Shear stress-induced vasodilation in porcine coronary conduit arteries is independent of nitric oxide release

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Shear stress-induced vasodilation in porcine coronary conduit arteries is independent of nitric oxide release. Am J Physiol Heart Circ Physiol 280: H2581–H2590, 2001.—The present study was performed to determine the importance of nitric oxide in eliciting epicardial coronary artery dilation during sustained increases in shear stress in the absence of pulsatile flow. Isolated first-order porcine epicardial coronary conduit arteries (~500 μm) were preconstricted (U-46619) and subjected to steady-state changes in flow in vitro. Nonpulsatile flow (shear stress range from 0 to ~100 dyn/cm²) produced a graded dilation of epicardial arteries. Inhibiting nitric oxide synthase with 10⁻⁵ M Nω-nitro-L-arginine methyl ester (l-NAME) blocked bradykinin-induced vasodilation but did not affect the flow-diameter relation or the maximum change in diameter from static conditions (67 ± 10 μm in control vs. 71 ± 8 μm after l-NAME, P = not significant). The addition of indomethacin (10⁻⁵ M) had no effect on flow-mediated vasodilation. Depolarizing vascular smooth muscle with KCl (60 mM) or removing the endothelium blocked bradykinin vasodilation and completely abolished flow-mediated responses. The K⁺ channel blocker tetraethylammonium chloride (TEA; 10⁻⁴M) attenuated flow-mediated vasodilation (maximum diameter change was 110 ± 18 μm under control conditions vs. 58 ± 10 μm after TEA, P < 0.001). These data indicate that epicardial coronary arteries dilate to steadystate changes in nonpulsatile flow via a mechanism that is independent of nitric oxide production. The ability to completely block this with KCl and attenuate it with TEA supports the hypothesis that epicardial coronary arteries dilate to steady levels of shear stress through hyperpolarization of vascular smooth muscle. This may be secondary to the release of an endothelium-dependent hyperpolarizing factor.

endothelium-dependent hyperpolarizing factor; endothelium-dependent relaxing factor; steady flow; pulsatile flow transduction mechanisms. In support of the latter possibility, Canty and Schwartz (1) found evidence that the release of nitric oxide (NO) appears to importantly modulate coronary diameter after interventions that alter the pulsatile characteristics of flow (e.g., reactive hyperemia after coronary occlusion and pacing), whereas it does not appear to mediate epicardial vasodilation to steady-state increases in mean flow when heart rate is kept constant. The role of steady versus pulsatile flow mechanisms may vary among species as a function of vessel size within an organ or perhaps also with the in vivo pulsatile characteristics of flow to which the endothelial cells are chronically subjected. In this regard, the endothelial cells in conduit arteries of the coronary circulation may be unique because they are subjected to large cyclical variations in shear stress with flow approaching zero during systole in each cardiac cycle.

Addressing the role of steady versus pulsatile flow in vivo is complicated because it is not possible to completely dissociate mean and pulsatile flow experimentally. We therefore turned to an in vitro preparation where the effects of graded steady-state changes in mean shear stress in eliciting flow-mediated vasodilation could be studied with the use of isolated epicardial coronary arteries. Porcine conduit arteries were perfused with a physiological salt solution (PSS), and shear stress was varied over the physiological range encountered in vivo. Pulsatile flow was eliminated by controlling perfusion with the use of a reservoir apparatus, which had the advantage of keeping distending pressure within the vessel constant by using matched micropipettes on the inflow and outflow end of the vessel, and video microscopy was used to assess external diameter. The results indicate that blocking NO synthase or cyclooxygenase has no affect on epicardial artery dilation to changes in mean shear stress. Vasodilation was completely abolished by removing the endothelium or depolarizing the vascular smooth muscle with KCl, and it was attenuated by administering the K⁺ channel antagonist tetraethylammonium chloride (TEA). These results support the hypothesis that...
the effects of steady flow are mediated by an endothelium-dependent relaxing factor that causes vascular smooth muscle hyperpolarization.

METHODS

All experiments were conducted in Yorkshire pigs of either sex (nominal weight 10 kg, \( n = 42 \)) and performed in accordance with institutional guidelines for the care and use of animals. One coronary conduit vessel was isolated from each heart. Pigs were sedated with a Telazol (50 mg/ml tiletamine-50 mg/ml zolazepam)-100 mg/ml xylazine mixture (0.037 ml/kg im). They were subsequently anesthetized with pentobarbital sodium (15 mg/kg) given through an ear vein, intubated, and ventilated with room air. We exposed the heart through a median sternotomy. After the animals were heparinized (5,000 units administered via the left atrium), the heart was electrically fibrillated, rapidly excised, and immediately placed into PSS, which was buffered to a pH of 7.40 at 4°C. The PSS solution contained (in mM) 141.9 NaCl, 4.7 KCl, 2.79 CaCl\(_2\), 1.71 MgSO\(_4\), 1.18 KH\(_2\)PO\(_4\), 5.0 dextrose, 1.0 pyruvate, 0.51 EDTA, and 10.0 HEPES. All PSS solutions contained 0.1% bovine serum albumin, which was felt to stabilize the endothelium during vessel isolation (3). The techniques used to isolate coronary vessels are similar to those previously described (11, 18). Briefly, first-order branches of the epicardial coronary conduit arteries were visualized by perfusing them with 0.3 ml of an india ink-gelatin-PSS mixture. This was prepared by adding 0.2 ml of india ink (Koh-I-Noor; Bloomsbury, NJ) and 0.36 g of porcine skin gelatin (Sigma; St. Louis, MO) to 10 ml of warm PSS as previously described (11). Because the heart was cooled to 4°C during this time, the india ink-gelatin-PSS mixture solidified and delineated the arterial vasculature. Previous studies (10, 12) have demonstrated that this isolation technique does not interfere with endothelial function.

A portion of the myocardium was then transferred to a silicone-lined ice-cooled dissection chamber containing filamented PSS-albumin buffered to a pH of 7.4 at 4°C. First-order branches of epicardial arteries having a nominal diameter of \( \sim 500 \) \( \mu \)m were dissected free from adventitial tissue. The vessels were transferred to a perfusion chamber (Living Systems; Burlington, VT) containing PSS-albumin at room temperature. One end of the vessel was cannulated with a glass pipette (nominal external tip diameter 300 \( \mu \)m) and secured with an 11-0 ophthalmic suture. The india ink column was gently flushed out at a low pressure (<10 mmHg), after which the opposite end of the vessel was cannulated and secured to the outflow pipette. The separation between the pipettes was adjusted to stretch the vessel at its in situ length. The vessel chamber was then carefully transferred to the stage of an inverted microscope (Nikon TMS/TMS-F; Tokyo, Japan) that was coupled to a video camera (Panasonic WV-1410; Secaucus, NJ). The output of the camera was interfaced to a video dimension analyzer (Living Systems). Coronary diameter was continuously monitored with the use of video microscopic techniques as previously described (5, 18). We assessed the external coronary artery diameter because epicardial arteries of this size could not consistently be transilluminated because of their wall thickness.

After the artery was cannulated, the outflow end of the chamber was closed, and the distending pressure was slowly increased to 50 mmHg over 3–5 min. Flow-through pressure transducers (Living Systems) were placed proximal to the inflow and distal to the outflow pipettes to measure the pressure gradient across each vessel. This was used as a relative index of flow. Vessels were leak tested and excluded from study if they were unable to keep a constant pressure under static conditions. The vessel was then gradually warmed to a temperature of 37°C. All perfusion tubing as well as the vessel bath was housed in an environmental chamber that was heated to 37°C for accurate temperature control of the circulating bath as well as the inflow perfusate.

After the vessel stabilized for 1 h, it was preconstricted with the thromboxane analog U-46619 (10\(^{-6}\)–10\(^{-8}\) M, depending on the protocol) administered in the bath water. Variations in steady flow were produced using a dual-reservoir system mounted on a pulley that has been previously reported by others (12). We matched the hydraulic resistances of the inflow and outflow of pipettes by determining their pressure-flow relations before cannulation with the use of known flow rates provided by a calibrated infusion pump (model 44, Harvard Apparatus). Steady-state changes in flow were produced by moving the inflow and outflow reservoirs in equal but opposite directions so that the pressure drop, which was halved to intraluminal flow, increased while the distending pressure within the coronary vessel remained constant at 50 mmHg. Flow was directly measured from timed outflow collection. Approximately 5–10 min were allowed for the diameter to equilibrate after each pressure step to ensure that a steady state had been reached. At the end of a series of pressure steps, flow was stopped by bringing the two reservoirs back to the same level. Endothelium-dependent dilation to pharmacological stimuli was assessed by applying single doses of bradykinin at selected concentrations (10\(^{-9}\)–10\(^{-7}\) M) in the extraluminal bath. At the end of each study, passive diameter was assessed using sodium nitroprusside (10\(^{-4}\) M).

**Experimental Protocols**

**Group 1:** effect of \( N^\text{G}-\text{nitro-L-arginine methyl ester} \) and U-46619 on flow-mediated epicardial coronary dilation (\( n = 10 \)). In the first group of experiments, we examined the effects of inhibiting NO synthase on flow-diameter relations. Control relations were obtained after vessels were preconstricted with 10\(^{-8}\) M U-46619 and repeated after a lower initial diameter was produced with 10\(^{-8}\) M U-46619. Flow-diameter relations were then obtained after equilibrating vessels for 20 min in the presence of \( N^\text{G}-\text{nitro-L-arginine methyl ester} \) (1-NAME; 10\(^{-5}\) M) at each of the previous two concentrations of U-46619.

**Group 2:** effect of KCl, \( N^\text{G}\)-monomethyl-L-arginine, and endothelial denudation on flow-mediated vasodilation (\( n = 10 \)). In a second series of experiments, we examined the effects of depolarization on the flow-diameter relation. Control flow-diameter relations were obtained after preconstricting the vessels with 10\(^{-7}\) M U-46619. This was washed out, the buffer was changed to one in which KCl (60 mM) replaced an equimolar amount of NaCl, and the flow-diameter relations were repeated. This KCl concentration has been shown to depolarize vascular smooth muscle and block hyperpolarization in epicardial coronary artery rings studied in vitro (16). Preconstriction with U-46619 was not required during this intervention because the high-K\(^+\) buffer constricted the vessels by \( \sim 70\% \) of their passive diameter. Subsequently, the blood vessels were washed with PSS and incubated with 10\(^{-5}\) M \( N^\text{G}\)-monomethyl-L-arginine \( \text{(L-NMMA)} \) for 20 min to block NO synthase, and the flow-diameter relations were repeated. Finally, the endothelium was removed by uncoupling the vessel from the outflow pipette and injecting air (5 ml) alternating with saline (5 ml) into the inflow pipette five times. The vessel was reconnected and equilibrated in PSS. The integrity of the vascular smooth muscle was confirmed by...
showing that U-46619 continued to constrict the vessel to the same extent as under control conditions. The completeness of endothelium removal was confirmed by showing that the response to 10^{-7} M bradykinin was abolished. If a residual vasodilation to bradykinin was present, the denudation procedure was repeated as required. Once denudation was confirmed, the flow-diameter relation was repeated.

Group 3: effect of intraluminal L-NAME, indomethacin, and TEA on flow-mediated vasodilation of epicardial coronary arteries (n = 13). In a third series of experiments, control flow-diameter relations were performed after vessels were preconstricted with 10^{-7} M U-46619. The vessels were then incubated with intraluminal L-NAME (10^{-5} M) and indomethacin (10^{-5} M) to evaluate the effect of cyclooxygenase inhibition on the flow-diameter relation. Finally, intraluminal TEA (10^{-4} M) was added to study the effects of K^+ channel blockade on epicardial conduit artery flow-mediated vasodilation.

Experiments Demonstrating Efficacy of NO Synthase Inhibition

To minimize the time period over which flow data were evaluated, the efficacy of the various interventions on endothelium-dependent vasodilation was evaluated with single doses of bradykinin in each vessel. In addition, we evaluated the effect of L-NAME, indomethacin, and TEA over a bradykinin concentration range of 10^{-10}-10^{-7} M in pressurized conduit arteries in group 3 animals.

We also performed an additional series of experiments to demonstrate the efficacy of NO synthase inhibition by 10^{-6} M L-NAME in the absence of indomethacin in conduit artery rings. Pairs of epicardial porcine conduit rings (n = 9 pigs) were isolated, connected to force transducers, and suspended in organ baths (HEPES buffer) heated to 37°C. The initial tension was gradually increased to a nominal value of 4 g, after which the vessels were preconstricted (10^{-7} M endothelin). Bradykinin dose-response curves were obtained at log concentrations between 10^{-11} and 10^{-7} M under control conditions and in the presence of 10^{-5} M L-NAME.

Drugs and Chemicals

Bradykinin, sodium nitroprusside, L-NAME, L-NMMA, TEA, indomethacin, U-46619, and HEPES were all obtained from Sigma. Porcine gelatin was also obtained from Sigma, and bovine serum albumin was obtained from either US Biochemical (Cleveland, OH) or from Sigma.

Data Analysis

All data were recorded on a Gould 2800 W recorder. Vessel diameters were normalized to the passive diameter obtained for each vessel in the presence of sodium nitroprusside. Normalized diameter and the percent dilation were calculated as follows: normalized diameter = \( \frac{D_{NP} - D}{D_{NP}} \), where D is the diameter at any given level of flow, \( D_{NP} \) is the initial diameter under static conditions (zero flow or in the absence of drugs), and \( D_{NP} \) is the passive diameter obtained during 10^{-4} M nitroprusside treatment. Percent relaxation after bradykinin was calculated by taking the difference in force or diameter after bradykinin compared with initial values and dividing it by the difference in force or diameter after sodium nitroprusside treatment compared with initial values. We estimated shear stress by assuming fully developed laminar flow in each vessel for a Newtonian fluid with the use of the following equation: \( \sigma = 4\eta Q/r^3 \), where \( \sigma \) is the shear stress, \( \eta \) is the viscosity (assumed to be 1 poise or equal to that of water), \( Q \) is the flow for each flow step, and \( r \) is the internal radius corresponding to each flow level under steady-state conditions. Because our preparation was only able to consistently measure external diameter, the internal radius that was used to calculate shear stress was estimated. Morphometric studies have found wall thickness-to-inner lumen ratios of 0.04 in normal diagonal branches of the left anterior descending coronary artery (21). Because wall thickness increases with tone, we assumed that internal diameter averaged 90% of the measured external diameter.

Statistics

Statistical comparisons within experimental groups and after different interventions were made using a repeated measures ANOVA for diameter at each level of pressure gradient studied. Paired (two tailed) t-tests were performed to detect differences between pharmacological responses under various conditions and were also used to compare differences in coronary diameters at each of the flow steps compared with the initial diameter. The Bonferroni correction was used to correct for multiple comparisons when appropriate.

RESULTS

A total of 41 vessels were successfully cannulated. Of these, five vessels were felt to be nonreactive because they did not contract after administration of the thromboxane analog U-46619 and were excluded from study. One vessel did not dilate to bradykinin under control conditions and was thought to be denuded during the isolation procedure. Two vessels became damaged during the denudation procedure and were excluded from analysis.

Efficacy of L-NAME in Inhibiting NO Synthase

Figure 1A summarizes the effects of 10^{-5} M L-NAME on the bradykinin dose-response curves. The vasodilation to bradykinin in epicardial porcine coronary artery rings was markedly attenuated after inhibiting NO synthase with L-NAME. Likewise, when the dose-response relation was evaluated in pressurized conduit arteries in group 3 animals (Fig. 1B), inhibiting NO synthase in the presence of indomethacin produced an ~1 log shift in the dose-response curve. The addition of TEA further reduced relaxation compared with L-NAME and indomethacin alone. Taken together, these data demonstrate that the dose of L-NAME that we employed in our studies significantly attenuated conduit artery dilation to pharmacological stimuli. In the majority of perfused vessel studies, only selected doses of bradykinin were used to confirm efficacy of NO synthase inhibition and minimize the time period that was required to examine the flow-diameter response during interventions.

Effect of Varying Initial Level of Tone and Blocking NO synthase with L-NAME on Flow-Diameter Relations (Group 1, n = 10)

Figure 2 summarizes the effects of L-NAME on the epicardial coronary flow-diameter relationship when
the initial diameters were varied with $10^{-8}$ and $10^{-6}$ M U-46619. Table 1 summarizes absolute measurements of diameter under static conditions and at the maximum pressure gradient studied along with the corresponding measurements of flow and shear stress at the two doses of U-46619 that were used to vary the initial level of tone. The passive sodium nitroprusside diameter in this group of vessels was 527 $\pm$ 25 $\mu$m. Inhibiting NO synthase with $10^{-5}$ M L-NAME reduced the relaxation to $10^{-5}$ M bradykinin (58.7 $\pm$ 10.3% in control vs. 18.6 $\pm$ 10.8% after L-NAME, $P < 0.01$). Initial diameters after L-NAME were similar to control conditions after $10^{-8}$ M U-46619 but were significantly lower than control after $10^{-6}$ M U-46619. Percent dilation and the flow-diameter relations under control conditions and after L-NAME were similar (Table 1). Even though the initial diameter under static conditions decreased from 452 $\pm$ 22 $\mu$m after $10^{-8}$ M U-46619 to 374 $\pm$ 21 $\mu$m after $10^{-6}$ M U-46619 with L-NAME ($P < 0.001$), the maximum change in diameter produced by a given change in flow did not vary significantly among interventions in the same vessel (Table 1). Because the change in diameter from baseline remained constant, the absolute maximum diameter that was achieved (gradient 50 mmHg, flow $\sim$4 ml/min) decreased with reductions in the initial diameter. The dilation to submaximal shear stress under control conditions was slightly reduced in vessels preconstricted with $10^{-6}$ M U-46619 versus that in vessels preconstricted with $10^{-8}$ M U-46619, but the difference did not reach significance. Because of potential variations in the flow-diameter responses at low levels of shear stress with different doses of U-46619, we did not attempt to match initial coronary artery diameter with variable concentrations of U-46619 in the subsequent protocols.

Effect of KCl and Endothelial Denudation on Flow-Diameter Relations (Group 2, $n = 10$)

Table 2 summarizes initial and maximal measurements of coronary artery diameter along with corresponding measurements of flow and shear stress for vessels studied after L-NMMA and KCl treatment and after removal of the endothelium. Figure 3 summarizes the flow-diameter relations after inhibiting NO synthase with L-NMMA. Pharmacological vasodilation to $10^{-9}$ M bradykinin was attenuated by L-NMMA (27.7 $\pm$ 11.9 vs. $-0.5 \pm 1.9\%$, $P < 0.05$). Diameters were lower after L-NMMA than under control conditions ($P < 0.05$, ANOVA), and there was a downward shift in the flow-diameter relation. Despite this, the percent dilation and change in diameter for a given level of flow after L-NMMA were the same as those under control conditions [maximum change 46 $\pm$ 11 $\mu$m under control conditions and 52 $\pm$ 10 $\mu$m after L-NMMA, $P = \text{not significant (NS)}$]. Thus the lack of effect of L-NMMA on flow-mediated vasodilation was similar to the result with L-NAME.

Figure 3 also summarizes the effect of 60 mM KCl and removal of the endothelium on the flow-diameter relations. Measurements under static conditions and at the maximal flow studied are summarized in Table 2. Depolarizing the vessel with KCl attenuated $10^{-7}$ M bradykinin-induced vasodilation (from 103 $\pm$ 14.6 to 42.8 $\pm$ 10.1%, $P < 0.001$) and completely blocked flow-mediated vasodilation (maximum flow-induced diameter 373 $\pm$ 22 $\mu$m vs. 363 $\pm$ 18 $\mu$m under static conditions, $P = \text{NS}$). After endothelial denudation, the vasodilation to $10^{-7}$ M bradykinin was completely abolished, and there was no significant change in coronary diameter with flow (365 $\pm$ 21 $\mu$m under static conditions vs. 371 $\pm$ 25 $\mu$m under maximum flow, $P =$
NS). Thus flow-mediated vasodilation was completely abolished by removing the endothelium as well as by depolarizing the vessel with KCl.

**Effect of Intraluminal L-NAME, Indomethacin, and $K^+$ Channel Blockade on Flow-Diameter Relations (Group 3, n = 13)**

Figure 4 summarizes the effect of administering inhibitors of flow-mediated vasodilation intraluminally. Table 3 summarizes initial and maximum diameters along with flow and calculated shear stress. Administering both L-NAME ($10^{-5}$ M) and indomethacin ($10^{-5}$ M) intraluminally attenuated the vasodilation to $10^{-8}$ M bradykinin (99.5 ± 1.8 vs. 53.5 ± 10.6%, *P* < 0.01) but did not alter the flow-diameter relation. Initial diameters were not significantly different from control (Table 3). With the addition of intraluminal TEA ($10^{-4}$ M), the dilation to bradykinin was further attenuated (27.6 ± 8.3%, *P* < 0.01 vs. L-NAME + indomethacin). As illustrated in Fig. 4, changes in diameter after increases in flow were partially but not completely attenuated by TEA. In this series of experiments, the maximum change in diameter decreased from 110 ± 18 μm under control conditions to 58 ± 10 μm after adding TEA (*P* < 0.01). These data indicate that intraluminal application of L-NAME and indomethacin blocks bradykinin-induced vasodilation but has no effect on the flow-diameter relationship. The partial attenuation of flow-mediated vasodilation after TEA supports the possibility that $K^+$ channels are involved in epicardial artery flow-mediated vasodilation in pigs.

**Relation Between Shear Stress Level and Diameter in Coronary Conduit Arteries**

Figure 5 summarizes the relation between shear stress and diameter from all of the vessels studied under control conditions. These are compared with results after NO synthase inhibition in the three groups. Results have been pooled because there were no differences between control responses and NO synthase inhibition in any of the groups. We found that the shapes of the flow-diameter relations were similar under control conditions and after blocking NO synthase. Specifically, low levels of shear stress appeared to affect maximal flow-induced changes in diameter (Table 1). At low levels of shear stress, however, the dilation after $10^{-6}$ M U-46619 was attenuated. This produced a more linear relation over the entire range of shear stress studied. Values are means ± SE; *n* = 10 arteries. *D*$_0$, diameter at any given level of flow; *D*$_D$, initial diameter under static conditions.

**Table 1. Effects of L-NAME and preconstriction on the flow-diameter relation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Static Conditions, μm</th>
<th>Maximum Flow Gradient, μm</th>
<th>ΔD, μm</th>
<th>Flow, ml/min</th>
<th>Shear Stress, dyn/cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$U$-46619 ($10^{-8}$ M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>452 ± 22</td>
<td>519 ± 24$^+$</td>
<td>67 ± 10</td>
<td>4.24 ± 0.18</td>
<td>68.2 ± 9.3</td>
</tr>
<tr>
<td>L-NAME</td>
<td>439 ± 23</td>
<td>511 ± 23$^+$</td>
<td>71 ± 8</td>
<td>4.20 ± 0.19</td>
<td>69.7 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>$U$-46619 ($10^{-6}$ M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>410 ± 23</td>
<td>493 ± 18$^+$</td>
<td>83 ± 14</td>
<td>4.24 ± 0.17</td>
<td>76.0 ± 8.0</td>
</tr>
<tr>
<td>L-NAME</td>
<td>374 ± 22$^+$</td>
<td>445 ± 23$^{*+}$</td>
<td>71 ± 11</td>
<td>3.91 ± 0.21</td>
<td>98.3 ± 11.4$^+$</td>
</tr>
</tbody>
</table>

Values are means ± SE; *n* = 10 porcine arteries. L-NAME, Nω-nitro-L-arginine methyl ester; ΔD, change in diameter. Normalized diameter was found with the use of a passive diameter obtained with $10^{-4}$ M nitroprusside (D$_{NP}$) of 527 ± 25 μm. *P* < 0.05 vs. corresponding control response; †$P$ < 0.001 vs. static conditions.
result in greater changes in diameter than higher shear stresses. Approximately one-half of the dilation occurred when shear stress was increased from static conditions to a nominal value of 8 dyn/cm². This value approximates the resting level of shear stress in the epicardial coronary artery. The maximum dilation reached with flow was always less than the maximum passive dilation after 10⁻⁴ M sodium nitroprusside. Thus regardless of whether absolute diameters or indexes of relaxation were used, inhibiting NO synthase had no affect on the vasodilation to increased shear stress.

**DISCUSSION**

There are a number of important new findings from our study. Epicardial porcine conduit arteries dilate in response to increases in shear stress under steady-flow conditions in the absence of the normal systolic-diastolic variations in coronary flow encountered in vivo. Whereas the vasodilation to mean shear stress was dependent on the endothelium, it was not affected by blocking NO synthase with the L-arginine analogs L-NMMA or L-NAME, in contrast to observations in porcine coronary arterioles, where the response to shear stress was completely abolished (12). This does not appear to be species dependent because epicardial coronary arteries also continued to dilate to changes in mean flow after blocking NO synthase in conscious chronically instrumented dogs (1, 20). Finally, although inhibiting cyclooxygenase did not affect flow-mediated vasodilation during nonpulsatile flow, it could be completely blocked by depolarizing the vessel with KCl and markedly attenuated by TEA. The latter findings support the hypothesis that nonpulsatile shear stress induces the release of an endothelium-dependent hyperpolarizing factor that may be a K⁺ channel opener.

**Relation to Previous Studies**

In contrast to other vascular beds, the coronary circulation is unique in that there is a marked variation in flow throughout each cardiac cycle, which is a manifestation of the compressive effects generated from left ventricular pressure development impeding systolic perfusion. This results in pronounced systolic-diastolic variations in pulsatile epicardial artery flow that are not present in other vascular beds and may provide independent physical stimuli (e.g., rates of change in shear) that are transduced by the endothelium (14, 19).

In support of this, Canty and Schwartz (1) previously demonstrated that inhibiting NO synthase in vivo blocked the flow-mediated increase in epicardial coronary artery diameter produced by increasing pulsatile flow by pacing and reactive hyperemia in conscious dogs. In the same study, however, changes in diameter produced by increasing coronary flow to a constant mean level were not affected by L-NAME. One possible explanation for this finding is that high levels of flow or shear overcome the inhibitory effects of L-arginine analogs on NO synthase. In a subsequent in vivo study, Smith et al. (20) demonstrated that this was not related to a dose dependency in the relation between shear stress and diameter. Inhibiting NO synthase reduced baseline diameter, but increases in mean flow above resting values elicited the same change in epicardial artery diameter before and after L-NAME. The present findings in pigs extend the in vivo observations in conscious dogs in two respects. First, they indicate that they can be generalized to the epicardial coronary arteries of another species and are not unique to dogs. Second, the NO-independent epicardial artery dilation occurs in vitro during steady-flow conditions when all of the cyclical variations in coronary flow are absent.

We found that L-arginine analogs did not affect the flow-diameter relation at low or high levels of shear stress. In contrast, steady-state flow-diameter relations in vivo demonstrated that there was a component of dilation at low levels of shear stress (i.e., below the resting flow level) that was NO dependent and attenuated by L-NAME (20). The most likely reason for this difference is that the pulsatile changes in coronary flow vary markedly as flow is reduced below resting values in vivo. As the coronary artery is progressively narrowed by a stenosis, the flow pattern becomes nonpulsatile. This reduction in pulsatile flow could attenuate NO release from epicardial arteries in a fashion similar to that of coronary resistance arteries (19). In the present study, flow was nonpulsatile at all levels of shear stress, and thus the component of flow-mediated dilation from NO was absent. Thus the epicardial artery flow-diameter relation in vivo is the net result of dilation from changes in mean flow that are independent of NO as well as of pulsatile flow changes that are NO dependent (1). Due to this complex interplay between two distinct mechanisms, in vivo studies need to examine flow-mediated vasodilation under circumstances where the mean flow level as well as pulsatile components (flow pulse frequency and amplitude) are

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**Table 2. Effects of KCl and endothelium removal on the flow-diameter relation**

<table>
<thead>
<tr>
<th>Static Conditions, μm</th>
<th>Maximum Flow Gradient, μm</th>
<th>ΔD, μm</th>
<th>Flow, ml/min</th>
<th>Shear Stress, dyn/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>419 ± 27</td>
<td>465 ± 26†</td>
<td>46 ± 11</td>
<td>4.26 ± 0.36</td>
</tr>
<tr>
<td>L-NMMA (10⁻⁵ M)</td>
<td>397 ± 23*</td>
<td>449 ± 25†</td>
<td>52 ± 10</td>
<td>4.24 ± 0.33</td>
</tr>
<tr>
<td>KCl (60 mM)</td>
<td>363 ± 18*</td>
<td>373 ± 22*</td>
<td>10 ± 9*</td>
<td>3.32 ± 0.41*</td>
</tr>
<tr>
<td>Endothelium denuded</td>
<td>365 ± 21*</td>
<td>371 ± 25*</td>
<td>6 ± 6*</td>
<td>2.84 ± 0.39*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 porcine arteries. L-NMMA, N⁷⁻-monomethyl-L-arginine. Normalized diameter was found with the use of a DNP of 516 ± 25 μm. *P < 0.05 vs. control; †P < 0.01 vs. static conditions.
Mechanism of Vasodilation to Steady Flow

Our results in porcine conduit arteries contrast with those of a previous study by Kuo et al. (12), who used a similar methodology to study isolated porcine resistance arteries. They found that resistance arteries subjected to steady nonpulsatile flow dilated via a mechanism that was completely abolished by removing the endothelium or incubating vessels with a 10^{-5} M concentration of the L-arginine analog L-NMMA. Flow-mediated vasodilation was not restricted to arterioles because it was subsequently demonstrated in resistance arteries as large as ~180 μm (13). Nevertheless, the endothelium dependence of these responses and the potential role of NO as a mediator in all sizes and classes of arterial vessels have not been established. We found that the extent of flow-mediated vasodilation was similar to what Kuo et al. (12) found in small arteries and that it was completely independent of NO because L-NMMA and L-NAME at similar doses and in a similar preparation failed to attenuate it. Whereas varying the level of preconstriction affected the initial and maximal diameter for any given flow, it had no effect on the relation between the maximum change in flow and change in diameter. Thus our results support
a longitudinal gradient in the mechanism responsible for vasodilation to steady-state levels of shear stress, with NO-mediated vasodilation being more important in resistance arteries than in epicardial conduit arteries.

A number of studies have demonstrated vasodilation in association with endothelial-dependent hyperpolarization in large as well as small coronary arteries. This mechanism appears to be particularly prominent in the coronary circulation as opposed to other vascular beds. Most studies have focused on demonstrating the release of a hyperpolarizing factor after agonist-mediated vasodilation. Bradykinin has been the prototype agonist used to study this because it elicits both NO-mediated and hyperpolarization-mediated vasodilation. Hyperpolarization of epicardial artery rings after bradykinin is dependent on the endothelium and is not altered by L-arginine analogs. It is completely blocked by KCl (8, 16), nonselective K⁺ channel blockers (16), and inhibitors of cytochrome P-450 (17). Although we did not measure membrane potential, the diameter responses we observed after bradykinin treatment are consistent with the notion that the mechanism of dilation in pigs involves both the release of an endothe-

Table 3. Effect of intraluminal L-NAME, indomethacin, and TEA on the flow-diameter relation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Static Conditions, μm</th>
<th>Maximum Flow Gradient, μm</th>
<th>ΔD, μm</th>
<th>Flow, ml/min</th>
<th>Shear Stress, dyn/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>433 ± 24</td>
<td>544 ± 33†</td>
<td>110 ± 18</td>
<td>3.39 ± 0.16</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>Indomethacin + L-NAME</td>
<td>421 ± 20</td>
<td>521 ± 29*†</td>
<td>100 ± 13</td>
<td>3.38 ± 0.16</td>
<td>62 ± 12</td>
</tr>
<tr>
<td>Indomethacin + L-NAME + TEA</td>
<td>418 ± 20</td>
<td>476 ± 28*†</td>
<td>58 ± 10*</td>
<td>3.09 ± 0.16</td>
<td>71 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 porcine arteries. TEA, tetraethylammonium chloride. Normalized diameter was found with the use of a \( D_{NP} \) of 557 ± 30 μm. *P < 0.01 vs. control; †P < 0.001 vs. static conditions.
lum-derived hyperpolarization factor (EDHF) as well as NO, because it was only partially attenuated by inhibiting NO synthase with l-NAME and L-NMMA.

In contrast to agonist-mediated vasodilation, there is very little information regarding the potential role of EDHF versus NO as a mechanism of flow-mediated vasodilation. While it is clear that NO is responsible for a portion of flow-mediated vasodilation, inhibiting NO synthase attenuates but does not eliminate flow-mediated vasodilation in epicardial coronary conduit arteries. Furthermore, as noted above, the responses observed appear to be related to the type of flow stimulus used to perturb the system (1). We were able to completely block flow-mediated vasodilation in isolated epicardial arteries by depolarizing the vessel with KCl, which supports the possibility that endothelium-dependent hyperpolarization or the release of a hyperpolarizing factor was entirely responsible for the dilation to steady-state changes in shear stress. In further support of hyperpolarization, we found that the nonselective K+ channel blocker TEA attenuated the vasodilation to shear stress. In addition, unlike some other vascular beds, cyclooxygenase inhibition with indomethacin had no effect (9). A limitation of our experimental preparation is that it does not allow us to determine whether the effects of KCl and TEA were on the vascular smooth muscle or the endothelium. Taken together, however, these results suggest that an EDHF may be one of the mechanisms involved in vasodilation to steady changes in shear stress. Although previous studies have not examined the role of flow or shear stress in producing EDHF in coronary conduit arteries, a recent study (4) in human coronary resistance arteries has demonstrated that vasodilation to steady flow may also be mediated by a hyperpolarizing factor. The available data in human resistance arteries suggest that the mediator may be a metabolite of the cytochrome P-450 pathway (15). Further studies will be required to more specifically characterize the nature of this factor in porcine conduit arteries.

Methodological Considerations

Our major conclusion is based on the assumption that we inhibited NO synthase in the epicardial conduit arteries. We employed bradykinin to test the efficacy of NO synthase blockade because previous studies have established that it causes relaxation by stimulating NO synthase as well as by releasing a hyperpolarizing factor that can be blocked by depolarizing the vascular smooth muscle membrane. Thus we would not anticipate that bradykinin-induced vasodilation would be completely attenuated by inhibiting NO synthase alone, as our results confirm. Previous studies have employed a variety of L-arginine analogs to block NO production in porcine coronary vessels, with concentrations that have varied between $10^{-5}$ and $10^{-3}$ M. Our quantitative results indicate a somewhat more prominent effect of l-NAME on bradykinin-induced vasodilation than found using even higher concentrations of other L-arginine analogs. For example, in porcine resistance arteries, Tschudi et al. (22) found only small shifts in the bradykinin dose-response curve after inhibiting NO synthase with l-NAME and L-NMMA. Cowan and Cohen (2) found small insignificant changes in bradykinin dose-response curves (but abolished cGMP production) after 3 $\times$ $10^{-4}$ M L-NMMA in porcine epicardial coronary artery rings. The residual dilation could be markedly attenuated by KCl. In canine epicardial rings, Illiano et al. (7) found a more prominent blockade of vasodilation with $3 \times 10^{-5}$ M nitro-L-arginine than demonstrated in the previous studies, with the residual vasodilation abolished by adding KCl. We performed limited dose-response relations in perfused conduit arteries and found a similar pharmacological response as in these previous studies. Both l-NAME and L-NMMA produced a modest reduction in conduit artery vasodilation to bradykinin, which was further inhibited by KCl or TEA. Our dose-response curves conducted in porcine epicardial artery rings support the efficacy of NO synthase blockade over a wider bradykinin concentration. The effects of l-NAME on bradykinin-induced vasodilation appeared to be more prominent than the other L-arginine analogs examined in these previous studies, supporting the possibility that it may be more potent when administered at a concentration of $10^{-5}$ M. Thus the lack of effect of inhibiting NO synthase on flow-mediated vasodilation was not related to an ineffective dose of l-NAME.

All of our data were obtained after preconstricting the vessels with the thromboxane analog U-46619, because the epicardial coronary arteries did not develop spontaneous tone. To exclude this as a confounding variable, we demonstrated that the maximum extent of flow-mediated vasodilation was independent of the level of initial tone, which has also been demonstrated in coronary resistance arteries that develop spontaneous tone or have been preconstricted with acetylcholine (10). Furthermore, the diameter changes we found in vitro were of a similar magnitude as those occurring in the epicardial coronary artery in vivo (1). The similarity of the findings with those in conscious dogs where mean flow was varied at a constant heart rate in vivo support the notion that preconstriction could not explain the failure of L-arginine analogs to affect the flow-diameter relation. Likewise, although it was not possible to evaluate sodium nitroprusside dose-response curves between the various interventions, another study (6) has demonstrated that L-arginine analogs, endothelium denudation, and KCL and TEA treatment have no effect on the dose-response relation.

Finally, the attenuation of flow-mediated vasodilation by TEA occurred during the coadministration of l-NAME and indomethacin. While L-NAME and indomethacin had no effect on the dilation to steady flow, we cannot exclude the possibility that there was synergy between TEA and these other agents that resulted in reduced conduit artery vasodilation. Further studies with more selective calcium-activated K+ inhibitors administered alone will be required to address the
importance of this pathway on conduit artery dilation to steady-state flow.

In summary, our data support the hypothesis that differential mechanisms are involved in transducing mechanical stimuli by the coronary endothelium of epicardial conduit arteries. While pulsatile flow dilates epicardial arteries via the production of NO, changes in mean shear stress appear to be transduced by a mechanism that is independent of NO synthase. Further studies will be needed to determine whether similar mechanisms exist in vascular beds not subjected to the pronounced pulsatile variations in flow unique to the coronary circulation.

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