Human Coronary Arteriolar Dilation to Arachidonic Acid Depends on Cytochrome P-450 Monooxygenase and Ca\(^{2+}\)-Activated K\(^+\) Channels

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Abstract—Endothelium-dependent hyperpolarization of vascular smooth muscle cells (VSMCs) plays a crucial role in regulating vascular tone, especially in resistance vessels. It has been proposed that metabolites of arachidonic acid (AA), formed by cytochrome P-450 monooxygenase (P450), are endothelium-derived hyperpolarizing factors (EDHFs). These metabolites have been reported to mediate dilation to endogenous vasoactive compounds, such as bradykinin and acetylcholine. However, it is not known whether these metabolites of AA contribute to dilation of human resistance vessels. This is important since it has been proposed that EDHF serves as a compensatory mechanism to maintain dilation in disease states. Therefore, we studied the effect of AA on vessel diameter and VSMC membrane potential in isolated human coronary microvessels. Arterioles (81±5 \(\mu\)m, \(n=70\)) were dissected from right atrial appendages at the time of cardiac surgery and cannulated at a distending pressure of 60 mm Hg and zero flow. Changes in internal diameter were recorded with videomicroscopy. Some vessels were impaled with glass microelectrodes to measure membrane potential of VSMCs while internal diameters were simultaneously recorded. After constriction (47±2\% with endothelin-1, AA (10\(^{-10}\) to 10\(^{-5}\) mol/L) induced substantial dilation of human coronary microvessels, which was abolished by removal of the endothelium. Treatment with 17-octadecynoic acid (17-ODYA, 10\(^{-5}\) mol/L; a P450 inhibitor) attenuated maximal dilation to AA (49±9\% versus 91±4\% [control]; \(P<0.05\) versus control), whereas indomethacin (INDO, 10\(^{-5}\) mol/L; a cyclooxygenase inhibitor) and N\(^{n}\)-nitro-\(L\)-arginine methyl ester (L-NAME, 10\(^{-4}\) mol/L; a NO synthase inhibitor) were without effect. Both 17-ODYA and miconazole (10\(^{-3}\) mol/L, a chemically distinct P450 inhibitor) further reduced the dilation to AA in the presence of INDO. The presence of 40 mmol/L KCl or charybdotoxin (10\(^{-5}\) mol/L, a blocker of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels) impaired dilation to AA (19±9\% [KCl versus 76±5\% [control] and 47±6\% [charybdotoxin versus 91±3\% [control]; \(P<0.05\) for both). After depolarization with endothelin-1 (−26±1 mV from −48±3 mV [before endothelin]), AA (10\(^{-5}\) mol/L) in the presence of INDO and L-NAME induced hyperpolarization of VSMCs (−57±5 mV). In the presence of 17-ODYA and miconazole (10\(^{-3}\) mol/L), endothelin produced similar depolarization (−26±2 mV from −48±3 mV), but hyperpolarization to AA was reduced (−33±2 mV; \(P<0.05\) versus absence of 17-ODYA). AA metabolites formed primarily by P450 produce potent endothelium-dependent dilation of human coronary arterioles via opening of Ca\(^{2+}\)-activated K\(^+\) channels and hyperpolarization of VSMCs. These findings support an important role for P450 metabolites in the regulation of human coronary arteriolar tone. (Circ Res. 1998;83:501-507.)

Key Words: arachidonic acid ■ vasodilation ■ coronary circulation ■ human ■ K\(^+\) channel

Vascular endothelial cells contribute to the regulation of vascular tone by releasing at least 3 vasoactive compounds: endothelium-derived relaxing factor (identified as NO), prostacyclin (PGI\(_2\)), and endothelium-derived hyperpolarizing factor (EDHF).\(^{1-4}\)

A common intracellular response to agonist stimulation is activation of endothelial phospholipases, which release arachidonic acid (AA) from the cell membrane.\(^{5}\) AA may then be metabolized by cyclooxygenase, lipoxygenase, or cytochrome P-450 monooxygenase (P450) to vasoactive substances.\(^{6-10}\) A number of these substances, including the P450-derived epoxygenesatrienoic acids, can effect vasorelaxation by hyperpolarizing vascular smooth muscle cells (VSMCs).\(^{11,12}\) Thus, P450 metabolites of AA are excellent candidates for EDHF. However, whether AA can elicit dilation in human coronary arterioles is not known. Furthermore, the potential role of metabolites of AA in coronary microvascular responses has not been investigated. The contribution of hyperpolarization to endothelium-dependent vasorelaxation increases inversely with vessel size, whereas the contribution of NO decreases.\(^{13-15}\) Furthermore, it has been proposed that in disease states EDHF may compensate for the loss of NO-mediated dilation.\(^{16,17}\) Thus, in resistance vessels, particularly vessels from patients with coronary
502 Human Coronary Arterioles, Cytochrome P-450, and AA

disease, factors other than NO play a major role in endothelium-dependent vasodilation.

The purpose of the present study was to investigate the role of P450, cyclooxygenase, and lipooxygenase metabolites in AA-induced dilation of coronary arterioles from humans undergoing cardiopulmonary bypass and to determine whether the mechanism of dilation involves hyperpolarization of VSMCs consequent to activation of K⁺ channels.

Materials and Methods

General Preparation

Human coronary arterioles were dissected from fresh pieces of human right atrial appendage obtained from 39 patients (60 ± 13 years of age; male, 30 patients; female, 9 patients) undergoing valve replacement (aortic, n = 2; mitral, n = 1) and/or coronary bypass graft surgery (n = 36). After surgical removal, the atrial appendage was placed in cold oxygenated Krebs buffer solution (4°C) composed of (mmol/L) NaCl 118, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 20, Na₂EDTA 0.026, and dextrose 11, pH 7.4, and transported to the laboratory. Under microscopic guidance, coronary arterioles (internal diameter, 29 to 161 μm; mean, 81 ± 5 μm; n = 70) were carefully dissected from the endocardial surface of the atrial appendage and transferred to a 20-mL organ chamber containing Krebs solution. One end of the vessel was secured to a glass micropipette filled with Krebs buffer using 10-0 ophthalmic suture (Ethicon, Inc). The other end was cannulated with a second micropipette, and 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). Internal vascular diameters were measured throughout the experiment with the manually adjusted video micrometer. Micropipettes were connected to independent hydrostatic reservoirs at 60 mm Hg without flow. The Krebs solution in the chamber was continuously recirculated at 30 mL/min (Masterflex, Cole Parmer Instrument Co), aerated with 20% O₂, 5% CO₂, and 75% N₂, and maintained at 37°C by an external heat changer. All pharmacological agents were added to the external bathing solution.

After 30 minutes of equilibration, vessels were transiently constricted with 75 mmol/L KCl to determine vessel viability. Vessels that failed to constrict >30% were discarded. Vessels with the appropriate response to KCl showed endothelium-dependent vasodilation to ADP (10⁻⁵ mol/L), confirming the integrity of endothelial function (82 ± 5%, n = 8). Experimental Protocols

Human coronary arterioles were set to their in situ length at 60 mm Hg without flow. After a stabilization period of 60 minutes, vessels were constricted to 30% to 60% of resting diameter with endothelin-1. The vascular responses to increasing concentrations of AA (10⁻¹⁰ to 10⁻³ mol/L) were examined in the presence and absence of Nω-nitro-L-arginine methyl ester (L-NAME, 10⁻⁴ mol/L; NO synthase inhibitor), indomethacin (INDO, 10⁻⁶ mol/L; cyclooxygenase inhibitor), miconazole (10⁻⁶ mol/L; 17-OXY, 10⁻⁵ mol/L; P450 inhibitor), or baicalin (10⁻⁶ mol/L; lipooxygenase inhibitor). In some experiments, combinations of ≥2 of these inhibitors were introduced into the organ bath 30 minutes before constriction with endothelin.

To examine whether K⁺ channels contribute to AA dilation, KCl (~40 mmol/L) rather than endothelin-1 was used to constrict vessels by 30% to 60%. Since large-conductance Ca²⁺-activated K⁺ channels (BKCa channels) may be involved in EDHF-induced vasodilation, the effect of charybdotoxin (CTX, 10⁻⁶ mol/L; a selective BKCa channel blocker) on AA dilation was also examined in the presence of INDO.

We tested also the effect of 5,8,11,14-eicosatetraynoic acid (ETYA, 10⁻⁶ to 10⁻³ mol/L) to evaluate the possibility that AA may directly produce vasodilation through the activation of K⁺ channels. This AA congener is not metabolized yet has been demonstrated to directly activate K⁺ channels.

Endothelial Denudation

In some experiments, the endothelium was mechanically denuded. This was accomplished by gently moving a human hair back and forth through the vessel lumen 4 or 5 times. Then after attaching one end of the vessel to a pipette, 0.1 to 0.5 mL of air was slowly passed through the vessel. Denudation was confirmed by preservation of dilation to sodium nitroprusside (SNP) and marked reduction in dilation to ADP (10⁻⁴ mol/L). In these experiments, dilation to SNP was calculated as a percentage of the difference between the constricted diameter and the maximal pressurized diameter in cooled Krebs buffer.

Measurement of Membrane Potential

In separate experiments, we simultaneously examined changes in vessel diameter and VSMC membrane potential (Em) in response to AA. Em was measured with glass microelectrodes filled with 3 mol/L KCl and connected to a high-impedance biological amplifier (Axo-clamp). Microelectrodes had impedances of 40 to 90 MΩ, with estimated tip sizes of 0.1 to 0.2 μm and tip potentials of ≤5 mV.

Arterioles were cannulated, pressurized, and suspended in a tissue bath for diameter measurement. The microelectrode was secured to a glass micropipette filled with Krebs buffer using 10-0 ophthalmic suture (Ethicon, Inc). The other end was cannulated with a second micropipette, and 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). Internal vascular diameters were measured throughout the experiment with the manually adjusted video micrometer. Micropipettes were connected to independent hydrostatic reservoirs at 60 mm Hg without flow. The Krebs solution in the chamber was continuously recirculated at 30 mL/min (Masterflex, Cole Parmer Instrument Co), aerated with 20% O₂, 5% CO₂, and 75% N₂, and maintained at 37°C by an external heat changer. All pharmacological agents were added to the external bathing solution.

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Figure 1. Endothelium-dependent dilation to AA. A, Dose-response curve of human coronary arterioles to AA or vehicle. In endothelin-constricted vessels, AA produced potent dilation of human coronary arterioles (n = 22), with an ED₅₀ of 7.2 ± 0.2. In the vehicle group (n = 5), constriction with endothelin-1 was maintained throughout the experiment. In this comparison, vessels were not paired for analysis. B, Effect of mechanical removal of the endothelium on coronary arteriolar dilation to AA, ADP, and SNP. Although dilation to SNP was not altered, dilation to AA was abolished and dilation to ADP was markedly attenuated, indicating endothelial dependence of the response. Values represent mean ± SE.
with 1% bovine serum albumin. L-NAME, CTX, SNP, and ADP were dissolved in distilled water. All concentrations represent the final molar concentrations in the organ chambers. The addition of pharmacological agents produced <1% change in the volume of the circulating bath. None of the pharmacological antagonists produced significant changes in baseline vessel diameter.

Patient demographic data and diagnoses were obtained from hospital patient information recorded at the time of surgery.

Statistical Analysis

Vessels from each patient were studied with vehicle (control) or with a pharmacological inhibitor. This allowed for paired analysis, thereby minimizing the effects of factors such as underlying diseases, age, and sex. Results are expressed as percent dilation, with 100% representing the change in diameter to SNP (10^{-4} mol/L) from the diameter elicited by endothelin-1. Statistical comparisons of the percent of vasodilation under different treatments were performed by 2-way ANOVA with repeated measures, followed by the Bonferroni test to detect individual differences. To compare the sensitivities of the agents used, ED_{50} values (negative logarithm of the molar concentration of vasodilator that produced 50% of the maximal dilation to the agonist) were calculated. Percent maximal dilations, ED_{50} values, and Em were compared by the Student t test. Multiple stepwise regression analyses were likewise carried out to detect the influence of underlying diseases, age, and sex on the vasodilation to AA. All procedures were carried out by using programs (proc mixed and proc reg) of SAS for Windows, version 6.12. Statistical significance was defined as P<0.05. All data were described as mean±SEM. For all data, n indicates the number of patients.

Results

AA produced concentration-dependent dilations in human coronary arterioles. Maximal dilation to AA was 82±3% (Figure 1A). Baseline demographic data are summarized in the Table. By multiple stepwise regression analysis, vasodilation to AA was not influenced by underlying disease (diabetes, hypertension, hypercholesterolemia, or congestive heart failure), sex, or age. Since all but 3 patients had coronary disease, we cannot exclude an effect of conduit coronary atherosclerosis on microvessel dilation to AA.

Pretreatment with L-NAME (10^{-4} mol/L), a NO synthase inhibitor, did not alter dilation to AA (maximum dilation, 85±6% versus 86±4% [control]; P=NS). INDO (10^{-5} mol/L), a cyclooxygenase inhibitor, did not alter the response to AA (maximum dilation, 68±4% versus 76±5% [control]; P=NS) (Figure 2).

K+ channels have been reported to mediate vasodilation to AA metabolites produced by the P450 pathway. When KCl instead of endothelin-1 was used to constict vessels, markedly reduced vasodilation to AA was observed in the presence of INDO (maximum dilation, 19±9% versus 76±5% (Figure 2A).
[endothelin-1]; P<0.05) (Figure 3A), whereas dilator responses to 10^{-4} mol/L SNP were unchanged (82±11% versus 86±9% [endothelin-1]; P=NS). This suggests that human coronary arteriolar dilation to AA involves a hyperpolarization mechanism mediated by K^+ channel activation. However, a direct interaction between AA and endothelin cannot be excluded.

We also tested the effect of CTX, a selective BK_{Ca} channel blocker. In the presence of INDO, CTX (10^{-4} mol/L) attenuated vasodilation to AA (maximum dilation, 47±6% versus 91±3% [control]; P<0.05) (Figure 3B).

Since recent animal studies indicate that P450 metabolites of AA dilate vessels through hyperpolarization,^5,11 we tested the effect of 17-ODYA, a P450 inhibitor, on AA-induced dilation. 17-ODYA (10^{-5} mol/L) reduced vasodilation to AA (maximum dilation, 49±9% versus 91±4% [control]; P<0.05) (Figure 4). In the presence of INDO, 17-ODYA further inhibited the maximal dilation to AA (maximum dilation, 32±8% versus 78±7% [control]; P<0.05) (Figure 4C). We also studied the effect of miconazole, a chemically distinct inhibitor of P450. In the presence of INDO, addition of miconazole (10^{-5} mol/L) inhibited maximal coronary arteriolar dilation to AA (27±4% versus 75±7% [control]; P<0.05) (Figure 4D) to an extent similar to that with 17-ODYA.

The effect of baicalein, a selective inhibitor of lipoxygenase, on human coronary arteriolar dilation to AA was tested. Vessels were treated with INDO to block cyclooxygenases, since this may increase flux through the lipoxygenase pathway.^9 At a dose that is reported to inhibit lipoxygenase in vitro,^20 baicalein (10^{-5} mol/L) had no effect on the coronary arteriolar response to AA in the presence of INDO (maximum dilation, 54±10% versus 68±6% [control]; P=NS).

In some experiments, all 3 pathways of AA metabolism were inhibited. The combination of INDO and baicalein did not affect the dilation to AA. Addition of 17-ODYA to INDO and baicalein reduced vasodilation to AA (maximum dilation, 30±4% versus 54±10% [INDO+baicalein]; P<0.05) (Figure 4B). Dilation to SNP (10^{-4} mol/L) was similar in the presence or absence of each antagonist used.

The role of the endothelium in the coronary arteriolar dilation to AA was tested in vessels from 4 patients (Figure 1B). In vessels from which the endothelium was removed, dilation to AA (10^{-6} mol/L) was abolished, whereas dilation to the known endothelium-independent dilator, SNP, was not altered. This indicates that the denudation procedure did not produce nonspecific damage to the vessel. Dilation to ADP, previously shown to be endothelium dependent in human coronary arterioles,^13 was also markedly reduced by mechanical removal of the endothelium. Thus, dilation to AA is endothelium dependent in human coronary arterioles.

It is possible that AA may directly produce vasodilation through activation of K^+ channels, independent of conversion to vasoactive metabolites. Therefore, we tested the effect of ETYA (10^{-10} to 10^{-5} mol/L), an AA congener that is not further metabolized. ETYA has been demonstrated to directly affect the vasodilation to AA (10^{-5} mol/L, a cyclooxygenase inhibitor) and baicalein (10^{-5} mol/L, a lipoxygenase inhibitor) did not alter the response to AA (10^{-10} to 10^{-5} mol/L). Addition of 17-ODYA (10^{-5} mol/L, a P450 inhibitor) attenuated vasodilation to AA. ED_{50} was 6.7±0.6 vs 6.9±0.4 (INDO+baicalein) (P=NS, n=5). C, The presence of INDO (10^{-5} mol/L) potentiated the inhibitory effect of 17-ODYA (10^{-5} mol/L, n=6) on AA (10^{-10} to 10^{-5} mol/L)-induced vasodilation. D, Vessels were incubated with miconazole (10^{-5} mol/L, a chemically distinct P450 inhibitor; n=6), which produced a similar inhibition of vasodilation to AA (10^{-5} to 10^{-5} mol/L) as that produced by 17-ODYA. Neither miconazole nor 17-ODYA affected the ED_{50} values for AA-induced dilation in the presence of INDO (6.6±0.5 [miconazole] vs 7.0±0.5 [control] and 7.0±0.2 [17-ODYA] vs 7.3±0.5 [control]; P=NS for each). Values represent mean±SE. #P<0.05 vs endothelin-1 (A) or control (B).
activate K⁺ channels. Vasodilation to ETYA was minimal (maximum dilation, 18±5% versus 76±5% [AA]; P<0.05) (Figure 5), confirming that dilation to AA is mediated primarily through conversion to metabolites.

Membrane hyperpolarization can only be inferred by pharmacological approaches using K⁺ channel closing agents. Therefore, we directly examined the effect of AA on Em. Figure 6 shows sample Em traces from microelectrode impalements of VSMCs. Data are summarized in Figure 7. In vessels treated with L-NAME+INDO and constricted and depolarized with endothelin-1, AA hyperpolarized and dilated arterioles in a dose-dependent manner. Subsequent addition of 17-ODYA attenuated both the hyperpolarization and vasodilation to AA, whereas incubation with 17-ODYA did not alter resting vascular tone. This suggests that P450 metabolites of AA contribute to human coronary arteriolar dilation to AA through VSMC hyperpolarization.

Discussion
The present study is the first to examine human coronary arteriolar vasodilation and hyperpolarization to AA, a potentially important mediator of endothelium-dependent vasodilation. The major new findings are 3-fold. First, AA is a potent endothelium-dependent dilator of human coronary arterioles. Second, vasodilation to AA is much more dependent on metabolism by P450 than by cyclooxygenase or lipoxygenase or activation of NO synthase. Third, AA produces VSMC membrane hyperpolarization and dilation of human coronary resistance arteries consequent to the opening of BKCa channels. Taken together, these findings indicate that endothelium-derived P450 metabolites of AA mediate human coronary arteriolar dilation through a hyperpolarization of vascular smooth muscle, providing evidence of a role for metabolites of P450 as an EDHF in regulating myocardial perfusion in humans.

A common mechanism for vasodilation to endogenous agonists involves activation of phospholipases, resulting in liberation of AA from cell membranes. Products of AA metabolism through any of 3 major pathways (cyclooxygenase, lipoxygenase, or P450) can elicit dilation. This mechanism of dilation has been well documented in conduit arteries. However, relatively little is known regarding the presence or mechanism of AA-induced dilation in resistance vessels. Koller and Kaley demonstrated that in skeletal muscle arterioles, cyclooxygenase products are responsible for dilation to AA. AA also produces dilation of canine arterioles in vivo, and recent preliminary data suggest that epoxyeicosatrienoic acids, specific metabolites of P450, are
potent dilators of canine coronary arterioles.\textsuperscript{27} We have extended these observations by demonstrating that AA dilates human coronary arterioles and that the mechanism involves metabolic conversion through P450 in the endothelium. These findings may have important implications for endogenous mechanisms of vasodilation to agonists such as bradykinin\textsuperscript{28} or to shear stress,\textsuperscript{29} both of which activate phospholipase A\textsubscript{2}, liberating AA in endothelial cells. Preliminary data from our laboratory support a role for EDHF in dilation of human coronary arterioles to both shear\textsuperscript{30,31} and to bradykinin.\textsuperscript{32}

Recent studies suggest that metabolites of AA induce vasodilation by opening BK\textsubscript{Ca} channels in VSMCs.\textsuperscript{5,11} In the present study, AA induced potent dilation of human coronary arterioles, which was inhibited by nonspecifically blocking K\textsuperscript{+} channel activation with extraluminal KCl or by endothelial denudation. Using the selective inhibitor CTX, we determined that BK\textsubscript{Ca} channels on VSMCs play an important role in the coronary dilation to AA. This is consistent with the involvement of EDHF.

Pfister and Campbell\textsuperscript{13} showed that inhibitors of NO synthase have no effect on AA-induced relaxation in rabbit aorta. In addition, AA relaxed rabbit aorta without changes in cAMP and cGMP levels.\textsuperscript{33} The vasodilator effect of P450 metabolites also is not associated with increased tissue levels of cAMP and cGMP.\textsuperscript{11} These studies are consistent with our finding that L-NAME did not alter human coronary arteriolar dilation to AA.

Vascular endothelial cell cyclooxygenases metabolize AA to prostaglandins and thromboxanes, which can modulate coronary arteriolar tone.\textsuperscript{34,35} In the present study, however, indomethacin, a specific cyclooxygenase inhibitor, did not alter the response to AA, suggesting that cyclooxygenase metabolism does not play a major role in regulating human coronary arteriolar tone to AA.

Inhibition of cyclooxygenase has been reported to potentiate vasorelaxation to AA in rabbit aorta by increasing flux through the lipoxygenase pathway.\textsuperscript{8} However, in the presence of indomethacin, we observed no further inhibition of dilation to AA after administration of baicalein, a lipoxygenase inhibitor. Interestingly, the combination of baicalein and indomethacin modestly attenuated dilation to AA compared with control responses without either inhibitor. This suggests a minor role for products from both metabolic pathways in mediating dilation to AA, since no inhibition was observed by blocking either pathway alone. Thus, the majority of the response was due to another metabolite of AA.

Potential Problems
In all studies using pharmacological antagonists, specificity is critical. This is especially true for inhibitors of P450 enzymes, since numerous isoforms of the enzyme exist in mammalian cells, and many inhibitors are not selective. Thus, a limitation of the present study is the possibility of nonspecific effects of antagonist compounds. However, for 2 reasons we believe that this possibility has been minimized. First, 2 chemically distinct inhibitors of P450 were used. Both miconazole and 17-ODYA attenuated the human coronary arteriolar responses to AA. Although imidazole derivatives like miconazole can block K\textsuperscript{+} channels directly,\textsuperscript{39} such an action for 17-ODYA has not been reported. Second, changes in Em paralleled changes in vasodilation in the presence of inhibitors. Thus, we conclude that the attenuated dilation of human coronary arterioles to AA is likely due to inhibition of the conversion of AA to P450 products.

AA can directly activate K\textsuperscript{+} channels in vascular smooth muscle cells.\textsuperscript{31,37} In the present study, ETYA, a nonmetabolizable analogue of AA that can also directly activate K\textsuperscript{+} channels in coronary smooth muscle cells,\textsuperscript{21,38} produced minimal vasodilation. This, together with the inhibitory effects of 17-ODYA and miconazole, indicates that human coronary arteriolar dilation to AA is more dependent on P450 metabolites than on a direct effect of AA. Nevertheless, a residual dilation to AA was observed after inhibition of all 3 pathways. This dilation could result from submaximal doses of blocking agents, a direct vasodilator effect of AA (Figure 5), or involvement of a fourth metabolic pathway, as suggested by Lonigro et al.\textsuperscript{7}

The importance of an intact endothelium on the response to AA was demonstrated in the present study. This is consistent with the dependence on P450, which is much more prominent in endothelial cells than in VSMCs,\textsuperscript{39,40} and with previous investigations showing that endothelial denudation abolishes AA-induced vasorelaxation.\textsuperscript{5,39}

We studied a variety of patients with diseases that can affect vasodilator responses. However, neither coronary risk factors (hypercholesterolemia, diabetes mellitus, hypertension, and smoking), age, nor sex affected the coronary arteriolar dilation to AA. Previous studies in humans demonstrated that NO-mediated dilation is reduced in hypercholesterolemia,\textsuperscript{41} whereas the reactive hyperemia response is normal. This can be explained by the observation that in hypercholesterolemia, hyperpolarizing mechanisms may be preserved or even enhanced.\textsuperscript{6,17} Thus, the results of the present study may have important implications for myocardial perfusion in coronary atherosclerosis, since hyperpolarizing mechanisms contribute substantially to endothelium-dependent responses in this vascular segment.

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References


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