20-Hydroxyeicosatetraenoic acid is a potent dilator of mouse basilar artery: role of cyclooxygenase

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Fang, Xiang, Frank M. Faraci, Terry L. Kaduce, Shawn Harmon, Mary L. Modrick, Shanming Hu, Steven A. Moore, J. R. Falck, Neal L. Weintraub, and Arthur A. Spector. 20-Hydroxyeicosatetraenoic acid is a potent dilator of mouse basilar artery: role of cyclooxygenase. Am J Physiol Heart Circ Physiol 291: H2301–H2307, 2006. First published June 16, 2006; doi:10.1152/ajpheart.00349.2006.—20-Hydroxyeicosatetraenoic acid (20-HETE), an arachidonic acid (AA) metabolite synthesized by cytochrome P-450 ω-oxidases, is reported to produce vasconstriction in the cerebral circulation. However, we find that like 14,15-epoxyeicosatrienoic acid (14,15-EET), 20-HETE produces dilation of mouse basilar artery preconstricted with U-46619 in vitro. Indomethacin inhibited the vasodilation produced by 20-HETE but not by 14,15-EET, suggesting a cyclooxygenase (COX)-dependent mechanism. Metabolic studies indicated several mechanisms that may play a role in this process. Mouse brain endothelial cells (MBEC) converted 20-HETE to 20-0H-PGE2, which was as potent as PGE2 in dilating the basilar artery. 20-HETE also stimulated AA release and PGE2 and 6-keto-PGF1α production in MBEC. Furthermore, the basilar artery converted 20-HETE to 20-COOH-IAA, which also produced COX-dependent dilation of the basilar artery. 20-COOH-IAA increased AA release and PGE2 and 6-keto-PGF1α production by the MBEC, but to a lesser extent than 20-HETE. Whereas the conversion of 20-HETE to 20-OH-PGE2 and production of endogenous prostaglandins probably are primarily responsible for vasodilation, the production of 20-COOH-IAA also may contribute to this process.

20-hydroxy-prostaglandin E2; 20-carboxy-arachidonic acid; prostaglandins; cerebral vascular tone

20-HYDROXYECOSATETRAENOIC acid (20-HETE) is a bioactive eicosanoid synthesized from arachidonic acid (AA) by cytochrome P-450 ω-oxidases. 20-HETE modulates vascular tone and cell proliferation, and it appears to play a role in the regulation of blood pressure and myogenic responses (33). Several different intracellular signaling pathways mediate these biological responses to 20-HETE. For example, 20-HETE can activate protein kinase C, mitogen-activated protein kinase, Rho-kinase, and cytosolic phospholipase A2 (20, 25, 33). 20-HETE also elicits the phosphorylation of myosin light chain (24), causes the subcellular translocation of Ras (31), activates L-type Ca2+ channels (15), and inhibits opening of some K+ channels (26). A major metabolite of 20-HETE is 20-carboxy-arachidonic acid (20-COOH-IAA), which has been shown to dilate porcine coronary microvessels and inhibit ion transport in kidney tubular cells (7, 8, 19).

The cerebral circulation is one of the important sites of 20-HETE function. 20-HETE is synthesized by cat cerebral microvessels (17) and by cultured rat cerebral vascular muscle (15), and it produces constriction of cerebral blood vessels and may contribute to autoregulation of cerebral blood flow during changes in arterial pressure (16). The concentration of 20-HETE in cerebrospinal fluid is increased after subarachnoid hemorrhage in rats, and this appears to contribute to the resulting acute fall in cerebral blood flow (3). Furthermore, an inhibitor of 20-HETE synthesis was shown to reduce infarct size in an ischemic stroke model (22). Therefore, agents that interfere with the actions of 20-HETE may have therapeutic benefit by preserving cerebral blood flow in the setting of stroke and acute subarachnoid hemorrhage (38).

To explore possibilities for this type of therapeutic approach, we have investigated the metabolism and functional responses to 20-HETE in an experimental model, the mouse basilar artery. As opposed to what has been reported in the rat middle cerebral, rat basilar, and canine basilar arteries (16, 26, 36), we find that 20-HETE produces potent, concentration-dependent dilation of the mouse basilar artery after submaximal constriction with U-46619. Additional studies were done to identify mechanisms responsible for the unexpected 20-HETE-mediated dilation in this preparation.

MATERIALS AND METHODS

Materials. 20-HETE, 14,15-epoxyeicosatrienoic acid (14,15-EET), 20-hydroxy-PGE2 (20-OH-PGE2), and enzyme immunoassay (EIA) kits for PGE2 and 6-keto-PGF1α were purchased from Cayman Chemicals (Ann Arbor, MI). Indomethacin, calcium ionophore A-23187, and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma (St. Louis, MO). 20-COOH-IAA and 20-[3H]HETE (16.7 μCi/nmol) were synthesized as described previously (9, 19).

Cerebral arteries in vitro. After an overdose of anesthesia (pentobarbital sodium, 200 mg/kg ip), the mouse (C57BL/6) brain was rapidly removed and placed in ice-cold Krebs buffer. As described previously (14, 37), the basilar artery was isolated using a dissecting microscope, cannulated onto glass micropipettes filled with Krebs buffer in an organ chamber, and secured with nylon monofilament suture. Arteries were pressurized to 60 mmHg. With the use of a microscope and a video camera, vessel images were projected on a video monitor. An electronic dimension analyzer was then used to measure lumen diameter. Experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Iowa, and mice were handled in a manner that meets the guidelines for the National Society of Medical Research and guidelines for the care and use of laboratory animals.

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Once prepared for study, basilar arteries were allowed to equilibrate for at least 30 min at a distending pressure of 60 mmHg before protocols were initiated. The basal diameters of the basilar arteries averaged between 134 and 143 μm. We examined changes in diameter of the basilar artery in response to KCl (50 mM) and cumulative doses of agents of interest (20-HETE, etc.). For studies in which vasodilator responses were examined, basilar arteries were constricted by ~30% (~60% of the response to 50 mM KCl) with the thromboxane mimetic U-14,418 from 1 × 10^{-7} to 4 × 10^{-7} M.

Cell culture. Murine cerebral microvascular endothelial cells (MBEC) were prepared as described previously (23). The cells were grown in modified medium 199 with 20% fetal bovine serum and maintained until confluent at 37°C in a humidified atmosphere containing 5% CO₂, 95% room air. Stocks were subcultured weekly into 100-mm-diameter dishes or six-well tissue culture plates by trypsinization, and the experiments were performed on culture passages 8–20.

Metabolism of 20-HETE and AA. The metabolic studies were done with either MBEC or an isolated mouse brain vascular preparation consisting of the basilar artery and the circle of Willis with the middle cerebral arteries. The method of incubation and analysis was similar in both cases. The tissues were incubated with 0.1 μM 20-[³H]HETE, or MBEC were incubated with 20-[³H]HETE or [³H]AA for various times. After incubation, the medium was collected, and the tissues were washed twice with cold phosphate-buffered saline solution and harvested. Radioactivity contained in an aliquot of the medium and in the tissue lipid extract was assayed by liquid scintillation counting. The remainder of the medium was extracted with H₂O-saturated ethyl acetate and separated by reverse-phase HPLC. A C₁₈ 5-μm 4.6 × 150-mm column was used to separate metabolites. For separation of AA metabolites, an elution profile, consisting of water adjusted to pH 4 with formic acid and an acetonitrile gradient that increased from 30% to 100% over 65 min at a flow rate of 0.7 ml/min was used. An acetonitrile gradient that increased from 10% to 100% over 60 min at a flow rate of 0.7 ml/min was used to separate 20-HETE metabolites (7). The column effluent was combined with scintillator solution and was passed through an in-line flow scintillation detector (IN/US System, Tampa, FL) to determine the distribution of radioactivity (7, 9, 12).

Prostaglandin determination. MBEC were incubated with 0.1 μM [³H]AA for 4 h. After the medium was removed and the cells washed, the incubation was continued for an additional 30 min in a medium containing 0.1 μM BSA and either vehicle, 2 μM 20-HETE, or 20-COOH-AA in the vehicle. The medium was collected and extracted, and the radiolabeled metabolites that accumulated in the medium were analyzed by reverse-phase HPLC. In additional experiments, the cells were incubated with 2 μM 20-HETE or 20-COOH-AA in medium 199 containing 0.1 μM BSA for 4 h. The medium was collected, and PGE₂ and 6-keto-PGF₁α, a stable metabolite of prostacyclin, were measured using PGE₂ and 6-keto-PGF₁α ELISA kits purchased from Cayman. The antibody against PGE₂ is specific for PGE₂, and it does not cross-react with 20-HETE-PGE₂ (specificity for PGE₂ is 100%; for 20-OH-PGE₂, <0.01%).

Identification of 20-HETE metabolites. The structure of lipid metabolites contained in the medium was identified by liquid chromatography combined with mass spectrometry (LC/MS) using a Hewlett-Packard 1100 MSD LC/MS system (19). HPLC separation was done with a C₁₈ 5-μm 4.6 × 150-mm column and mobile phase solvents consisting of water: formic acid (100:0.03, vol/vol; solvent A) and acetonitrile (solvent B) at a flow rate of 0.7 ml/min. The gradient was maintained at 30% solvent B for the first 2 min and then linearly increased to 57% solvent B at 20 min, 65% at 40 min, 70% at 45 min, and 95% at 50 min. Negative ion electrospray was used with the fragmentor voltage set at 110 V to produce in-source collision-induced decompositions (CID). N₂ nebulizing gas was maintained at 60 bar, whereas the N₂ drying gas was set at a flow rate of 10 l/min.
basilar artery to 20-HETE were completely inhibited by 10 μM indomethacin, a nonselective COX inhibitor. By contrast, indomethacin did not affect the vasodilation produced by 14,15-EET, indicating that the inhibitory effect was selective for 20-HETE.

**Production and function of 20-COOH-AA.** Metabolic studies were done with 20-HETE to investigate the mechanism of the vasodilator response. When 20-[3H]HETE was incubated in the medium in the absence of tissues for 24 h, a single radiolabeled peak with a reverse-phase HPLC retention time (RT) of 43 min, which comigrated with a standard 20-[3H]HETE, was detected (Fig. 2A). When a preparation consisting of mouse cerebral arteries was incubated with 20-[3H]HETE, a radiolabeled metabolite with a RT of 41 min was detected in the medium (Fig. 2B). This product was previously identified as 20-COOH-AA, a ω-oxidation product of 20-HETE (7, 19). 20-[3H]COOH-AA accounted for 5% of the total radioactivity recovered from the medium after incubation for 3 h. Formation of 20-[3H]COOH-AA continued to increase as the incubation progressed, accounting for 35% of total radioactivity in the medium at the end of the 24-h incubation. The increase in 20-COOH-AA was associated with a concomitant decrease in 20-[3H]HETE (Fig. 2C).

Previous studies (19) indicated that 20-COOH-AA produced dilation of porcine coronary microvessels. Therefore, studies were done to determine whether it might have a similar effect in the mouse cerebral vasculature. As shown in Fig. 2D, 20-COOH-AA produced dilation of the basilar artery, but the response was somewhat smaller than that produced by 20-HETE. However, as was observed with 20-HETE, the vasodilation produced by 20-COOH-AA was completely inhibited by 10 μM indomethacin (Fig. 2D).

**Prostaglandin production.** The inhibition of dilation produced by indomethacin suggested that 20-HETE and 20-COOH-AA produce vasodilation through a COX-dependent mechanism. This would be consistent with previous results demonstrating that COX-1 and COX-2 are expressed in the MBEC (5, 11). To assess this possibility, we investigated whether these compounds stimulate production of vasodilator prostaglandins in mouse cerebrovascular tissue. These studies were done with cultures of MBEC because the amount of endothelium present in intact blood vessels that can be obtained from the mouse is too small to produce detectable amounts of prostaglandins.

The cultures of MBEC were incubated with 0.1 μM [3H]AA for 4 h, washed, and subsequently incubated in a fresh medium containing either vehicle (control), 2 μM 20-HETE, or 2 μM 20-COOH-AA. The results are shown in Fig. 3. A radiolabeled compound with a RT of 15 min, which is identical to the RT of a PGE2 standard in this HPLC gradient, was detected in the control cultures (Fig. 3A). This radiolabeled product was also observed when the MBEC were incubated with 20-HETE, together with a second product with a RT corresponding to a 6-keto-PGF1α standard (9 min) (Fig. 3B). Although radiolabeled material with the RT of PGE2 was also detected in the cultures incubated with 20-COOH-AA, a distinct component corresponding to 6-keto-PGF1α was not observed (Fig. 3C).

To obtain quantitative results, additional cultures of MBEC were incubated with 2 μM 20-HETE or 20-COOH-AA, and the medium was collected and assayed for PGE2 and 6-keto-PGF1α by EIA. When compared with the control cultures, PGE2 production was sevenfold higher when the MBEC were incubated with 20-HETE and 4.5-fold higher when incubated with 20-COOH-AA (Fig. 4A). Likewise, when compared with the control cultures, 6-keto-PGF1α production increased 3.9-fold and 1.8-fold during incubation with 20-HETE and 20-COOH-AA, respectively (Fig. 4B).

To investigate the mechanism of the increases in prostaglandin production, we determined whether 20-HETE and 20-COOH-AA increased the expression of COX-2 in MBEC. As shown in Fig. 5, neither 20-HETE nor 20-COOH-AA increased COX-2 protein expression in the control cultures, 6-keto-PGF1α production increased by 10.2 ± 0.3 on October 15, 2017

![Fig. 2. Formation of 20-COOH-arachidonic acid (AA) and its effect on vascular tone. A and B: representative chromatograms showing radiolabeled compounds present in medium after 24 h of incubation of 0.1 μM 20-[3H]HETE in the absence (A) or presence (B) of cerebral artery preparation. C: time-dependent changes in radiolabeled 20-HETE and 20-COOH-AA contained in the medium. These data were converted to values (in pmol) using the specific activity of radiolabeled 20-[3H]HETE added to cultures. D: changes in diameter of basilar artery produced by 20-COOH-AA in the absence or presence of 10 μM indomethacin (Indo) in U-46619-preconstricted arteries. Procedure was the same as described in Fig. 1. Data in in D are means ± SE obtained from 3 to 4 separate experiments. **P < 0.01 compared with 20-COOH-AA in the presence of Indo at same concentration.

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The cultures were then incubated for 30 min in a fresh medium containing either 0.1 μM BSA as a control or BSA with either 2 μM 20-HETE or 20-COOH-AA. The results, which also are illustrated in Fig. 5, show that both compounds increased [3H]AA release compared with that of the control cultures. The MBEC incubated with 20-HETE released 2.3-times more radioactivity than the corresponding controls, whereas those incubated with 20-COOH-AA released 35% more than the controls. These results suggest that the increase in prostaglandin formation produced by 20-HETE and 20-COOH-AA is secondary to increased AA mobilization from endothelial lipids.

Fig. 3. Effects of 20-HETE and 20-COOH-AA on prostaglandin formation. Mouse brain endothelial cells (MBEC) were incubated with 0.1 μM [3H]AA for 4 h. After incubation, cells were washed and then incubated with medium containing 0.1 μM BSA and either vehicle (A), 2 μM 20-HETE (B), or 2 μM 20-COOH-AA (C) for 30 min. Medium was collected and radiolabeled products analyzed by reverse-phase HPLC. Representative chromatograms are shown.

Fig. 4. Effects of 20-HETE and 20-COOH-AA on endogenous PGE2 (A) and 6-keto-PGF1a production (B). MBEC were treated with either vehicle, 2 μM 20-HETE, or 20-COOH-AA. After 4 h of incubation, medium was collected, and the amounts of PGE2 and 6-keto-PGF1a in medium were assayed by enzyme immunoassay. Bars in graph are means ± SE obtained from 3 separate cultures. *P < 0.05 compared with control.

Fig. 5. Effect of 20-HETE and 20-COOH-AA on expression of cyclooxygenase (COX)-2 protein and AA release. MBEC were incubated with 2 μM 20-HETE or 20-COOH-AA or 100 nM phorbol 12-myristate 13-acetate (PMA) for 4 h. Medium was removed, and cells were harvested and sonicated. COX-2 protein in cell lysates was detected by Western blot analysis using a specific antibody against COX-2. For AA release experiments, MBEC were incubated with 0.1 μM [3H]AA for 4 h. After incubation, cells were washed and then treated with medium containing 0.1 μM BSA and either vehicle (C) or 2 μM 20-HETE (20-H) or 2 μM 20-COOH-AA (20-C) for 30 min. Medium was collected and radioactivity measured by liquid scintillation counting. Values are results obtained from 5 separate cultures, expressed as means ± SE. Specific activity of added [3H]AA was used to calculate amounts (in pmol). *P < 0.05 compared with control. Std, standard.
The chemical structure of compound X was identified by LC/MS using in-source CID (Fig. 7A). The mass spectrum contained a molecular ion (M-H)/H11002 mass/charge (m/z) 367 and fragmented with loss of H2O (m/z 349), loss of 2H2O (m/z 331), loss of CO2 and 2H2O (m/z 287), loss of C6H13O2 (m/z 233), and loss of C7H13O4 (m/z 189). This fragmentation pattern is identical to that obtained with authentic 20-OH-PGE2 under the same LC/MS conditions (Fig. 7B).

To determine whether 20-OH-PGE2 formation might play a role in the observed COX-dependent vasodilatation produced by 20-HETE, we compared effects of the PGE2 and 20-OH-PGE2 in the basilar artery. In arteries preconstricted with U-46619, PGE2 produced dose-dependent vasodilatation when the concentration was raised from 50 to 500 nM, but dilatation partially abated when the concentration was raised to 1 μM PGE2 (Fig. 8A). In contrast, 50 nM to 1 μM 20-OH-PGE2 produced only concentration-dependent vasodilatation (Fig. 8B). To determine whether this dilatation might be due to a PGE2- or 20-OH-PGE2-induced increase in AA mobilization, these compounds were incubated with MBEC previously loaded with [3H]AA. Unlike 20-HETE and 20-COOH-AA, neither 20-OH-PGE2 nor PGE2 increased radiolabeled AA mobilization when compared with control cultures. However, calcium ionophore A-23187 produced a 2.5-fold greater increase in radiolabeled AA release from MBEC (Fig. 8C).

Fig. 6. Conversion of 20-HETE to 20-OH-PGE2: effect of Indo. MBEC were treated with or without Indo (10 μM) for 30 min. Medium was removed, and incubation was continued in fresh medium containing 2 μM 20-[3H]HETE in the absence (A) or presence (B) of Indo. Medium was collected and radiolabeled products analyzed by reverse-phase HPLC. Representative chromatograms are shown, but similar results were obtained from 2 additional cultures in each case. 16-OH-16:3, 16-OH-hexadecatrienoic acid.

Fig. 7. Mass spectra of 20-HETE metabolite. Mass spectra were obtained by liquid chromatography combined with mass spectrometry (LC/MS) with in-source collision-induced decompositions (CID). To obtain sufficient quantities of products for structural identification, it was necessary to utilize 75-cm2 cultures of MBEC. HPLC analysis indicated that pattern of metabolite formation by endothelial cell cultures was similar to that shown in Fig. 5. Major polar metabolite (A) exhibited a mass spectrum that was identical to that of a 20-OH-PGE2 standard (B).

Fig. 8. Effects of 20-OH-PGE2 and PGE2 on mouse basilar artery vasoactivity and on AA release from MBEC. Cumulative concentration-response relationships were determined for PGE2 (A) and 20-OH-PGE2 (B). Data are presented as means ± SE obtained from 4 separate experiments. For AA release experiments (C), MBEC were incubated with 0.1 μM [3H]AA for 4 h. After incubation, cells were treated with medium containing 0.1 μM BSA and either vehicle (control), 2 μM 20-OH-PGE2, 2 μM PGE2, or 1 μM A-23187 for 30 min. Medium was collected and radioactivity measured by liquid scintillation counting. Values are expressed as means ± SE of 3 separate cultures of MBEC. Specific activity of added [3H]AA was used to calculate amounts (in pmol). **P < 0.01 compared with control.
AA release compared with that of the controls cultures (Fig. 8C).

**DISCUSSION**

In the present study, we found that 1) 20-HETE produced potent dilation of the mouse basilar artery; 2) 20-HETE was converted to 20-COOH-AA by the artery, and the 20-COOH-AA also produced dilation of the basilar artery; 3) indomethacin inhibited this response in both cases; 4) 20-HETE and 20-COOH-AA increased AA release and the production of PGE2 and 6-keto-PGF1α in MBEC; 5) 20-HETE was converted to 20-OH-PGE2 by MBEC; and 6) similar to 20-HETE, 20-OH-PGE2 also produced potent dilation of the basilar artery. These findings suggest that the vasodilation produced by 20-HETE in the mouse basilar artery occurs through a COX-dependent mechanism.

20-HETE is a potent vasoconstrictor in several vascular beds, including kidney, cerebral, and coronary arteries (33). In contrast, EETs, which are cytochrome P-450 epoxygenase metabolites of AA, are vasodilators in most vascular beds (33, 35). These findings have led to the generally accepted view that the balance between these two classes of cytochrome P-450 eicosanoid products contributes to the regulation of vascular tone. For example, the formation of 20-HETE antagonizes endothelium-derived hyperpolarization factor-mediated relaxation in porcine coronary arterioles (32). Consistent with previous results, we found that 14,15-EET caused potent dilation of the mouse basilar artery. Surprisingly, 20-HETE also caused dilation of this artery. However, the mechanism differs in these two cases because unlike 20-HETE, responses to 14,15-EET were not inhibited by indomethacin.

20-HETE is generally viewed as a vasoconstrictor, and cerebral vasoconstrictor effects of 20-HETE were observed in the cat, rat, and dog (33). However, there are systems where 20-HETE functions as a vasodilator. For example, 20-HETE produces vasodilation in the rabbit kidney, bovine coronary, and bovine pulmonary arteries (4, 30, 39). These vasodilatory responses have been attributed to nitric oxide release (39) and prostacyclin formation (4, 30, 39). In the present study, we found that the addition of 20-HETE to mouse brain endothelial cultures increased the production of PGE2 and prostacyclin (measured as the stable product 6-keto-PGF1α). A mechanism involving an increase in COX expression seems unlikely because COX-1 and COX-2 are constitutive in MBEC (5, 11), and the expression of COX-2 was not increased when the cells were incubated with 2 μM 20-HETE. A more likely possibility is that prostaglandin formation resulted from the increased release of AA, perhaps triggered by a 20-HETE-mediated increase in intracellular free Ca2+ (24). It should be noted that some previous studies (1, 38) demonstrating constrictor effects of 20-HETE in the cerebral artery were performed in the presence of indomethacin, which would mask a vasodilatory effect of 20-HETE that was dependent on COX activity.

PGE2 and prostacyclin are potent dilators in the cerebral circulation (21, 29). In agreement with these results, we observed that PGE2 caused marked dilation of the mouse basilar artery at concentrations between 50 to 500 nM. Thus increased production of PGE2 and prostacyclin might contribute to 20-HETE-induced vasorelaxation in the basilar artery. In addition, COX-dependent production of reactive oxygen species has been reported to produce dilation of cerebral blood vessels in several species (13). Whether such an oxidative mechanism also might contribute to 20-HETE-induced dilation of the mouse basilar arteries remains to be determined.

COX regulates renal arterial 20-HETE levels, and the metabolism of 20-HETE by COX is proposed to be a regulatory mechanism in vascular tone (2, 6, 18, 34). 20-HETE is converted to 20-OH-PGG2 and 20-OH-PGH2 by COX in ram seminal vesicle microsomes. However, these COX metabolites produce contraction of the rat aorta (34). Rabbit airway epithelial cells also convert 20-HETE to a COX metabolite that produces bronchial relaxation (18). In addition, lung microsomes convert 20-HETE to a prostanoid product (2). Consistent with these results, we found that 20-HETE is converted to 20-OH-PGE2 by the COX-dependent mechanism in MBEC. The production of 20-OH-PGE2 was enhanced by treatment of the cells with PMA, suggesting the involvement of COX-2 in this process. Like PGE2, 20-OH-PGE2 causes vasodilation in the kidney and increases renal cortical and medullary blood flow (28). Likewise, we observed that 20-OH-PGE2 produced potent dilation of the basilar artery. However, unlike 20-HETE, 20-OH-PGE2 did not increase AA mobilization in MBEC. Because PGE2 also did not increase AA mobilization, it is likely that both compounds produced vasodilation by a direct effect rather than indirectly through AA mobilization.

We found that 20-COOH-AA, like 20-HETE, produced dilation of the basilar artery and increased formation of PGE2 and 6-keto-PGF1α. Although these results raise the possibility that conversion to 20-COOH-AA might also contribute to 20-HETE-induced vasodilation, this seems less likely for several reasons. 20-COOH-AA was less potent than 20-HETE in producing these effects, and we were unable to detect the formation of a radiolabeled prostaglandin derivative when the brain endothelial cultures were incubated with 20-[3H]COOH-AA (data not shown). In addition, it is unlikely that 20-COOH-AA mediates the rapid vasodilatory response to 20-HETE, because 3 h was the earliest time that radiolabeled 20-COOH-AA was detected when the isolated cerebral artery preparation was incubated with 20-[3H]HETE. However, it is possible that 20-COOH-AA may prolong the vasodilatory response produced by 20-HETE in the basilar artery.

In summary, these findings indicate that 20-HETE produces dilation of the mouse basilar artery through a COX-dependent mechanism. The most likely possibilities are conversion of 20-HETE to 20-OH-PGE2 or increased production of PGE2 and prostacyclin due to mobilization of AA. While 20-HETE is generally considered to be a cerebral vasoconstrictor, modulation of its actions by COX could play an important counter-regulatory role in the cerebral circulation in some species or under some conditions.

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