Flow-Induced Dilation of Human Coronary Arterioles
Important Role of Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Channels

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Background—Flow-induced vasodilation (FID) is a physiological mechanism for regulating coronary flow and is mediated largely by nitric oxide (NO) in animals. Because hyperpolarizing mechanisms may play a greater role than NO in the microcirculation, we hypothesized that hyperpolarization contributes importantly to FID of human coronary arterioles.

Methods and Results—Arterioles from atria or ventricles were cannulated for videomicroscopy. Membrane potential of vascular smooth muscle cells (VSMCs) was measured simultaneously. After constriction with endothelin-1, increases in flow induced an endothelium-dependent vasodilation. Nω-Nitro-L-arginine methyl ester \(10^{-4}\) mol/L modestly impaired FID of arterioles from patients without coronary artery disease (CAD), whereas no inhibition was seen in arterioles from patients with CAD. Indomethacin \(10^{-5}\) mol/L was without effect, but 40 mmol/L KCl attenuated maximal FID. Tetraethylammonium \(10^{-3}\) mol/L but not glibenclamide \(10^{-6}\) mol/L reduced FID. Charybdotoxin \(10^{-8}\) mol/L impaired both FID (15\% \pm 3\% versus 75\% \pm 12\%, \(P<0.05\)) and hyperpolarization (\(-32\pm 2\) mV [from \(-28\pm 2\) mV after endothelin-1] versus \(-42\pm 2\) mV [\(-27\pm 2\) mV], \(P<0.05\)). Miconazole \(10^{-6}\) mol/L or 17-octadecynoic acid \(10^{-5}\) mol/L reduced FID. By multivariate analysis, age was an independent predictor for the reduced FID.

Conclusions—We conclude that shear stress induces endothelium-dependent vasodilation, hyperpolarizing VSMCs through opening Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in human coronary arterioles. In subjects without CAD, NO contributes to FID. NO and prostaglandins play no role in patients with CAD; rather, cytochrome P450 metabolites are involved. This is consistent with a role for endothelium-derived hyperpolarizing factor in FID of the human coronary microcirculation. (Circulation. 2001;103:1992-1998.)

Key Words: blood flow \(\text{\ circ}^{+}\) vasodilation \(\text{\ circ}^{+}\) microcirculation

Flow-induced vasodilation (FID) is a physiologically important mechanism regulating coronary microvascular tone. FID has been demonstrated in a variety of vessels from different species,\textsuperscript{1-5} including humans.\textsuperscript{6,7} An endothelium-dependent mechanism for FID has been demonstrated,\textsuperscript{1-5} although the endothelial factor varies, depending on species, vascular bed, and vessel size. Three principal dilator compounds are released from endothelial cells: nitric oxide (NO), prostacyclin (PGI\textsubscript{2}), and endothelium-derived hyperpolarizing factor (EDHF).\textsuperscript{8-10} Animal studies have reported that FID is mediated by either NO,\textsuperscript{2-4} PGI\textsubscript{2},\textsuperscript{5} or both,\textsuperscript{11} FID is prominent in resistance vessels as well as conduit vessels.\textsuperscript{9} NO or other factors contribute to FID of large epicardial coronary arteries in patients.\textsuperscript{12,13} In the human coronary microcirculation, agonist-induced dilation largely involves mechanisms independent of the formation of NO.\textsuperscript{10,14} There is little direct evidence that FID is endothelium-dependent in humans.

This study investigates the role of endothelial factors in the vascular response to flow in human coronary arterioles, focusing on the contributions of NO, prostaglandins, and K\textsuperscript{+} channel activation, because K\textsuperscript{+} channels are normally responsible for membrane hyperpolarization in vascular smooth muscle cells (VSMCs).

Methods

Fresh specimens of right atrial appendage and ventricle, obtained from 77 patients who were undergoing cardiac surgery, were placed in oxygenated Krebs solution (4°C) of the following composition (in mmol/L): NaCl 118, KCl 4.7, CaCl\textsubscript{2} 2.5, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 20, Na\textsubscript{2}EDTA 0.026, and dextrose 11, pH 7.4. Under microscopic guidance, coronary arterioles were carefully dissected from the endocardial surface of atrial appendage or from the epicardial surface of ventricle. Arterioles were transferred to a 20-mL organ chamber containing Krebs solution, cannulated with glass micropipettes (30 to 40 μm; identical internal diameters), and secured with opthalmic suture (10-0). The preparation was transferred to the stage of an inverted microscope (CK2, Olympus) coupled to a CCD camera (WV-BL200, Panasonic) and video micrometer (VIA-100K, Boeckeler Instruments, Inc). Micropipettes were connected to independent hydrostatic reservoirs, each raised 81.6 cm H\textsubscript{2}O (60 mm Hg) above the vessel. Krebs solution in the

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chamber was continuously recirculated at 30 mL/min (Masterflex pump, Cole Parmer Instrument Co), aerated with 20% O₂, 5% CO₂, and 75% N₂, and maintained at 37°C. Intraluminal flow was monitored with a flowmeter (model 4552, Gilmore Instruments, Inc.). Vessel preparations with leaks (flow>0) were discarded.

Flow was produced by simultaneously changing the heights of the reservoirs in equal and opposite directions to generate a pressure gradient, as described previously by Kuo et al.¹ Because the tip sizes of the 2 pipettes were similar, simultaneous changes in the heights of the reservoirs induced increases in flow without change in intraluminal pressure according to Hagen-Poiseuille’s law. The pressure gradients used in these studies correspond to those used by others.³

After 30-minute equilibration, vessels were transiently constricted with 75 mmol/L KCl. Vessels that failed to constrict >50% were discarded.

At the end of each experiment, ADP 10⁻⁸ mol/L (an endotheli-um-dependent vasodilator) was applied to confirm endothelial integrity.¹⁵ Maximal vascular diameter was determined by addition of sodium nitroprusside (SNP, 10⁻⁴ mol/L).

**Experimental Protocols**

After 30 minutes of stabilization, endothelin-1 10⁻¹⁰ to 5×10⁻¹⁰ mol/L was added to increase resting tone to 30% to 50% of passive diameter, because human coronary arterioles develop varying degrees of spontaneous myogenic tone (10% to 50%). Internal vessel diameter was then examined at different flows corresponding to pressure gradients of 5, 10, 20, and 100 cm H₂O in the absence or presence of Nω-nitro-L-arginine methyl ester (L-NAME) 10⁻⁴ mol/L (an NO synthase inhibitor) or indomethacin (INDO) 10⁻⁵ mol/L (a cyclooxygenase inhibitor). In some experiments, miconazole 10⁻⁶ mol/L (a cytochrome P450 inhibitor) or 17-octadecynoic acid (17-ODYA) 10⁻⁶ mol/L (a chemically distinct and selective cytochrome P450 inhibitor) was applied.

To examine whether membrane hyperpolarization contributed to FID, KCl 40 mmol/L rather than endothelin-1 was used to constrict vessels. In separate studies, the effect of tetraethylammonium (TEA) 10⁻⁴ mol/L (a blocker of Ca²⁺-activated K⁺ channels [KᵥCa]) or glibenclamide 10⁻⁴ mol/L (a blocker of ATP-sensitive K⁺ channels [KᵥATP]) was tested.

After the second flow-response relationship was completed, in the presence of SNP 10⁻⁴ mol/L, the diameter at a gradient of 100 cm H₂O was measured first in one direction, then with the gradient reversed. Vessel diameter always varied by <2 μm, verifying matched cannula resistances.

**Measurement of Membrane Potential**

Flow-induced changes in diameter and membrane potential (Eₘ) of VSMCs were examined simultaneously, as described previously.¹⁰ Eₘ was measured with a glass microelectrode (50 to 100 MΩ) filled with 3 mol/L KCl and connected to a high-impedance amplifier (Axoprobe, Axon Instruments). The microelectrode was manually advanced perpendicular to the long axis of the vessel and through the adventitial side.

Criteria for successful impalements included an abrupt drop in Eₘ, a steady-state value for >2 seconds, and a rapid return to baseline on electrode withdrawal.¹⁰ Successful impalements of ≥3 cells were averaged to obtain Eₘ for a single experimental protocol.¹⁰,¹⁶

To examine the role of KᵥCa flow-induced changes in Eₘ and/or diameter were measured in the presence of charybdotoxin (CTX) 10⁻⁸ mol/L.

**Endothelial Denudation**

Endothelium was mechanically denuded by injection of 2 mL of air through the vessel, then flushing with Krebs solution. Denudation was confirmed by observation of preserved dilation to SNP and markedly reduced dilation to ADP 10⁻⁴ mol/L.¹⁵

**Materials**

Endothelin-1 was obtained from Peninsula Laboratories. Other agents were obtained from Sigma Chemical Co. All pharmacological agents except 17-ODYA (applied intraluminally) were added to the external bathing solution, and the concentrations stated represent the final molar concentrations in the organ chamber.

**Patient Data**

Demographic data and diagnoses were obtained from hospital patient information recorded at the time of surgery. Protocols were approved by both the University of Iowa and Medical College of Wisconsin Institutional Review Boards.

**Statistical Analyses**

FID or dilation to SNP is expressed as a percentage, with 100 representing the change from the constricted diameter (endothelin-1 or KCl) to the maximal diameter. Statistical comparisons of maximal percent vasodilation and Eₘ values under different treatments were performed by paired or unpaired Student’s t test. A 2-factor repeated-measures ANOVA was used to compare dose-response relationships between treatment groups. Corollary dose-specific contrasts were tested with the Bonferroni-adjusted t test whenever the interactions were statistically significant. Multiple stepwise regression analyses were used to detect the influence of underlying diseases, age, and sex on FID. Regression models were constructed for all doses. All procedures were performed with “proc mixed” and “proc reg” programs of SAS for Windows, version 8. Statistical significance was defined as a value of P<0.05. All data are described as mean±SEM. For all data, n indicates the number of patients.

**Results**

A total of 99 atrial and 4 ventricular vessels with a mean internal diameter of 91±4 μm and a range of 40 to 212 μm were dissected (n=103).

Figure 1 shows that flow is a potent dilator of both atrial and ventricular coronary arterioles. Pressure gradients of 5, 10, 20, and 100 cm H₂O elicited intraluminal flows of 1.1±0.3, 2.9±0.6, 5.4±1.1, and 13.7±1.9 μL/min, respectively. Corresponding vasodilation was 9±2%, 27±4%, 49±4%, and 69±4% in atrial tissue and 9±6%, 36±12%, 63±15%, and 72±13% in ventricular tissue (P=NS, ventricular versus atrial).

Patient demographics and diagnoses are summarized in the Table. FID was significantly reduced by aging but not by underlying diseases (diabetes, hypertension, hypercholesterolemia, congestive heart failure, coronary artery disease [CAD], and myocardial infarction) or sex (P<0.05).
The effect of endothelial denudation on FID is shown in Figure 2. FID in human coronary arterioles was abolished in denuded vessels, which established the necessary role of the endothelium. Denudation was confirmed by reduced dilation to ADP (4 ± 6%, n = 5, P < 0.05 versus intact vessels, 86 ± 5%, n = 14, unpaired t test).

The effect of inhibiting NO synthase is shown in Figure 3. Treatment with L-NAME (10⁻⁴ mol/L) did not reduce FID (68 ± 8% versus control 60 ± 7% at 100 cm H₂O; P = NS, n = 11). In the subgroup of patients without CAD, however, L-NAME modestly decreased FID (Figure 3A; dilation at 5, 10, 20, and 100 cm H₂O gradient = 20 ± 11%, 46 ± 8%, 56 ± 8%, and 73 ± 7% before and 2 ± 2%, 19 ± 6%, 31 ± 7%, and 69 ± 9% after L-NAME, P < 0.05, n = 5). L-NAME did not affect FID in the subgroup of patients with CAD (Figure 3B; 3 ± 2%, 26 ± 10%, 47 ± 9%, and 49 ± 10% before and 10 ± 3%, 27 ± 7%, 48 ± 10%, and 67 ± 10% after L-NAME, P = NS, n = 6). Thus, NO synthesis contributes partially to FID in “normal” human coronary arterioles, whereas other factors may compensate for the loss of NO in the presence of CAD.

Because cyclooxygenase products mediate FID in rat cremaster muscle arterioles,⁵ we tested the effect of INDO. INDO did not alter FID (Figure 4A, 63 ± 7% versus control 67 ± 15% at 100 cm H₂O gradient, P = NS).

Arachidonic acid can be metabolized by cytochrome P450 to produce EDHF.⁸,⁹,¹⁷ We examined the effect of inhibiting cytochrome P450 on FID. Miconazole reduced FID (Figure 4B, maximum dilation reduced from 72 ± 8% to 18 ± 12%, P < 0.05). 17-ODYA markedly reduced FID in the presence of L-NAME and INDO (Figure 4C, 24 ± 5% versus control 69 ± 8% at 100 cm H₂O gradient, P < 0.05).

Activation of K⁺ channels with concomitant hyperpolarization of VSMCs has been described as an important mechanism underlying vasodilation, especially in arterioles.⁸,¹⁰,¹⁸ When KCl instead of endothelin-1 was used to constrict vessels to 30% to 50% of resting diameter, FID was reduced (Figure 5. P < 0.05 at 20 and 100 cm H₂O). Dilation to SNP was similar when vessels were constricted with either KCl (86 ± 6%) or endothelin-1 (95 ± 2%; P = NS).

We next evaluated the contribution of both Kₐ₅ and Kₐ₆ channels to FID. Both Kₐ₅ and Kₐ₆ channels are present on human coronary arteries.¹⁹ Glibenclamide, however, had no effect on FID (Figure 6A; 74 ± 8% after glibenclamide versus control 72 ± 9% at 100 cm H₂O gradient, P = NS). Treatment...
with TEA reduced FID ($P<0.05$ at 10, 20, and 100 cm H$_2$O, Figure 6B) but did not alter vasodilation to SNP (92±3% versus control 88±3%, $P=\text{NS}$). These findings suggest that FID involves activation of K$_{\text{Ca}}$ in human coronary arterioles.

To examine the role of hyperpolarization, we simultaneously measured changes in both $E_m$ and vascular diameter. Figure 7A shows a series of sample $E_m$ traces from microelectrode impalements of VSMCs. Endothelin-1 constricted the vessel and caused a depolarization of VSMCs. Flow produced graded vasodilation and hyperpolarization, with the maximal effect occurring at 100 cm H$_2$O. In separate vessels, addition of CTX attenuated both hyperpolarization and vasodilation (Figure 7B). CTX inhibited both effects of flow but did not alter the vasodilation to SNP (87±4% versus control 94±3%, $P=\text{NS}$).

**Discussion**

This study is the first to directly examine FID in human coronary arterioles. The major new findings are 4-fold. First, flow induces a potent vasodilation in both atrial and ventricular coronary arterioles. Second, FID is endothelium-dependent and requires cytochrome P450 metabolites but not prostaglandins. NO contributes modestly to FID in arterioles from patients without CAD but does not play a role in arterioles from patients with CAD. Third, FID occurs as a result of membrane hyperpolarization consequent to the opening of K$_{\text{Ca}}$ in VSMCs. Fourth, age is significantly correlated with reduced FID. Taken together, these findings indicate that endothelium-derived vasodilator metabolites of
cytochrome P450 play an important role in FID in human coronary arterioles. This substance is most likely EDHF.

Although other studies have examined FID in several vascular beds, both animal and human, 1–7,11–13 this is the first direct demonstration of FID in human coronary resistance vessels. This is also the first demonstration of FID due to EDHF in the coronary microcirculation. In vivo experiments on the coronary microcirculation are complicated by the confounding influences of cardiac metabolism, extravascular compressive forces, and neurohumoral agents. Our findings provide clear evidence that FID is an intrinsic property of both atrial and ventricular coronary arterioles.

FID Is Endothelium-Dependent

Animal studies have demonstrated that an intact endothelium is necessary for FID. 1–5,11 Effluent from a porcine coronary arteriole with an intact endothelium subjected to flow induces vasodilation in an endothelium-denuded vessel. 4 The perfusate from flow-stimulated cultured endothelial cells also evokes vasorelaxation in rabbit iliac arteries. 20 These findings confirm that an endothelium-derived transferable factor is responsible for FID.

NO, 4,7,13 PGI₂, 5 or both 11 have been proposed as mediators of FID. In the rat, NO is the mediator of FID in cerebral arterioles, 21 and PGI₂ is the mediator in cremaster muscle arterioles, 5 and both contribute to FID in gracilis muscle arterioles. 11

In the present study, we demonstrated that L-NAME slightly reduced endothelium-dependent FID of arterioles from patients without CAD, whereas no inhibition was seen in patients with CAD. Development of CAD decreases NO formation at rest and in response to acetylcholine in the human coronary circulation. 22 A recent study in vivo shows that vasorelaxing factors other than NO primarily mediate FID in nonatherosclerotic human conduit coronary arteries. 12 INDO did not affect FID in the present study, suggesting that some factor other than NO or PGI₂ is involved in FID in human coronary arterioles. We speculate that this factor may act as a compensatory mechanism for impaired bioavailability of NO in diseases.

Endothelium-Derived Hyperpolarizing Factor

Endothelium-dependent vasodilation is often mediated by a transferable substance distinct from NO or PGI₂. This unidentified vasoactive substance has been called “endothelium-derived hyperpolarizing factor” (EDHF) because the dilation is associated with hyperpolarization of VSMCs. EDHF activates K⁺ channels, leading to hyperpolarization and vasodilation. 8,10,17 Although the chemical nature of EDHF is not firmly established, strong evidence suggests that it is a metabolite of arachidonic acid derived from cytochrome P450. 8–10,17 Inhibition by miconazole and 17-ODYA in the present study is consistent with the concept that EDHF mediates FID in the diseased human heart. Inhibition of FID by blockers of K⁺, and demonstration of flow-induced hyperpolarization supports a role for EDHF. 10

K⁺ Channels

Several lines of evidence implicate K⁺ in the response to flow. FID was greatly attenuated when KCl instead of endothelin-1 was used to constrict the vessels. By depolarizing the membrane, KCl may have already activated voltage-dependent Ca²⁺ channels that were involved in the flow-induced response. In addition, flow-induced activation of K⁺ channels in the presence of high external K⁺ concentration may have been less effective in causing membrane hyperpolarization, because the K⁺ equilibrium potential was lowered to a value close to the resting E_m. FID was also attenuated by TEA and CTX but not glibenclamide. Nevertheless, TEA-sensitive K⁺ channels probably do not account fully for FID in human coronary arterioles, because substantial dilation remained in the presence of the inhibitor. Distinct K⁺ channels or other mechanisms (eg, Na⁺-K⁺ exchange, alternative arachidonic acid derivatives 23 ) may play a role in the dilation.

Potential Limitations

Animal studies have demonstrated that NO-mediated FID is impaired in coronary arterioles of diet-induced atherosclerotic swine. 24 Human in vivo studies have also reported that FID is impaired in atherosclerotic conduit coronary arteries. 6
Because most vessels used in these experiments were from patients with CAD, our results may have been skewed toward identifying a reduced role of NO and a more prominent, perhaps compensatory, role for EDHF in FID. This would be consistent with the observation that hyperpolarizing mechanisms may be preserved or even enhanced in hypercholesterolemia, where NO bioavailability is reduced. This idea is also supported by the observation that in subjects without CAD, there is a significant contribution of NO to FID of coronary arterioles.

It is reported that aging selectively causes endothelial dysfunction of coronary arterioles, whereas endothelial dysfunction is restricted to conduit coronary arteries in age-matched patients with angiographic evidence of coronary atherosclerosis. In most of our patients, NO did not contribute substantially to FID; thus, it may be expected that risk factors would not have the same detrimental influence on FID.

It is possible that the prominent FID in human coronary arterioles from subjects with CAD indicates that we are interrogating a portion of the vasculature that contributes minimally to resistance. In conditions in which flow reserve is reduced, such as maximal dilation or in the presence of an epicardial stenosis, resistance may be shifted to small-caliber vessels. Although this possibility cannot be excluded, the vessels we examined are identical in size to those that contribute most to coronary resistance in animal models.

Hyperpolarizing mechanisms may be especially important in the microvasculature, because the contribution of EDHF to endothelium-dependent vasorelaxation increases inversely with vessel size. Thus, in human coronary resistance vessels, EDHF may contribute more than NO to FID. It is important to note that although FID was observed in both atrial and ventricular arterioles, because of tissue availability, the mechanisms involved were tested only in atrial tissue. We cannot exclude the possibility that a different mechanism of dilation occurs in ventricular arterioles.

It is possible that NO or PG12 may have contributed to vasodilation and hyperpolarization in this preparation because of insufficient inhibition of NO synthase or cyclooxygenase. This is unlikely, however, because the concentrations of inhibitors used were sufficiently high to inhibit these enzymes in similar in vitro studies. It has been reported that millimolar doses of NO synthase inhibitors may be necessary to block formation of NO in some vessels. Even 10^{-7} mol/L L-NAME, however, did not alter FID in vessels from subjects with CAD (data not shown).

The K^+ channel blockers used in these experiments, such as CTX, may act on the endothelium as well as VSMCs, potentially blocking the release or synthesis of endothelium-derived relaxing factors. We cannot exclude this possibility in the present study. Flow-stimulated increases in endothelial K^+ efflux and [Ca^{2+}], are not altered, however, by high K^+ concentrations or K^+ channel–blocking agents, such as tetraethylammonium and CTX. Furthermore, CTX does not inhibit shear stress–dependent release of NO in rabbit femoral arteries.

In addition to inhibition of cytochrome P450, miconazole has been reported to inhibit K^+ channels and tyrosine kinase activity, suggesting the nonspecific effects of miconazole on FID. In contrast, 17-ODYA does not directly affect these activities.

Clinical Implications
Porcine coronary resistance arteries between 80 and 150 μm in diameter are more sensitive to flow than either larger or smaller arteries. Furthermore, the magnitude of FID in large conduit arteries appears to be less than that in resistance arteries. The vessels used in this study had an average diameter of 91 μm and were quite sensitive to flow, consistent with an important role for flow in the regulation of human coronary vascular resistance.

The prominent role of EDHF in coronary arteriolar FID distinguishes the human from the animal models and suggests a mechanism for preserving myocardial perfusion in conditions such as diabetes, CAD, and hypertension, in which NO bioavailability is reduced. This is in contrast to conduit vessels, in which the presence of similar disease states results in reduced FID.

Conclusions
An increase in luminal flow induces potent endothelium-dependent vasodilation of human coronary arterioles. The human coronary microvasculature appears to be unique in that FID is associated largely with hyperpolarization of VSMCs and K_E, opening.

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