Human Coronary Arteriolar Dilation to Arachidonic Acid Depends on Cytochrome P-450 Monoxygenase and Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Channels

Hiroto Miura, David D. Gutterman

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 monoxygenase (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\textsuperscript{-10} to 10\textsuperscript{-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\textsuperscript{-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\textsuperscript{-5} mol/L; a cyclooxygenase inhibitor) and \( N\)-nitro-L-arginine methyl ester (L-NAME, 10\textsuperscript{-4} mol/L; a NO synthase inhibitor) were without effect. Both 17-ODYA and miconazole (10\textsuperscript{-5} 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\textsuperscript{-3} mol/L, a blocker of large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} 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\textsuperscript{-5} mol/L) in the presence of INDO and L-NAME induced hyperpolarization of VSMCs (−57±5 mV). In the presence of 17-ODYA together with INDO and L-NAME, 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\textsuperscript{2+}-activated K\textsuperscript{+} 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\textsuperscript{+} 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\textsubscript{2}), and endothelium-derived hyperpolarizing factor (EDHF).\textsuperscript{1-4} A common intracellular response to agonist stimulation is activation of endothelial phospholipases, which release arachidonic acid (AA) from the cell membrane.\textsuperscript{5} AA may then be metabolized by cyclooxygenase, lipoxygenase, or cytochrome P-450 monoxygenase (P450) to vasoactive substances.\textsuperscript{6-10} A number of these substances, including the P450-derived epoxyeicosatrienoic acids, can effect vasorelaxation by hyperpolarizing vascular smooth muscle cells (VSMCs).\textsuperscript{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.\textsuperscript{13-15} Furthermore, it has been proposed that in disease states EDHF may compensate for the loss of NO-mediated dilation.\textsuperscript{16,17} Thus, in resistance vessels, particularly vessels from patients with coronary disease, hyperpolarization may be an important compensatory mechanism...
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 maintained at 37°C by an external heat changer. All pharmacological agents were added to the external bathing solution.

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 (10⁻⁵ to 10⁻³ mol/L) were examined in the presence and absence of Nω-nitro-L-arginine methyl ester (L-NAME, 10⁻³ mol/L; NO synthase inhibitor), indomethacin (INDO, 10⁻³ mol/L; cyclooxygenase inhibitor), miconazole (10⁻³ mol/L), 17 octadecynoic acid (17-ODYA, 10⁻³ mol/L; P450 inhibitor), 20 or baicalein (10⁻³ 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, 21 the effect of charybdo toxin (CTX, 10⁻⁵ 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 10⁻⁵ 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. 22

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 the response to AA (10⁻⁵ mol/L; P450 inhibitor), 19 or baicalein (10⁻³ mol/L; lipooxygenase inhibitor). In some experiments, the endothelium was mechanically denuded. In separate experiments, we simultaneously examined changes in vessel diameter and VSMC membrane potential (Em) in response to ADP (10⁻⁴ mol/L; P450 inhibitor), 19 or baicalein (10⁻³ mol/L; lipooxygenase inhibitor). In some experiments, the endothelium was mechanically denuded.

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 by a 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 (10⁻⁴ mol/L), confirming the integrity of endothelial function (82±5%, n=8). 18

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 human 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.
Demographics (N=35)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>26/9</td>
</tr>
<tr>
<td>Age, y</td>
<td>59±11*</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>32</td>
</tr>
<tr>
<td>Valve replacement</td>
<td></td>
</tr>
<tr>
<td>Aortic</td>
<td>2</td>
</tr>
<tr>
<td>Mitral</td>
<td>1</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>33</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>12</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>9</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3</td>
</tr>
<tr>
<td>None of the above</td>
<td>1</td>
</tr>
</tbody>
</table>

N indicates total number of patients studied; n, number of patients in group; CABG, coronary artery bypass graft; and CAD, coronary artery disease.

*Mean±SD.

a piezoelectric microdrive perpendicular to the long axis of the vessel and advanced through the adventitial side in 0.5-μm increments while monitoring tip potential. Multiple successful impalements included an abrupt drop in potential to a new steady-state value that was maintained for a minimum of 10 seconds and a sudden return to the original baseline when the electrode was pulled from the VSMCs. Data were obtained only from cells estimated to be within the medial portion of the vessel (based on visual assessment of vessel wall thickness and measured depth of penetration). Multiple successful impalements of at least 3 distinct VSMCs were made to obtain average Em values for a single experimental protocol. The effect of 17-ODYA on simultaneous changes in Em and diameter to AA was tested in the presence of L-NAME and INDO.23 At the end of each experiment, maximal vascular diameter was determined with SNP (10⁻⁴ mol/L).

Materials

AA (sodium salt) was obtained from Nu Check Prep, Inc. Endothelin-1 was from Peninsula Laboratories, Inc. The others were from Sigma Chemical Co. AA was prepared in distilled water previously sparged with nitrogen. ETYA and baicalene were prepared in 100% dimethyl sulfoxide. Both AA and ETYA were stored under a nitrogen atmosphere at −70°C. The stock solution and dilutions of AA and ETYA were made fresh for each experiment and kept on ice. Miconazole and 17-ODYA were dissolved in 100% ethanol. INDO was dissolved in saline with 1% NaOH, and pH was adjusted to 7.4 with 0.1N HCl. Endothelin-1 was prepared in saline 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, ED₅₀ values (negative logarithm of the molar concentration of vasodilator that produced 50% of the maximal dilation to the agonist) were calculated. Percent maximal dilations, ED₅₀ 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 constrict vessels, markedly reduced vasodilation to AA was observed in the presence of INDO (maximum dilation, 19±9% versus 76±5%.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Effect of inhibiting NO synthase (A) or cyclooxygenase (B) on the dilation of human coronary arterioles to AA. Vessels were treated with 10⁻⁴ mol/L L-NAME (A) or 10⁻⁵ mol/L INDO (B), constricted with endothelin-1, and then given incremental doses of AA (10⁻⁸ to 10⁻⁴ mol/L). Neither inhibitor altered dilation to AA (n=5, respectively). For panel A, ED₅₀ was 6.7±0.4 vs 7.1±0.4 (control) (P=NS), and for panel B, ED₅₀ was 7.3±0.4 vs 7.4±0.4 (control) (P=NS). Values represent mean±SE.
blocker. In the presence of INDO, CTX (10⁻⁴ mol/L, 20 baicalein (10⁻⁴ mol/L) reduced vasodilation to AA (maximum dilation, 47.6% versus 54.6% [control]; P<0.05) (Figure 4). In the presence of INDO, 17-ODYA (10⁻⁵ mol/L), a lipoxygenase inhibitor) did not alter the response to AA (10⁻⁵ to 10⁻² mol/L). Addition of 17-ODYA (10⁻⁵ mol/L, a selective BKCa channel blocker) inhibited the vasodilation to AA (10⁻⁵ to 10⁻² mol/L) in the presence of INDO. ED₅₀ values were 6.2±0.2 (CTX) and 7.0±0.4 (control) (P<0.05, n=5). Values represent mean±SE. #P<0.05 vs endothelin-1 (A) or control (B).

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, 49.4% 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 (10⁻³ 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 lipooxygenase, on human coronary arteriolar dilation to AA was tested. Vessels were treated with INDO to block cyclooxygenases, since this may increase flux through the lipooxygenase pathway.5 At a dose that is reported to inhibit lipooxygenase in vitro,20 baicalein (10⁻⁴ 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, 30.6% versus 54±10% [INDO+baicalein]; P<0.05) (Figure 4B). Dilation to SNP (10⁻⁶ 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,18 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

Figure 3. Effect of blocking K⁺ channel activation with KCl or CTX on human coronary arteriolar dilation to AA. A, Constricting vessels with 40 mmol/L KCl instead of endothelin-1 abolished vasodilation to AA (10⁻¹⁰ to 10⁻⁵ mol/L) in the presence of INDO (10⁻⁴ mol/L, a cyclooxygenase inhibitor; n=5). ED₅₀ value was unchanged (6.7±0.6 vs 6.9±0.4 [endothelin-1]; P=NS). B, CTX (10⁻⁸ mol/L, a selective BKCa channel blocker) inhibited the vasodilation to AA (10⁻¹⁰ to 10⁻⁵ mol/L) in the presence of INDO. ED₅₀ values were 6.2±0.2 (CTX) and 7.0±0.4 (control) (P<0.05, n=5). Values represent mean±SE. #P<0.05 vs endothelin-1 (A) or control (B).

Figure 4. Effect of inhibiting AA metabolism on dilation to AA.

A, In vessels constricted with endothelin-1, 17-ODYA (10⁻⁵ mol/L, inhibitor of P450) attenuated vasodilation to AA (10⁻¹⁰ to 10⁻⁵ mol/L). The ED₅₀ value was not changed (7.1±0.5 vs 7.3±0.4 [control]; P=NS, n=5). B, The combined treatment with INDO (10⁻⁵ mol/L, a cyclooxygenase inhibitor) and baicalein (10⁻⁵ mol/L, a lipooxygenase inhibitor) did not alter the response to AA (10⁻¹⁰ to 10⁻⁵ mol/L). Addition of INDO (10⁻⁵ mol/L, a cyclooxygenase inhibitor; 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 (10⁻³ mol/L, a chemically distinct inhibitor of P450) to an extent similar to that with 17-ODYA. Neither miconazole nor 17-ODYA altered the ED₅₀ 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. In panel A, #P<0.05 vs control. In panel B, #P<0.05 vs no inhibitors and vs INDO+baicalein. In panels C and D, #P<0.05 vs control.
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. 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 or to shear stress, both of which activate phospholipase A₂, liberating AA in endothelial cells. Preliminary data from our laboratory support a role for EDHF in dilation of human coronary arterioles to both shear and to bradykinin.

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

Pfister and Campbell 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. The vasodilator effect of P450 metabolites also is not associated with increased tissue levels of cAMP and cGMP. 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 arterial tone. Inhibition of cyclooxygenase has been reported to potentiate vasorelaxation to AA in rabbit aorta by increasing flux through the lipoxygenase pathway. 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.

Inhibition of cyclooxygenase has been reported to potentiate vasorelaxation to AA in rabbit aorta by increasing flux through the lipoxygenase pathway. 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 non-specific 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⁺ channels directly, 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⁺ channels in vascular smooth muscle cells. In the present study, ETYA, a nonmetabolizable analogue of AA that can also directly activate K⁺ channels in coronary smooth muscle cells, 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 Longiro et al.

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 and with previous investigations showing that endothelial denudation abolishes AA-induced vasorelaxation.

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, 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. 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.

Acknowledgments

This study was supported by a Fellowship Grant from the American Heart Association, Iowa Affiliate, Inc, by a VA Merit Review award, and by a grant from NHLBI (NIH HL-52869). Dr Guterman is the recipient of an American Heart Association Established Investigator Award. We wish to acknowledge the expert technical assistance of Sara Manthei, Michael Breu, and Diann M. McCoy and the secretarial help provided by Lucinda Van Ark. We also thank Drs Neal Weintraub and Kevin Dellsperger for their critical review of this manuscript.

References

by guest on October 19, 2017 http://circres.ahajournals.org/ Downloaded from


24. Deleted in proof.


Human Coronary Arteriolar Dilation to Arachidonic Acid Depends on Cytochrome P-450 Monooxygenase and Ca$^{2+}$-Activated K$^+$ Channels
Hiroto Miura and David D. Gutterman

Circ Res. 1998;83:501-507
doi: 10.1161/01.RES.83.5.501

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/83/5/501

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/