Pathophysiological Consequences of Atherosclerosis Extend Into the Coronary Microcirculation

Restoration of Endothelium-Dependent Responses by L-Arginine

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The goals of this study were 1) to quantitate the effects of atherosclerosis on physiological and pharmacological endothelium-dependent vasoactive responses in coronary arterioles downstream from arterial lesions and 2) to determine if administration of L-arginine, the precursor for endothelium-derived relaxing factor, would restore normal endothelium-dependent function. Dietary-induced atherosclerosis was induced in pigs, and vasomotor responses of isolated, cannulated coronary arterioles (30–70 μm in diameter) were assessed by measuring diameter changes in vitro. To assess pharmacological alterations of endothelium-dependent responses, dose–response curves were constructed to ADP, serotonin, and histamine. To assess physiological alterations in endothelial function, different flow rates were established across the vessel. Arteriolar diameters were measured in vessels from normal and atherosclerotic pigs under control conditions, after administration of L-arginine, and after endothelial denudation. In arterioles from normal pigs, administration of serotonin, histamine, or ADP produced dose-dependent vasodilation, which was abolished by endothelial denudation. In arterioles from atherosclerotic pigs, administration of histamine, serotonin, and ADP produced dilation at only the highest doses (10^-6–10^-7 M), and the extent of dilation was only 20–30% of that observed in arterioles from normal pigs. Initiation of flow also produced vasodilation in arterioles from normal pigs that was completely abolished after endothelial denudation. In arterioles from atherosclerotic pigs, flow-induced responses were absent. These abnormal physiological and pharmacological responses (i.e., blunted vasodilation to pharmacological stimulation and to flow) were restored after administration of L-arginine for 40 minutes. The vascular responses after administration of L-arginine were not different from those observed under control conditions in arterioles from normal pigs. In addition, L-arginine did not restore vasodilation to the endothelium-dependent agonists in denuded segments. From these data in arterioles downstream from atherosclerotic lesions, we conclude that 1) the ED₅₀ and maximal responses of endothelium-dependent vasodilation to ADP, histamine, and serotonin are attenuated; 2) the physiological response to flow, that is, flow-mediated endothelium-dependent vasodilation, is absent; and 3) the abnormality in arteriolar responsiveness during large vessel disease involves an impairment of the synthesis and/or release of endothelium-derived relaxing factor. (Circulation Research 1992;70:465–476)

KEY WORDS • coronary circulation • coronary microcirculation • endothelium-dependent responses • atherosclerosis • vasodilation, flow-induced • arteriole • endothelium-derived relaxing factor

Atherosclerosis is known to potentiate the vasoconstrictor responses of arteries to several agonists. The mechanisms underlying this enhanced responsiveness have not been unequivocally elucidated but potentially include impaired endothelium-dependent relaxation, increases in numbers of adrenergic and serotonergic receptors, and altered calcium regulation by vascular smooth muscle. Despite abundant evidence showing that atherosclerosis alters vasomotor control of arteries, only recently have studies suggested that the effects of atherosclerosis may extend into the microcirculation. Specifically, small arteries or resistance vessels in the coronary circulation that do not exhibit gross atherosclerotic lesions show altered responses to pharmacological stimulation by endothelium-dependent agonists. Microvessels that do not develop

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lesions are nevertheless exposed to the abnormally high levels of circulating lipid and circulating cholesterol incorporated into specific lipoproteins, which may be a sufficient pathological stimulus to produce the observed changes in microvascular reactivity. This suggestion is corroborated by evidence demonstrating that hypercholesterolemia per se, without accompanying atherosclerosis, is a sufficient pathology to alter endothelium-dependent vasoactive responses in large arteries. Moreover, it has been recently reported that hypercholesterolemia also influences resistance vessel responses to endothelium-dependent agonists.

The goals of this study were (1) to quantitate the effects of atherosclerosis on physiological and pharmacological endothelium-dependent vasoactive responses in coronary arterioles downstream from arterial lesions and (2) to determine if administration of L-arginine, the precursor for endothelium-derived relaxing factor (EDRF), would restore normal endothelium-dependent function. These hypotheses were tested by measuring pharmacological and physiological responses of isolated coronary arterioles from control and atherosclerotic pigs.

Materials and Methods

General Preparation

Twenty-six domestic miniswine (of either sex, 4–6 months old) were randomly divided into two groups. The control group (32.2±6 kg, n=13) was fed a regular chow, and the atherosclerotic group (40±7 kg, n=13) was fed the regular chow supplemented with fat and cholesterol (sodium cholate, 1.5%; cholesterol, 3%; corn oil, 5.5%; grain, 18%; dried milk, 20%; lard, 24%; and sugar, 28%) for 16–20 weeks. The high lipid portion constituted 30–50% of the total diet. After the appropriate period on the diet, the pigs were sedated with ketamine (15–20 mg/kg i.m.) and Rompun (0.1 mg/kg i.m., Haver-Lockhart, Cutter Laboratories, Shawnee, Kan.) and masked; anesthesia was induced with 4–5% isoflurane. After induction of a stable plane of anesthesia, the pig was intubated and maintained with a 1% isoflurane–99% oxygen mixture. After a left thoracotomy, heparin (1,000 units/kg) was administered into the left atrium, and the heart was electrically fibrillated, excised, and immediately placed in cold (4°C) saline solution.

The techniques for identification and isolation of coronary arterioles were described previously. In brief, a mixture of India ink and gelatin in physiological salt solution (PSS) was perfused under a low pressure (30–50 cm H2O) into the left anterior descending artery and the circumflex artery to visualize the coronary microvessels. At 4°C, arteriolar branches from left anterior descending or circumflex arteries (0.6–1.0 mm in length and 30–70 μm i.d.) were selected and dissected from the surrounding cardiac tissue and transferred for further dissection to a dish (4°C) containing filtered PSS-albumin solution at pH 7.4. After careful removal of any remaining cardiac tissue, an arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution equilibrated with room air at ambient temperature. One end of the arteriole was cannulated with a glass micropipette (with a tip diameter of 40 μm i.d. and filled with filtered PSS-albumin solution), and the outside of the arteriole was securely tied to the pipette with 11-0 ophthalmic suture. The ink-gelatin column inside the vessel was flushed out at low perfusion pressure (<20 cm H2O). Any small side branches were ligated; then the other end of the vessel was cannulated with a second micropipette and secured with suture. Electrical resistances (measured by LCR Bridge Circuit, model LCR-740, Leader Electronics Corp., Japan) of the two pipettes were matched (±0.5%).

Instrumentation

After the vessel was cannulated, the preparation was then transferred to the stage of an inverted microscope (model IM35, Carl Zeiss, Inc., Thornwood, N.Y.) coupled to a camera (67M Newvicon, Dage-MTI, Michigan City, Ind.), video micrometer, and video recorder (Panasonic). Internal diameters were measured manually throughout the experiment using video microscopic techniques.

To study the flow-induced responses independent of luminal pressure changes, a dual-reservoir system was used to maintain a constant intraluminal pressure over a wide range of flows. The micropipettes were connected to independent reservoir systems, and intraluminal pressures were measured through side arms of the two reservoir lines by low-volume displacement strain-gauge transducers (Statham P23Db, Gould, Cleveland, Ohio). The isolated vessels were pressurized without flow by setting both reservoirs at the same hydrostatic level (without a pressure gradient between the reservoirs). The intraluminal pressure of the vessel should equal the hydrostatic pressure of reservoirs if there are no leaks in the vessel. Leaks were detected by differences between reservoir pressure and luminal pressure. Any preparations with leaks were excluded from the data analysis. Flow was initiated by simultaneously moving the reservoirs in opposite directions and generating a pressure gradient (ΔP). Because the resistances of both cannulation pipettes were equivalent, simultaneous movement of the reservoirs in equal and opposite directions did not change midpoint luminal pressure. Thus, flow-induced responses could be studied by perfusing the vessel at different flow rates (i.e., at various values of ΔP) without changing intraluminal pressure.

Endothelial Denudation

The technique for mechanical removal of endothelium in isolated coronary arterioles was described previously. In brief, a concentric micropipette with an irregular tip and long shank was advanced into the lumen of the cannulated vessel. A negative luminal pressure (−20 cm H2O) was produced to collapse the vessel and to allow the abrasive pipette to closely contact the endothelial cells. The endothelial cells were disrupted by passing the abrasive pipette back and forth several times. The viability of vascular smooth muscle after this procedure was determined by the development of spontaneous tone and the response to acetylcholine, an endothelium-independent vasoconstrictor in the porcine coronary microcirculation. The efficacy of denudation was verified by the abolition of vasoconstrictor responses to bradykinin (10−6 M), an endothelium-dependent vasodilator in porcine coronary arterioles. The success rate of denudation in this study was ~85%.
Morphological Studies

From three control (normal) and four atherosclerotic pigs, a segment of right coronary artery and a small piece of myocardium were dissected and drop-fixed in a glutaraldehyde-formaldehyde solution for electron microscopy. The small piece of myocardium was obtained for morphological examination of small arteries and arterioles. After fixation, all tissue samples were embedded in plastic and sectioned; vessels were examined to 1) verify the presence of the endothelium and 2) detect if intimal proliferation (an atherosclerotic lesion) was apparent. In addition, from six control and eight atherosclerotic pigs, samples of the right coronary artery were drop-fixed in 10% neutral-buffered formalin, embedded in plastic (glycol-methacrylate) or paraffin, sectioned, and stained with hematoxylin and eosin; the von Kossa method was used to detect calcium. Also, formalin-fixed frozen sections of right coronary artery were subjected to the oil red O technique to detect lipid (neutral fat). The extent of the atherosclerotic lesions was assessed from the size of the lesion (amount of thickening), deposition of lipid (from the oil red O stain), and presence of calcification (von Kossa method).

Protocol 1: Physiological Studies of Flow-Induced Responses

To examine the function of the endothelium in the control of flow-induced responses, the flow–diameter relation of arterioles from normal and atherosclerotic pigs was studied before and after mechanical removal of the endothelium. For the control study, the cannulated arteriole was bathed in the chamber containing PSS–albumin solution, and the temperature was maintained at 36–37°C by an external heat exchanger. The vessel was set to its in situ length as described previously and allowed to develop spontaneous tone at 60 cm H2O luminal pressure without flow. This internal pressure is comparable to the pressure in arterioles of this size in vivo. After the vessel developed spontaneous tone, flow was initiated by equal and opposite movements of the two reservoirs. Diameter was measured at each level of flow corresponding to ΔP values of 4, 10, 20, 40, and 60 cm H2O and again at zero flow (ΔP = 0). According to our previous study in vessels of this size, flow (Q, nl/sec) is proportionally related to ΔP in arterioles of this size and can be calculated from the equation:

\[ Q = \frac{0.58 \text{ nl/sec} \times \text{ cm H}_2\text{O}(\Delta P) + 0.9}{\text{nl/sec}} \]

Because L-arginine is the physiological precursor to EDRF and because atherosclerotic arteries demonstrate an impaired ability to release EDRF, we hypothesized that administration of L-arginine would restore normal endothelium-dependent function. Both groups of vessels were incubated with L-arginine (3 mM) and allowed to reestablish spontaneous tone for 40 minutes. The same steps of ΔP values as described above were performed to examine the effect of L-arginine on flow-induced responses. To study the role of endothelium in the flow-induced response, vessels were denuded (control, n = 13; atherosclerotic, n = 4), and vascular responses to flow were reexamined. In some arterioles denuded of endothelium (control, n = 5; atherosclerotic, n = 4), L-arginine was administered to ascertain if the effects of the amino acid precursor were mediated by the endothelium.

Protocol 2: Pharmacological Studies

To directly assess the role of the endothelium in mediating responses to serotonin, histamine, and ADP in arterioles from normal and atherosclerotic pigs, the following protocols were performed. Initially, the cannulated arterioles were set to 60 cm H2O intraluminal pressure without flow. After the vessels developed spontaneous tone, the dose-dependent vasomotor responses were examined by construction of cumulative dose–response curves at a constant pressure (60 cm H2O). To evaluate the effects of L-arginine on arterioles from normal and atherosclerotic pigs, vessels were incubated with L-arginine (3 mM) for 40 minutes, and the dose–response curves to the agonists were reestablished. Endothelial dependence of these responses was examined by selective removal of endothelial cells. To assess the function of vascular smooth muscle in normal and atherosclerotic vessels, acetylcholine or nitroprusside was administered into the bath cumulatively, and dose–response curves of these two groups of vessels were compared. At the end of each experiment, each vessel was relaxed with nitroprusside (10^{-4} M) in PSS–albumin solution to obtain its maximum diameter at 60 cm H2O. All drugs, except those specifically mentioned, were purchased from Sigma Chemical Co., St. Louis, Mo.

Data Analysis

In this study, the viability of each vessel was examined according to the criteria for reactive isolated arterioles: 1) development of spontaneous tone, 2) myogenic responses, and 3) sensitivity to vasoactive agents. In the present experiments, arterioles had to satisfy these criteria in order to be included for data analysis. For analysis of flow-induced responses, arteriolar diameter was normalized to the passive diameter (in the presence of 10^{-4} M nitroprusside) at 60 cm H2O intraluminal pressure. For analysis of the effect of drugs on vascular tone, the diameters of the vessels were normalized to their control diameter (i.e., their initial diameters with spontaneous tone). To assess vascular smooth muscle function, responses to acetylcholine were expressed as percentage reductions in luminal diameter. Normalized diameters were averaged at each step of flow or drug administration. All data are reported as mean±SEM. Statistical comparisons between groups and within groups were made with factorial or repeated-measures analysis of variance tests with Fisher’s least significant difference multiple-range tests when appropriate. Significance was accepted at p<0.05.

Results

Vessel Morphology

Figure 1A shows typical light micrographs of right coronary arteries (hematoxylin and eosin stain) from a normal pig, and Figure 1B shows the artery from an atherosclerotic pig. In the coronary arteries from eight atherosclerotic pigs, seven pigs demonstrated raised lesions (10–50% encroachment into the lumen) with an increase in collagenous fibers, synthetic vascular smooth muscle, and scattered areas of necrosis, whereas one pig showed what appeared to be only the beginning of a lesion formation. Of the seven pigs with demonstrable lesions, five showed foam cells with lipid deposits primarily in the tunica intima with some in the tunica...
media, and two of these pigs showed calcium deposits in the tunica intima near the tunica medial border.

Electron micrographs of arterioles are shown in Figures 2A and 2B. Figure 2A is an arteriole (65 μm) from a normal pig, and Figure 2B is an arteriole (73 μm) from an atherosclerotic pig. Although areas of intimal proliferation were not evident in any of the arterioles from atherosclerotic pigs, there were areas of the internal elastic lamina that commonly appeared thickened and damaged (i.e., fractured areas). The arteriolar endothelium was intact in both groups of vessels, but it is worth noting that, in vessels from atherosclerotic pigs, the endothelium often contained large vacuoles and/or lipid droplets.

**Protocol 1: Physiological Studies of Flow-Induced Responses**

The responses of isolated coronary arterioles (normal and atherosclerotic) to flow are shown in Figures 3 and 4. In normal vessels with intact endothelium, vasodilation to flow (ΔP) was observed in all vessels. On the average, the vessels dilated from 68% (spontaneous tone) of maximal diameter at zero flow (ΔP=0) to 90% of maximal diameter when ΔP was increased to 20 cm H₂O (Figure 3A). No significant further dilatation was observed at the higher flows (40 and 60 cm H₂O). In normal vessels, flow-induced dilation was not significantly altered by l-arginine (3 mM) but was completely abolished by mechanical removal of endothelium (Figure 3A). The evidence for functional denudation of the endothelium is summarized in Figure 3B. With the endothelium intact, bradykinin (10⁻⁸ M) dilated the vessels from 70% of maximal diameter (spontaneous tone) to ~95% of maximal diameter (Figure 3B). After mechanical removal of the endothelium, the arterioles still exhibited the same magnitude of spontaneous tone, but the relaxation to bradykinin was completely abolished (Figure 3B). It is worth emphasizing that nitroprusside produced equivalent dilation of control and denuded vessels.

In contrast to the normal vessels, coronary arterioles isolated from atherosclerotic pigs did not vasodilate to flow (Figure 4A). Interestingly, three vessels slightly constricted with increases in flow (approximately a 5% decrease in diameter). Flow-induced dilation was observed in all (n=8) arterioles from atherosclerotic pigs after incubation with l-arginine (3 mM) for 40 minutes (Figure 4A). There were no significant differences in the magnitude and threshold of flow-induced dilation between normal and l-arginine-treated atherosclerotic vessels (Figures 3A and 4A). In the atherosclerotic vessels, l-arginine did not alter the spontaneous tone of the vessels, and dilation produced by bradykinin (10⁻⁸ M) was only observed when the vessels were pretreated with l-arginine (Figure 4B).

Although not shown in Figures 3 or 4, administration of l-arginine after denudation did not restore flow-induced vasodilation in arterioles from normal pigs (n=5) or from atherosclerotic pigs (n=4).

**Protocol 2: Pharmacological Studies**

Serotonin (10⁻⁹ to 5×10⁻⁶ M) produced a dose-dependent dilation of normal vessels with intact endothelium, causing a 30% increase in diameter above
control (Figure 5A). Denudation reversed the vasodilatory response to a vasoconstrictory response (Figure 5A). In atherosclerotic vessels, serotonin in low doses (<5×10⁻⁸ M) produced a slight vasoconstriction, but in high doses (>2×10⁻⁷ M), serotonin caused a slight vasodilation (Figure 5B). The magnitude of the vasodilation was significantly and substantially less than that in the normal vessels (Figure 5). L-Arginine (3 mM) significantly potentiated the vasodilatory responses to serotonin in atherosclerotic vessels (Figure 5B), which was comparable to that in the normal vessels (Figure 5A). Nitroprusside produced similar endothelium-indepen-
dent vasodilation (40–50% increase in diameter) in all the groups.

Histamine also produced a dose-dependent vasodilation in normal arterioles, causing a 47% increase in diameter above control at 5 × 10^{-6} M (Figure 6A). The vasodilation was reversed to a vasoconstriction at low doses (10^{-6}–10^{-7} M) when the endothelium was mechanically removed (Figure 6A). A vasodilatory response to histamine in denuded vessels was observed only at the highest dose (5 × 10^{-6} M), and the magnitude of dilation was significantly less than that in intact, normal vessels (Figure 6A). Atherosclerotic vessels also dilated to histamine, but the threshold concentration (5 × 10^{-7} M) was significantly higher than that for normal vessels (5 × 10^{-6} M). The maximal dilation induced by histamine (5 × 10^{-4} M) was quantitatively lower in atherosclerotic vessels (20% increase in diameter above control) than that in the normal vessels (47% increase in diameter) (Figures 6A and 6B). Incubation of vessels from atherosclerotic pigs with L-arginine resulted in a significant upward shift of the dose–response curve so that the dilation was similar to that of normal vessels with intact endothelium. All groups of arterioles demonstrated the same magnitude of vasodilation to nitroprusside.

ADP produced a dose-dependent vasodilation in control arterioles with a threshold of 5 × 10^{-9} M and a maximal response at 5 × 10^{-8} M (40% increase in diameter above
control) (Figure 7A). Mechanical removal of the endothelium abolished the dilation except at the highest dose of ADP (Figure 7A). The threshold (5×10⁻⁸ M) and magnitude (15% increase in diameter) for ADP-induced dilation were significantly attenuated in atherosclerotic vessels (Figure 7B). Again, L-arginine (L-Arg, 3 mM) significantly potentiated the magnitude of vasodilatory responses (ED₅₀, 10⁻⁷ M). *p<0.05 between Athero and Athero+L-Arg vessels at doses of 5-HT >10⁻⁸ M.

To test the contractile function and direct vasodilatory responses of coronary arteries from normal and atherosclerotic pigs, an endothelium-independent vasconstrictor and vasodilator, acetylcholine and nitroprusside, respectively, were administered (Figure 8). Acetylcholine produced dose-dependent constriction in both normal and atherosclerotic vessels with similar thresholds (5×10⁻⁸ M) and ED₅₀s (2×10⁻⁷ M). The maximum constriction occurred at 10⁻⁹ M, and there was no significant difference in contractile response between these two groups of vessels (Figure 8A). In addition, there were no significant differences in the threshold (10⁻³⁰ M), ED₅₀ (1.5×10⁻⁷ M), or maximum (1×10⁻⁴ M) vasodilatory responses to nitroprusside in vessels from normal and atherosclerotic pigs (Figure 8B).
Discussion
This study demonstrates that, during coronary artery disease, arterioles exhibit altered endothelium-dependent physiological and pharmacological reactions. Specifically, we have made three new observations in isolated coronary arterioles that do not exhibit vascular lesions (intimal proliferation): 1) There is blunted endothelium-dependent vasodilation to the agonists ADP, histamine, serotonin, and bradykinin. 2) The physiological response to flow (i.e., flow-mediated endothelium-dependent dilation) is also compromised. 3) Administration of l-arginine to isolated arterioles restores normal endothelium-dependent vasodilation to the pharmacological and physiological stimuli. From these data we conclude that the pathophysiological manifestations of atherosclerosis extend into the microcirculation and cause abnormal endothelium-dependent pharmacological responses to agonists and abolish flow-mediated vasodilation. We also conclude that the pathological alteration of arteriolar responses involves a deficiency in the synthesis and/or release of EDRF, because administration of the precursor to this substance, l-arginine, restores normal endothelium-dependent function. Critical to our conclusions and interpretations are several factors including 1) the experimental model and methodology, 2) endothelial modulation of arteriolar tone, and 3) influences of atherosclerosis and hypercholesterolemia on vascular function.

Critique of the Experimental Model and Methodology
Pigs with dietary-induced atherosclerosis were used as the experimental model in the present study. The

FIGURE 7. Graphs showing dose-dependent responses of coronary arterioles to ADP at a luminal pressure of 60 cm H₂O. Maximum diameters were obtained in the presence of nitroprusside (NP, 10⁻⁶ M). n, Number of vessels; d, average luminal diameter. Vertical bars denote mean ± SEM. Panel A: ADP produced a dose-dependent vasodilation (ED₅₀, 10⁻⁷ M) in normal arterioles (closed circles). Mechanical removal of the endothelium eliminated this vasodilatory response except at the highest dose of ADP (open circles). *p<0.05 between normal and denuded vessels at doses >7×10⁻⁸ M. Panel B: Atherosclerotic (Athero) vessels diluted (ED₅₀, 6.3×10⁻⁷ M) to ADP only at doses much higher than in normal vessels (closed circles). l-Arginine (L-Arg, 3 mM) significantly potentiated the vasodilatory response (ED₅₀, 10⁻⁷ M) to ADP (open circles). *p<0.05 between Athero and Athero+L-Arg vessels at doses >10⁻⁸ M.

FIGURE 8. Graphs showing dose-dependent responses of normal (open circles) and atherosclerotic (closed circles) vessels to acetylcholine and nitroprusside at a luminal pressure of 60 cm H₂O. n, Number of vessels. Panel A: Threshold and ED₅₀ were at 5×10⁻⁶ and 2×10⁻⁷ M, respectively. There were no significant differences in the contractile responses to acetylcholine between these two groups of vessels. Panel B: Threshold, ED₅₀, and maximum dilation were at 10⁻¹⁰, 1.5×10⁻⁷, and 1×10⁻⁴ M, respectively. The vasodilation to nitroprusside was not different in these two groups of vessels.
atherosclerotic lesions appear similar to those of humans, which are also characterized by intimal proliferation, presence of foam cells, lipid deposition, and areas of calcification.\textsuperscript{22,23} It is also worth noting that, in both pigs and patients, microvessels, usually <300 \( \mu \text{m} \) in diameter, do not exhibit growth of atherosclerotic lesions during the evolution of the disease process.\textsuperscript{24}

There is, however, a limitation of the experimental model of dietary-induced atherosclerosis in the pig as a representation for coronary artery disease in human patients. Specifically, the time for induction of atherosclerosis is much shorter in our experimental model than in patients with coronary artery disease (months versus years). Taken together, the results indicate that there may be some differences between the development of atherosclerosis in our porcine model versus that in humans, but there are many similarities, which give the overall impression that vascular complications associated with the disease process may be due to similar mechanisms.

We used isolated arterioles for the present studies in order to examine the microvascular consequences of large-vessel disease in a model of atherosclerosis. We elected to measure vasoactive responses to pharmacological stimulation and to flow in isolated arterioles for several reasons. First, because coronary arterioles exhibit an active myogenic response,\textsuperscript{16} it would be difficult in vivo to distinguish flow-induced from pressure-induced responses. Our dual-reservoir system, however, allowed us to change flow rate and intraluminal pressure independently; therefore, we could assess flow-induced responses independent of the myogenic response. For each experiment, the tip resistances of the cannulation pipettes were matched, and the major resistance of the system resides in the tips of these pipettes. Therefore, by simultaneously raising or lowering both reservoirs in equal and opposite directions, flow could be initiated without altering intraluminal pressure. In an earlier report, we confirmed that intraluminal pressures did not change if resistances of the pipettes were matched.\textsuperscript{15} Also, at the end of an experiment when the vessels were dilated with nitroprusside, passive changes in luminal diameter did not occur during initiation of flow; thus, there was no passive distention or collapse of the passive vessel, which indicated that intraluminal pressure did not change. It is also worth emphasizing that AP values of 4–60 cm H\(_2\)O used in the present study correspond to flow velocities of 1.2–16 mm/sec, which approximate the range of red blood cell velocities reported for coronary microvessels with tone in the beating heart.\textsuperscript{25,26} The isolated vessel technique also offers significant advantages for pharmacological studies. Specifically, the concentration of drugs to which the vessels are exposed should correlate well with the concentration of the drug in the bath. In the intact in vivo preparation, delivery of drugs by varying amounts of blood flow, the capillary barrier for diffusion of drug into the interstitium, partition coefficients, and metabolism all confound approximations of the drugs at the effector sites in arterioles. Also, the arteriolar endothelium imposes a barrier to drugs acting on vascular smooth muscle.\textsuperscript{27} In isolated arterioles, we were able to construct quantitative dose–response relations between agonist concentration and microvessel responses, because we were able to circumvent the above-mentioned problems. In the aggregate, the isolated arteriole preparation allows us to quantitatively assess alterations in endothelium-dependent mechanisms involved in the control of vasomotor tone.

We have also reported previously that mechanical abrasion has been demonstrated to be an effective way to selectively remove the endothelium in small coronary arterioles without damaging the overlying vascular smooth muscle (i.e., without impairing viability).\textsuperscript{17} Endothelial denudation was confirmed using two approaches. First, during luminal abrasion under bright field illumination, we observed cellular debris in the lumen and membrane remnants attached to the lumen wall, indicating that endothelial cells were disrupted. Second, pharmacological agonists known to produce endothelium-dependent vasodilation did not induce such responses; yet, vasoactive responses to endothelium-independent constrictors (acetylcholine) and dilators (nitroprusside) were not altered after mechanical disruption of the endothelium. It should be emphasized that acetylcholine in the pig is an endothelium-independent constrictor of coronary arterioles and arteries\textsuperscript{15–18}; thus, vascular responses to this drug can be used functionally to assess the status of smooth muscle. In the aggregate, we would argue that our experimental preparations are viable and that endothelial denudation selectively removes the endothelium without causing detectable changes in smooth muscle function.

**Endothelial Modulation of Coronary Arteriolar Tone**

Although it has been well established for almost 10 years that the endothelium is capable of modulating coronary arterial reactions to neurohumoral factors,\textsuperscript{3,7,12,13,28–32} only within the last few years has endothelial modulation of microvascular reactions to vasoactive substances in coronary resistance vessels been elucidated.\textsuperscript{10,11,14,33–36} The endothelium has also been found to mediate flow-induced vasodilation in the coronary microcirculation as well as a number of other organ systems.\textsuperscript{15,37,38} It is worth noting, however, that in some species, endothelium-independent vasoactive reactions to flow have been reported.\textsuperscript{39,40} Our results indicate that the endothelium of pig coronary arterioles mediates the vasoactive reaction to flow and to serotonin, histamine, ADP, and bradykinin. This conclusion is based on our observations that dilatation to these aforementioned factors was eliminated after endothelial denudation. In arterioles from atherosclerotic animals, there appears to be a defect in the production and/or release of EDRF, because these vessels also demonstrated impaired relaxation to the previously mentioned physiological and pharmacological factors.

Our results also provide insight into the chemical nature of EDRF in the coronary microcirculation. There is a substantial body of evidence in the literature indicating that EDRF is either nitric oxide or a nitroso compound.\textsuperscript{41–45} Moreover, it has been shown that the guanidino nitrogen group of L-arginine is enzymatically used for synthesis into nitric oxide.\textsuperscript{44} Further evidence indicating that L-arginine is the substrate for the synthesis of EDRF is provided by results from several studies indicating that a number of analogues of L-arginine behave as false substrates; that is, they compete with L-arginine for binding and prevent the formation of
EDRF. Also in a previous report, we found that \(^{N}^{14}\)-monomethyl \(L\)-arginine, a false substrate for the synthesis of EDRF, inhibited the flow-induced vasodilation in coronary arterioles.\(^{47}\)

This supported the hypothesis that the endothelium-derived vasodilatory factor in coronary arterioles is nitric oxide or a nitroso compound. Because of these observations, which supported a role for \(L\)-arginine in the synthesis of EDRF,\(^{44,46}\) and a recent report, which indicated that atherosclerotic vessels demonstrated an impaired ability to synthesize EDRF,\(^{20}\) we speculated that arterioles would also suffer from the same pathology as the diseased arteries (i.e., an inability to synthesize EDRF after a physiological or pharmacological challenge). Our present results were consistent with this hypothesis.

We also speculated that, if the defect in atherosclerotic vessels involves the impairment of production of EDRF, administration of the substrate \(L\)-arginine for the pathway would potentially reverse the pathological effects. Our experimental findings supported this hypothesis; that is, treatment of arterioles from atherosclerotic animals with \(L\)-arginine restored normal endothelium-dependent reactions both to flow and to pharmacological stimulation with serotonin, bradykinin, histamine, and ADP. It is important to emphasize that \(L\)-arginine not only has the ability to restore endothelium-dependent vasoactive reactions in isolated coronary arterioles but also has recently been reported to restore endothelium-dependent vasodilation in the peripheral circulation in vivo in hypercholesterolemic animals and patients. The effects of \(L\)-arginine appear to be endothelium-dependent, because in our studies of denuded vessels, administration of the amino acid did not restore pharmacological or flow-induced vasodilation. Collectively, it would appear that, in arterioles downstream from atherosclerotic lesions, endothelium-dependent vasoactive reactions are abnormal and that the abnormality involves a defect in the synthesis and/or release of arginine-derived relaxing factors.

**Influences of Atherosclerosis and Hypercholesterolemia on Vascular Function**

Many previous studies have found that atherosclerosis augmented vasoconstrictor responses and impaired vasodilator responses of arteries to a variety of vasoactive compounds. In monkeys, atherosclerosis potentiates vasoconstriction to numerous compounds including endothelin, \(^{50}\) thromboxane \(A_{2}\),\(^{51}\) and serotonin.\(^{51}\) In patients, atherosclerosis also potentiates vasoconstriction to a variety of compounds including acetylcholine,\(^{52}\) ergonovine,\(^{53}\) and histamine.\(^{54}\) Moreover, in patients, atherosclerosis inhibits flow-induced vasodilation.\(^{50}\) It also has been shown by a number of investigators that atherosclerosis is not necessary to alter endothelium-dependent vasoactive reactions; rather, hypercholesterolemia is a sufficient stimulus to augment vasoconstriction and blunt vasodilation of arteries.\(^{11-14}\) The effects of atherosclerosis and hypercholesterolemia on vascular reactions appear to be fairly selective for the endothelium, because vasoactive responses to endothelium-independent substances are generally found to be normal. Within this context, we found that arteriolar responses to the endothelium-independent dilator (nitroprusside) and constrictor (acetylcholine) were unaffected in arterioles from atherosclerotic animals.

The observations indicating that hypercholesterolemia and hyperlipidemia are sufficient pathological factors to induce dysfunction of endothelial modulation of vasoactive reactions underlie the plausibility that the endothelial modulation of arteriolar tone is abnormal during the development and presence of arterial disease. Specifically, microvessels would be exposed to the same levels of circulating lipids as arteries; therefore, it would appear reasonable that microvessels also demonstrate impairment of endothelium-dependent vasoactive function. There are a few reports in the literature that support this concept. In skeletal muscle of atherosclerotic rabbits, endothelium-dependent vasodilation to acetylcholine is impaired. Microvascular endothelial dysfunction in atherosclerosis is also supported by studies of resistance vessels and small arteries within the coronary microcirculation.\(^{10,11,14}\) We have previously reported that the normal vasodilator response of coronary resistance vessels to serotonin in control monkeys is reversed to a vasoconstrictory response in atherosclerotic monkeys.\(^{11}\) Also, in undiseased small coronary arteries from atherosclerotic monkeys, Selke et al found that endothelium-dependent vasodilation to bradykinin, acetylcholine, and the calcium ionophore A23187 were impaired. Furthermore, in small arteries, hypercholesterolemia is reported to impair endothelium-dependent relaxation.\(^{14}\) We would like to emphasize that the present study is the first to unequivocally show impaired endothelium-dependent vasoactive functions in coronary arterioles downstream from atherosclerotic lesions.

It is tempting to speculate about the pathophysiological significance of impaired endothelium-dependent responses to physiological and pharmacological stimuli in the coronary microcirculation. Within this context, there are some clinical studies that report augmented coronary constrictor responses to ergonovine in the absence of gross atherosclerotic lesions in epicardial coronary arteries.\(^{56,57}\) It was postulated by these investigators that the coronary microcirculation is hyperresponsive to ergonovine. It has been speculated that in patients with syndrome X,\(^{58,59}\) inappropriate microvascular reactions occur in response to physiological challenges, leading to impaired vasodilation and augmented vasoconstriction. Perhaps the microvascular defect in these patients is related to impaired endothelial function.

**Summary and Conclusions**

In this article we report that the pathophysiological consequences of atherosclerosis in the coronary circulation extend downstream to small coronary arterioles between 30 and 70 \(\mu\)m in diameter. This abnormality is evident because normal endothelium-dependent vasodilation to serotonin, histamine, bradykinin, and ADP are all impaired and the normal flow-induced vasodilation is completely abolished. The mechanism for this effect appears to involve the synthesis and/or production of EDRF, because administration of \(L\)-arginine can restore normal endothelium-dependent function in vessels from atherosclerotic animals.
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References


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