Nishikawa, Yasuhiro, David W. Stepp, and William M. Chilian. In vivo location and mechanism of EDHF-mediated vasodilation in canine coronary microcirculation. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1252–H1259, 1999.—Responses of epicardial coronary arterioles to ACh were measured using stroboscopic fluorescence microangiography in dogs (n = 38). ACh (0.1 and 0.5 µg·kg⁻¹·min⁻¹ ic) dilated small (<100 µm, 11 ± 2 and 19 ± 2%, respectively) and large (>100 µm, 6 ± 3 and 13 ± 3%, respectively) arterioles at baseline. Combined administration of N^ω-monomethyl-L-arginine (L-NMMA; 1.0 µmol/min ic) and indomethacin (10 mg/kg iv) eliminated ACh-induced dilation in large coronary arterioles but only partially attenuated that in small arterioles. Suffusion of a buffer containing 60 mM KCl (high KCl) completely abolished cromakalim-induced dilation in arterioles and in combination with L-NMMA plus indomethacin completely blocked ACh-induced dilation in small arterioles. This indicated that the vasodilation to ACh that persists in small arterioles after administration of L-NMMA and indomethacin is mediated via a hyperpolarizing factor. The ACh-induced vasodilation remaining after L-NMMA and indomethacin was completely blocked by the large-conductance potassium-channel antagonist iberiotoxin or by epicardial suffusion of miconazole or metyrapone, inhibitors of cytochrome P-450 enzymes. These observations are consistent with the view that endothelium-derived hyperpolarizing factor (EDHF) is a product of cytochrome P-450 enzymes and produces vasodilation by the opening of large-conductance potassium channels. We conclude that ACh-induced dilation in large coronary arterioles is mediated mainly by nitric oxide (NO), whereas, in small arterioles both NO and EDHF mediate dilation to ACh. These data provide the first direct evidence for an in vivo role of EDHF in small coronary arterioles.

The endothelium releases a variety of vasodilators, including nitric oxide (NO), prostacyclin (PGI₂), and the yet unidentified endothelium-derived hyperpolarizing factor (EDHF) (32). The existence of EDHF was initially based on observations that ACh caused hyperpolarization and relaxation of isolated vascular preparations obtained in the presence of NO synthase/cyclooxygenase blockade, including coronary (13, 16, 30, 34), mesenteric (5), and cerebral arteries (32).

Inhibitors of endothelial NO synthase completely abolished in vivo ACh-induced dilation of the human (19) and canine epicardial coronary arteries (23), suggesting that EDHF has no role in vivo ACh-induced dilation in epicardial conductance vessels. In contrast, in vitro studies of isolated epicardial coronary arteries report relaxation to ACh after inhibition of NO and PGI₂ production (13, 29, 34). In the coronary microcirculation, most studies have reported that inhibition of NO synthase did not block completely ACh-induced vasodilation in dogs (15, 17, 36) or humans (19). Interestingly, inhibition of NO synthase produced different effects on ACh-induced dilation in large and small arterioles (15, 17). Specifically, inhibition of NO synthase completely blocked ACh-induced dilation in large but not in small arterioles (17). Because a component of microvascular dilation to ACh seems to be resistant to NO synthase inhibition, it is not unreasonable to suggest that ACh-induced dilation may have different mediators at different sites within the coronary microcirculation (22).

Conventionally, the "residual" dilation to ACh that remains after inhibition of NO synthase and cyclooxygenase has been attributed to EDHF. However, more recently, some investigators have found that in arterial endothelial cells ACh-induced production of an endothelium-derived factor that produced hyperpolarization could be blocked by antagonists to the cytochrome P-450 enzyme family (7, 14). A recent report concluded that coronary arteriolar EDHF-induced dilation occurred in vivo, because the dilation was inhibited by antagonists of cytochrome P-450 (37). Importantly, the role of EDHF in the coronary microvascular dilation to ACh has not been unequivocally verified, because results consistent with hyperpolarization were not presented. We state this with conviction, because given the many differences in regulation between macrovascular and resistance microvessels, the assumption that a class of inhibitors (cytochrome P-450 enzyme antagonists) block the same vasodilator (EDHF) in the two classes of vessels should not be accepted without question. Moreover, a role for EDHF in the residual dilation to ACh appeared improbable, because Komaru et al. (17) found that tetraethylammonium (TEA), the large-conductance potassium-channel antagonist, did not block this dilation in vivo.

Thus, a physiological in vivo role of EDHF in coronary microvascular adjustments remains unclear. Unequivocal information of EDHF-mediated responses is best accomplished by measurement of membrane potentials, or potassium channel conductance, which is impossible to accomplish in the beating heart. However, we designed experiments to examine the role of EDHF in ACh-induced dilation by using interventions designed to "damp" membrane potential in a depolarized state to block the effects of a hyperpolarizing factor (elevated KCl) or to block the opening of large-conductance potassium channels (iberiotoxin). Therefore, the purpose of this study was to test the hypotheses that 1)
EDHF produces vasodilation of coronary microcirculation in vivo; 2) EDHF-mediated vasodilation occurs via activation of large-conductance calcium-activated potassium (K_Ca) channels; and 3) EDHF is a metabolite of P-450 enzymes. Our study has provided unequivocal evidence for the existence of EDHF in the coronary microcirculation and the mechanism by which it produces vasodilation.

METHODS

General Preparation

Surgical preparation. Adult mongrel dogs of either sex (7–12 kg) were anesthetized with pentobarbital sodium (35 mg/kg iv), intubated, and ventilated with room air. A femoral artery and a femoral vein were catheterized for measurement of arterial pressure, arterial blood gases, and pH and drug administration. A catheter was inserted into the carotid artery and advanced into the left ventricle (LV) for measurement of LV pressure and LV dP/dt. The heart was exposed via a left thoracotomy, and the pericardium was incised. The proximal left circumflex or anterior descending coronary artery and advanced into the left ventricle (LV) for measurement of arterial pressure, arterial blood gases, and pH and drug administration. After these procedures, the animal was ventilated on a high-frequency jet ventilator (supplemented with 100% O2 at a pressure of 9–12 pounds/square in.) synchronized to the cardiac cycle. A pressure regulator connected to a solenoid valve was triggered from the LV dP/dt and remained open for 30–40 ms each cardiac cycle. The advantage of this procedure is that respiratory influences on cardiac motion are eliminated because the jet ventilation system produces almost no pulmonary movement.

Arterial blood gases and pH were monitored throughout the study and were maintained within normal limits (pH 7.35–7.45, P CO2 25–40 mmHg, P O2 100–220 mmHg). All experimental procedures were performed to conform with the "Guiding Principles for Research Involving Animals and Human Beings". The protocol of this study was approved by the Animal Care and Research Committee in the Medical College of Wisconsin.

Microvascular Preparation

Intravital microscopy. The intravital microscope system consisted of a Leitz Ploemopak (Wild Leitz, Rockleigh, NJ) mounted on a vertical support over an X-Y adjustable table. The use of the X-Y adjustable table allowed for fine movements of the position of the heart within the field of view. The Ploem system was used with either a polarizing filter to minimize reflected light from the surface of the heart or filters for fluorescence microscopy. A total magnification of the video image of approximately ×200 was achieved by the microscope objective (Leitz L 10 × 10, numerical aperture 0.22) in conjunction with a ×10 magnification eyepiece and video display. The resolution of this configuration is 2 μm.

Illumination of the epicardial surface of the LV was accomplished with a xenon stroboscopic light source (Chadwick-Helmuth; 100-W Xenon Arc, El Monte, CA) synchronized with the cardiac cycle (1 pulse/cycle). A computer (Quadra 950; Macintosh) received input from the LV dP/dt and subsequently triggered the strobe at the same point in time (late diastole) per cardiac cycle. With this system, the heart and microvasculature appear to be "fixed," because the epicardium is in view for a short instant (15–25 ms) at the same time of cardiac cycle.

Cardiac motion was partially restrained by inserting two 22-gauge needles attached to a rod. By this method, the horizontal movement of the microvascular field was restricted, and vertical movements were nearly abolished. Without pinning, the majority of studies would be impossible because the vessels would move in and out of the field of view and therefore in and out of focus. It has been determined that both resting and maximal myocardial blood flow are the same in normal and "restrained" areas of the myocardium (10), indicating that resting vasomotor tone and vasodilator reserve are not compromised by this procedure.

Diameter measurements. Video images of blood vessels were made with a Cohu intensified charge-coupled device video camera (Cohu, San Diego, CA) and were recorded with a frame digitizer. Control of video acquisition was achieved with LabView software (National Instruments, Austin, TX). A series of camera frames were digitized and stored on the hard disk of a Macintosh Quadra 950. Images were later replayed on a high-resolution black and white video monitor for diameter analysis with a Power Mac computer utilizing image-processing software (Image 1.28c; NIH Research Services Branch, Bethesda, MD). The microvasculature was visualized using fluorescence video microscopy. Small bolus injections (50–100 ml) of fluorescein isothiocyanate-labeled dextran were made via the coronary catheter. The existence of a well-defined anatomic landmark (branching point, etc.) was the major criterion used in the selection of a blood vessel.

Experimental Protocol

Protocol 1: Verification that KCl suffusion blocks dilation by hyperpolarization (cromakalim-induced vasodilation). Five nanomoles of cromakalim in 2 ml saline solution were injected manually within 2 s into the coronary artery (n = 6). Microvascular measurements were performed within 2 min after the cromakalim injection. After 10 min, 10 nmol of cromakalim were injected. A 60 mM KCl solution (high KCl) was suffused continuously onto the microvascular field of interest. This dose of KCl should depolarize cells by lowering the equilibrium potential for the outward diffusion of potassium from cells. Fifteen minutes later, administration of cromakalim was repeated. This protocol was performed to ensure that the protocol for suffusion of the KCl solution could completely inhibit dilation induced by hyperpolarization. Microvascular responses to papaverine (2 mg ic), an agonist that signals independently of potassium channels, in the presence of the KCl suffusion were measured to verify that the microvasculature was still capable of dilation.

Protocol 2: Contribution of NO, PGI₂, and EDHF to ACh-induced vasodilation. Measurements were made during the following conditions (n = 9): 1) baseline; 2) ACh (0.1 and 0.5 μg·kg⁻¹·min⁻¹ ic for 5 min each); 3) baseline; 4) Nω-nitro-L-arginine (L-NMMA; 1 μmol/min ic for 10 min) and indomethacin (10 mg/kg iv) to block the production of NO and prostanoids, respectively; 5) ACh during L-NMMA plus indomethacin; 6) baseline; 7) suffusion of the high-KCl buffer onto the epicardium; 8) ACh (during L-NMMA + indomethacin + high KCl); 9) baseline; and 10) papaverine (2 mg ic). Papaverine was administered to verify that the vessels were responsive to agonists during the high-KCl suffusion. At least 5 min was placed between each ACh infusion. In another three animals, the same protocols were repeated with higher doses of L-NMMA (3 μmol/min ic) and indomethacin (30 mg/kg iv) to demonstrate that higher doses of these antagonists would not completely antagonize ACh-induced dilation of coronary arterioles.
Protocol 3: Role of ATP-sensitive and calcium-activated potassium channels to EDHF-induced vasodilation. Measurements were made during the following conditions (n = 9): 1 baseline; 2 ACh (0.1 and 0.5 μg·kg⁻¹·min⁻¹ i.v. for 5 min each); 3 baseline; 4 L-NMMA (1 μmol/min ic for 10 min) and indomethacin (10 mg/kg iv); 5 ACh (during L-NMMA + indomethacin); 6 baseline; 7 iberiotoxin [IBTX, selective inhibitor of high-conductance KCa channels, 1 μg·kg⁻¹·min⁻¹ i.v. for 5 min each]; 8 ACh (during L-NMMA + indomethacin + IBTX); 9 baseline; and 10 papaverine (2 mg ic). The dose of IBTX was shown to block bradykinin-induced increases in coronary blood flow during NO synthase antagonism (nitro-L-arginine methyl ester) without any effects on the cardiac contractile function. In six dogs, the same protocol was repeated with the substitution of glibenclamide (1 mg/kg iv) for IBTX.

Protocol 4: Role of P-450 metabolite pathway in EDHF-induced vasodilation. Measurements were made during the following conditions (n = 5): 1 baseline; 2 ACh (0.1 and 0.5 μg·kg⁻¹·min⁻¹ i.v. for 5 min each); 3 baseline; 4 L-NMMA (1 μmol/min ic for 10 min) and indomethacin (10 mg/kg iv); 5 ACh during L-NMMA plus indomethacin; 6 baseline; 7 miconazole suffusion (P-450 enzyme inhibitor, 30 mM, 20 min); 8 ACh during L-NMMA plus indomethacin plus miconazole; 9 baseline; and 10 papaverine (2 mg i.c.). In addition to its inhibitory action on cytochrome P-450 enzymes, miconazole can potentially inhibit other enzymes containing heme moieties that synthesize vasoactive substances, e.g., lipoxygenase-induced production of leukotrienes. Thus, in two dogs, the same protocol was repeated using metyrapone (10 mM, 2-methyl-1,2-di-3-pyridyl-1-propanone), which inhibits cytochrome P-450 enzymes. Metyrapone shows greater specificity for cytochrome P-450 enzymes than miconazole and does not bind to heme groups.

Drugs

ACh was prepared as a 10 μg/ml solution in 0.9% saline. Indomethacin was dissolved in 95% ethanol and made up to a 10 mg/ml solution in 0.9% saline. L-NMMA was prepared as a 0.27 mg/ml solution in 0.9% saline brought to a physiological pH (between 7.3 and 7.5) by addition of small aliquots of 1 mol/l NaOH immediately before use. Krebs solution (in mM): 142 NaCl, 5.4 KCl, 2.0 CaCl₂, 1.2 MgCl₂, 11.0 dextrose, and 18 bicarbonate) and high-KCl solution (in mM: 102 NaCl, 45.4 KCl, 2.0 CaCl₂, 1.2 MgCl₂, 11.0 dextrose, and 18 bicarbonate) were bubbled with 20% O₂-5% CO₂-75% N₂. Miconazole (metyrapone) was initially dissolved in 100% ethanol and was subsequently dissolved in the Krebs perfusate to provide a final concentration of 30 (10) μM. All drugs were obtained from Sigma Chemical.

Statistical Analysis

Microvascular diameters in response to ACh infusion in the presence of inhibitors for NO, prostaglandins, KCa channel, and/or P-450 enzymes were calculated as a percent change from the data before ACh infusion at each state. Thus +% and −% indicate dilation and constriction, respectively. Data were analyzed separately in view of the well-recognized differences in physiological behavior between vessels of these size classes (large arterioles >100 μm, small arterioles <100 μm). Two-way repeated measures of ANOVA were used to assess the effects of ACh infusion on the diameters and hemodynamics in each condition. If ANOVA showed significant difference, then a paired t-test was used. To show significant vasodilation to ACh, the percent changes in diameter were compared with zero using a paired t-test. The data are presented as means ± SE. P value <0.05 was considered significant.

RESULTS

Systemic and Microvascular Hemodynamics

Mean aortic blood pressure and heart rate did not change significantly during any of the interventions. All vessels studied were 38–258 μm in diameter. The average baseline diameters of large (>100 μm) and small (<100 μm) arterioles under control conditions were similar among the various protocols.

Protocol 1: Verification that KCl Suffusion Blocks Dilation by Hyperpolarization (Cromakalim-Induced Vasodilation)

Figure 1 shows the scatter plot of relation of diameter and the percent changes in diameter in response to cromakalim (5 and 10 nmol ic), which was used as a challenge to verify block of hyperpolarization-induced dilation by KCl suffusion. Five and ten nanomoles of cromakalim caused 4 ± 2 and 9 ± 2% increases in diameter in large arterioles and 8 ± 4 and 17 ± 6% increases in small arterioles (P < 0.05, respectively). The average percent change of diameter to high-KCl suffusion was −7 ± 2% (−24 ± 3%, P < 0.05 vs. 0) for small arterioles and 6 ± 1% (−17 ± 0.6%, P < 0.05 vs. 0) for large arterioles. Cromakalim-induced dilation was completely blocked by KCl suffusion. This suggested that the suffusion was effective in blocking hyperpolarization-mediated dilation by the ATP-sensitive potassium (Kₐtp) channel opener cromakalim.

Protocol 2: Contribution of NO, PGI₂, and EDHF to ACh-Induced Vasodilation

Figure 2 illustrates a scatter plot of the relation of baseline diameters to the percent changes in diameter during administration of low (A) and high (B) doses of ACh during 1 control conditions, 2 L-NMMA plus indomethacin, and 3 L-NMMA plus indomethacin plus high KCl. Figure 3 shows the average data of the percent changes in diameter to ACh at each condition in
large (A) and small (B) arterioles. Small arterioles
dilated by 11 ± 6 and 19 ± 8% at 0.1 and 0.5 µg·kg⁻¹·min⁻¹, respectively, and large arterioles
dilated by 6 ± 3 and 13 ± 3% (P < 0.05 vs. small arterioles). L-NMMA
and indomethacin caused small but significant constriction of the large (-5 ± 2%) and
small arterioles (-9 ± 5%). Baseline diameters did not
change in response to IBTX (-1 ± 2%), but the
L-NMMA- and indomethacin-resistant vasodilation to
ACh in small arterioles was blocked by administration
of IBTX.

Baseline diameter did not change in response to
glibenclamide (-3 ± 3%). In small coronary arterioles,
glibenclamide did not affect vasodilation to ACh (0.5
µg·kg⁻¹·min⁻¹) after L-NMMA and indomethacin (17 ±
2 vs. 16 ± 2% before and after glibenclamide, respec-
tively). Papaverine caused 16 ± 4 and 14 ± 5% in-

Fig. 2. Scatter plots showing the percent changes in diameter to 0.1
(A) and 0.5 (B) µg·kg⁻¹·min⁻¹ ic ACh. In large arterioles, N-
monomethyl-L-arginine (L-NMMA) with indomethacin (Indo) causes
almost complete blockade on ACh-induced vasodilation (○) compared
with control conditions (●). In contrast, small arteries still dilate to
ACh in the presence of L-NMMA and indomethacin, although to a
lesser degree. Additional administration of potassium suffusion with
L-NMMA and indomethacin causes complete blockade on ACh-
induced vasodilation (▼).

Fig. 3. Bar graphs showing averaged data of the percent changes in
diameter in control, L-NMMA + indomethacin (Indo), and L-NMMA +
indomethacin + potassium suffusion (K) in large arterioles (A) and
small arterioles (B).
creases in diameter of large and small arterioles, respectively ($P < 0.01$).

**Protocol 4: Role of P-450 Metabolite Pathway in EDHF-Induced Vasodilation**

Miconazole suffusion after L-NMMA and indomethacin increased the baseline diameter by 6%. Figure 6 shows the averaged data of percent changes in diameter to $ACh$ at each condition in large (A) and small (A) coronary arterioles. In small arterioles, L-NMMA and indomethacin partially blocked the $ACh$-induced vasodilation, and additional administration of miconazole with L-NMMA and indomethacin caused complete blockade of vasodilation in small arterioles. In five vessels (76 ± 5 µm) with metyrapone, $ACh$-induced L-NMMA/indomethacin-resistant vasodilation was significantly inhibited by metyrapone (14 ± 2 vs. 3 ± 1% before and after metyrapone, respectively, at 0.1 µg·kg⁻¹·min⁻¹ of $ACh$; 22 ± 6 vs. 6 ± 2% before and after metyrapone, respectively, at 0.5 µg·kg⁻¹·min⁻¹ of $ACh$).

**DISCUSSION**

The major new findings we have made are that the in vivo $ACh$-induced dilation of coronary microvessels is unequivocally mediated by a hyperpolarizing factor,
independent of NO and prostanoids. This factor appears to signal through large-conductance KCa channels but not via K_ATP channels. We also found that an inhibition of cytochrome P-450 blocks the EDHF response, which confirms a previous report (37). We conclude that EDHF, in the canine coronary microcirculation, is a product of cytochrome P-450 enzymes. Relevant to these conclusions are several issues concerning the limitations of the methodology, the role, mechanisms of action, and identity of EDHF, and physiological implications.

Limitations of Methodology

Because of technical problems of measuring membrane potentials in coronary microvessels in the beating heart preparation, which would unequivocally verify hyperpolarization, we suffused a buffer of 60 mM KCl onto the epicardium to clamp membrane potential in a depolarized state and thus prevent membrane hyperpolarization. This dose of KCl would depolarize many cell types on the epicardial surface, e.g., cardiac myocytes and nerve cells, in addition to smooth muscle cells. The relatively mild contraction that we observed (<10% decrease in diameter) could be due to the release of neurotransmitter or vasoconstrictor paracrine factors. However, we do not believe this possibility complicates our findings, because to our knowledge such factors do not prevent the actions of hyperpolarizing factors. In addition, suffusion of KCl did not cause any alterations in hemodynamics, which means that the effects of the suffusion were likely confined to the superficial subepicardium. Cromakalim-induced vasodilation was completely blocked by topical suffusion of the high-KCl buffer, indicating that the suffusate prevents vasodilation mediated by hyperpolarization, i.e., potassium channel opening. Thus this in vivo observation strongly supports that L-NMMA/indomethacin-resistant but KCl-sensitive vasodilation to ACh is mediated by a hyperpolarizing factor.

The specificity of miconazole and IBTX as antagonists of P-450 enzymes and KCa channels, respectively, is an important assumption in our experiment. Miconazole inhibits all cytochrome P-450 enzymes and can affect smooth muscle contraction (38). However, it caused only small changes in baseline diameter and completely inhibited L-NMMA/indomethacin-resistant dilation to ACh. Likewise, IBTX caused only minor changes in vessel tone but blocked the L-NMMA- and indomethacin-resistant ACh-induced dilation. Moreover, papaverine dilated the coronary microvessels in the presence of miconazole or IBTX. These results further suggest that data obtained using miconazole, metyrapone, and IBTX can be interpreted on the basis of inhibition of cytochrome P-450 enzyme activity and large-conductance potassium channels, respectively. Taken together, these observations indicate that miconazole and IBTX have negligible nonspecific effects on the reactivity of smooth muscle. Role of EDHF in Vasodilation

Nagao et al. (25) showed that nitro-L-arginine abolished relaxation to ACh in the aorta, pulmonary artery, and common iliac arteries in rats, whereas as much as 80% of the maximal relaxation persisted in the mesenteric and femoral arteries and the majority of the renal arteries studies. Chen et al. (9) showed that membrane hyperpolarization may account for 20–25% of the relaxation by ACh in large vessels such as pulmonary artery and aorta of the rat. Thus contribution of EDHF in the ACh-induced vasodilation varies in different parts of the circulation. It appears that a larger component of the relaxation is mediated by EDHF in the more peripheral and slightly smaller vessels. Our data, in the presence of EDHF-dependent vasodilation in small arterioles but not in large arterioles, support the concept that the small arterioles are more exquisitely controlled by KCa channels than the upstream vessels.

We presume that the hyperpolarizing factor is produced by the endothelium, and the factor is EDHF. Although we did not verify endothelial production, previously our laboratory found that in vivo ablation of coronary microvascular endothelium with CO2 gas completely abolished dilation to ACh (12). Thus we believe the hyperpolarizing factor is endothelium derived. This view is also consistent with a plethora of reports demonstrating that ACh-induced dilation is endothelium dependent.

Mechanisms of Action of EDHF

Although EDHF-mediated vasodilation has been suggested to occur by activation of potassium channels, the subtype of potassium channels involved in endothelium-dependent hyperpolarization remains uncertain in vivo in coronary microcirculation. We observed that glibenclamide, an antagonist of KCa channels, had no significant effect on EDHF-induced vasodilation, which is consistent with a previous observation in epicardial coronary arteries of the guinea pig (8). IBTX completely blocked L-NMMA/indomethacin-resistant vasodilation to ACh in small coronary arterioles, which was similar to the blockade produced by high-KCl suffusion. This strongly indicated that EDHF-mediated vasodilation was mediated through activation of KCa channels in the coronary microcirculation in vivo. One previous study (17) showed that TEA (topical administration, large-conductance KCa channel) failed to block ACh-induced vasodilation in canine small coronary arterioles in the presence of L-NMMA. However, the investigators did not ascertain the efficacy of blockade of potassium channels by epicardial suffusion of TEA. Our study is notably different from this previous work, because we found a role for hyperpolarization via opening of KCa channels in ACh-induced vasodilation. Specifically, we established that KCl suffusion inhibited the vasodilatory action of a hyperpolarizing factor by showing that this intervention prevented dilation via the K_ATP-channel agonist cromakalim. We also observed that the component of ACh-induced dilation remaining after combined blockade with L-NMMA plus indomethacin
was dependent on $K_{Ca}$ channel activation. This was concluded from experiments using intracoronary infusion of IBTX, a specific antagonist of large-conductance $K_{Ca}$ channels. This dose of IBTX was shown to block bradykinin-induced coronary vasodilation after inhibition of NO production (29). Finally, the ACh-induced dilation remaining after blockade with l-NMMA plus indomethacin was inhibited by suffusion with the highKC1 solution. Our data are compatible with previous in vitro data showing the importance of $K_{Ca}$ channels in EDHF-induced relaxation in isolated coronary arteries (2, 8, 20) and suggest a role for EDHF in ACh-induced dilation of coronary microvessels.

In contrast to NO, a role of EDHF in the regulation of resting vasomotor tone in vivo has not been clearly demonstrated. Depolarization of vascular smooth muscle after removal of endothelium (4, 26, 35) suggested that there might be tonic release of EDHF at the resting state, whereas others (9, 26) do not show that removal of endothelium does not affect the resting membrane potential in isolated blood vessels. These contradictory results may be explained by the possibility of damage of the smooth muscle cells during the removal procedure. Recently, one study in an open-chest anesthetized canine model showed that IBTX did not change resting myocardial blood flow in nonischemic myocardium (29). In our study, the microvascular diameter did not change after administration of IBTX with l-NMMA. This indicates that, unlike NO, EDHF probably is not released in a tonic manner in the coronary circulation of a beating heart. This may be explained by the results of Bauersachs et al. (3), who reported that NO exerts a feedback inhibition on EDHF; thus vasodilation due to EDHF is most prevalent only after NO production has been blocked.

Identity of EDHF

A recent study has shown that EDHF activity may be attributed to the action of epoxyeicosatrienoic acids (EETs) formed from arachidonic acid by the action of cytochrome P-450 (7). The chemical nature of EDHF remains obscure, and patch-clamp studies have demonstrated that EETs increase the activity of the $K_{Ca}$ channels in coronary arterial smooth muscle cells (14), suggesting that activation of $K_{Ca}$ channels plays a role in EDHF-mediated vascular dilatation. Because we found that EDHF production was blocked after inhibiting cytochrome P-450 and that its dilation was antagonized by IBTX, we suggest that EDHF is an EET or a metabolite of this pathway.

Physiological Significance and Conclusions

The endothelial production of NO is impaired in a variety of pathological conditions, such as hypertension, diabetes, heart failure, and hyperlipidemia (11). Endothelial vasomotion controlled by NO will be lost with progression of the disease process. In contrast, it remains unclear whether these diseases cause similar impairments in EDHF-mediated vasodilation. Endothelium-dependent hyperpolarization is present in coronary arteries form patients with different cardiac diseases (28). Carotid arteries in rabbits with a high-cholesterol diet suggest an increased EDHF-mediated vasodilation in the presence of reduced production of NO (27). Recent several studies have shown that hypertension results in a compensatory increase in the activity of potassium channels (21, 31), and possibly, increased synthesis/release of the putative hyperpolarizing factors (1, 6). These data suggest that the EDHF-mediated response is spared or even augmented in the presence of endothelial dysfunction of NO production.

In conclusion, we have demonstrated that ACh produces dilation of small coronary arterioles via activation of $K_{Ca}$ channels and that EDHF appears to be a cytochrome P-450 metabolite. Because at rest 55% of total coronary resistance is distal to the 100-μm arterioles (10) and EDHF appears to have its major action confined to these small resistance vessels, it has the potential to greatly modify total coronary resistance and flow. An improved understanding of the physiological mechanisms for EDHF-mediated vasomotor adjustment will help in clinical evaluation and therapy for patients with coronary heart disease.

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