,23 The difference in transient and steady-state diameter responses to increased flow in arteriolar and epicardial arteries suggests that the mechanisms responsible for flow-mediated vasodilation in each class of vessel are also different, with nitric oxide–dependent mechanisms predominating in coronary resistance vessels.

A limited number of in vivo studies have examined the effects of sustained increases in mean flow on the diameter of conduit arteries in other vascular beds in acutely instrumented anesthetized animals. Kaiser et al24 found that the femoral artery dilated to increased flow and that this dilation could be attenuated by applying topical methylene blue. This can inactivate guanylate cyclase as well as bind nitric oxide. The former mechanism appears to be the most important because they could not reverse the inhibition of methylene blue by suffusing the vessel with buffer. Although their results are compatible with the notion that changes in steady flow dilated the femoral artery by increasing cyclic GMP (cGMP) (presumably through increased nitric oxide production), the flow variations were produced by varying the speed of a roller pump. As a result, effects of pulse frequency could not be excluded and were also accentuated as the speed and flow rate through the pump were increased. In the cerebral circulation, Fuji et al25 increased basilar artery flow using unilateral and bilateral carotid occlusion in anesthetized rats. They found that basilar artery dilation (resting diameter, ≈250 μm) to sustained increases in flow was not affected by topical application of a variety of antagonists, including L-NMMA in a preparation in which changes in pulse frequency appear to have been minimal. Our results with L-NAME are similar to the findings of Fuji et al and indicate that changes in coronary diameter as steady flow is varied at a constant heart rate (ie, constant pulse frequency) are also not dependent on nitric oxide production in the coronary circulation of unanesthetized dogs.

Attention of Flow-Mediated Vasodilation to Changes in Pulse Frequency

A number of in vitro studies performed in isolated conduit vessels from other vascular beds have demonstrated that one or more EDRFs are released in response to changes in pulsatility as well as mean flow. Pohl et al26 demonstrated that pulsatile perfusion enhanced the release of prostacyclin in isolated, perfused vessels. Inhibition of EDRF by hemoglobin and dithiothreitol attenuated the associated vasodilation. The effects of inhibiting prostaglandin synthesis was not studied. Rubanyi et al26 used a bioassay to demonstrate that excised femoral arteries released a transferable substance that relaxed isolated coronary artery rings. Although coronary vascular smooth muscle relaxed to a twofold increase in mean flow, the relaxation to an increase in pulse frequency at constant mean flow was much more pronounced. As in the study of Pohl et al, increases in pulse frequency and mean flow increased prostacyclin release. Indomethacin reduced the release of prostacyclin, but it did not change the degree to which coronary vascular smooth muscle relaxed, indicating that the vasodilation was not mediated by cyclooxygenase products. More recently, LaMontagne et al27 compared the effects of steady flow and pulsatility on the release of cGMP (an indirect assay of nitric oxide release). While the effects of inhibiting nitric oxide production were not studied, they found that external compression of isolated femoral arteries produced a much more pronounced increase in effluent cGMP than did approximately eightfold changes in wall shear stress produced by constricting the vessel with endothelin. Hutcheson and Griffith28 demonstrated frequency-dependent vasodilation in isolated perfused aortas in vitro. The relation between pulse frequency and relaxation of a donor ring under control conditions was nonlinear and reached a peak at 4 Hz. Frequency-dependent vasodilation was completely abolished by inhibiting nitric oxide production with L-NAME. Responses to variations in steady flow were not examined. The in vivo significance of all of these studies is complicated by the fact that none of these preparations were blood perfused. It is conceivable that they could release much larger concentrations of nitric oxide into the effluent because it cannot bind to hemoglobin. Furthermore, most experimental preparations were perfused at flow rates and/or pressures that were substantially lower than those found under normal conditions in vivo. Thus, the effects of changes in flow, shear, or pulsatility on arterial dilation of a recipient bioassay may be different than responses that occur in intact animals.

In concert with these in vitro studies, we also found that changes in pulse frequency had an effect on large coronary artery tone in conscious dogs that was dependent on nitric oxide production. Macho et al29 previously found that pacing-induced increases in flow produced vasodilation of the large epicardial artery but did not dissociate the role of mean flow and pulse frequency on the responses. In a subsequent study, Hintze and Vatner2 showed that pacing-induced increases in coronary artery diameter continued to occur when mean flow was kept constant by inflating a coronary occluder, indirectly suggesting that pulse frequency affected epicardial tone in the absence of a change in mean flow. Our results also demonstrate that pulse frequency is an important determinant of epicardial tone in conscious dogs. Pacing caused modest but comparable increases in flow before and after L-NAME. Under control conditions, we found that epicardial diameter increased by 170 μM in response to a ≈35 mL/min increase in mean
flow and twofold increase in pulse frequency. The changes in diameter from control values that we observed for pacing-induced dilation and distal intracoronary adenosine infusion are plotted versus changes in mean flow in Fig 7. Like the results from in vitro studies,26-28 the degree of epicardial artery dilation to pacing-induced changes in pulse frequency under control conditions was disproportionately large in relation to the change in mean flow. Holtz et al12 have suggested that relations between flow and diameter are linear in the coronary circulation. Thus, the accentuated increase in diameter after pacing-induced increases in flow may be secondary to the additional effects of pulse frequency on conduit artery tone. After inhibiting nitric oxide production, the diameter increase during pacing was attenuated (54 μm) and more proportional to the relative changes in mean flow from baseline (Fig 7, hatched circles).

There are several alternative explanations for the disparity in the effects of inhibiting nitric oxide production on epicardial artery dilation to pacing and intracoronary infusion of adenosine. Although previous studies have suggested that the coronary flow–diameter relation is linear, the effects of inhibiting nitric oxide production on the relation are unknown. Conceivably, high levels of flow may be able to overcome the blockade of nitric oxide production produced by L-NAME in a dose-dependent fashion, whereas lower levels of mean flow, such as those encountered during pacing, cannot. Flow-diameter relations performed over a wide range of flow will be required to address this question. Another possibility is that adenosine dilates the epicardial artery through propagated responses from distal to more proximal vessels. Making this less likely, however, is the finding that conduit artery vasodilation continues to occur normally after transecting vessels from the distal vascular bed,30 as well as the lack of an effect of adenosine on coronary resistance vessels >150 μm from in vivo microcirculatory studies.31 Finally, although we have interpreted the inhibition of epicardial dilation during pacing to indicate a coupling of nitric oxide production to pulse frequency, other components of the pulsatile waveform, including pulse amplitude and the rate of change in flow, are altered when mean coronary flow is varied in vivo. Perfusion of isolated coronary conduit vessels with more controlled flow and pressure waveforms will be required to elucidate whether any of these factors are involved in eliciting flow-mediated vasodilation.

While our results indicate that nitric oxide production is not required for flow-mediated vasodilation of epicardial conduit arteries, we did not define the other mediators that may be responsible for these responses. They are probably produced by the endothelium in light of previous in vivo studies that demonstrate that flow-mediated effects are abolished by denuding the endothelium in vitro25 as well as in epicardial arteries in conscious dogs in vivo.6 Although prostaglandins may affect basal tone, previous studies have shown that they are unlikely to be involved in epicardial artery dilation to changes in flow.3 In addition, all of the animals in our study chronically received aspirin to maintain patency of the catheters. Several laboratories have demonstrated that a soluble hyperpolarizing factor (endothelium-derived hyperpolarizing factor) is released in response to a variety of endothelium-dependent agonists.32,33 While this factor has not been identified, it could explain the persistent epicardial artery dilation we observed to sustained increases in mean coronary flow as well as the residual vasodilation we observed to acetylcholine after inhibiting nitric oxide production. Another possibility is that epicardial vasodilation to sustained changes in mean flow is mediated by direct hyperpolarization of the endothelial cell by luminal shear stress.34 This mechanism has been demonstrated in cultured endothelial cells, but its importance in vivo remains to be defined.

In summary, the role of nitric oxide in mediating epicardial conduit artery dilation to changes in flow is complex and appears to differ from that observed in coronary resistance vessels. Vasodilation of large epicardial arteries to sustained as opposed to transient changes in flow is not dependent on nitric oxide production. Nevertheless, dilation to changes in pulse frequency appears to be mediated by nitric oxide because it is inhibited by the arginine analog L-NAME. Under some circumstances, the epicardial artery dilation to changes in pulse frequency appears to be quantitatively similar to that resulting from large increases in mean flow. This may have particular significance in the coronary circulation due to the systolic-diastolic variations in epicardial flow. Based on our findings, future studies designed to determine the mechanisms responsible for flow-mediated vasodilation will need to carefully control and characterize mean flow as well as flow pulse frequency.

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