ω-Oxidation of 20-Hydroxyeicosatetraenoic Acid (20-HETE) in Cerebral Microvascular Smooth Muscle and Endothelium by Alcohol Dehydrogenase 4∗

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20-Carboxyeicosatetraenoic acid (20-COOH- AA) is a bioactive metabolite of 20-hydroxyeicosatetraenoic acid (20-HETE), an eicosanoid that produces vasoconstriction in the cerebral circulation. We found that smooth muscle (MSMC) and endothelial (MEC) cultures obtained from mouse brain microvessels convert [3H]20-HETE to 20-COOH-AA, indicating that the cerebral vasculature can produce this metabolite. The [3H]20-COOH-AA accumulated primarily in the culture medium, together with additional radiolabeled metabolites identified as the chain-shortened dicarboxylic acids 18-COOH-18:4, 18-COOH-18:3, and 16-COOH-16:3. N-Heptoflumamide, a potent inhibitor of alcohol dehydrogenase (ADH), decreased the conversion of [3H]20-HETE to 20-COOH-AA by the MSMC and MEC and also by isolated mouse brain microvessels. Purified mouse and human ADH4, human ADH3, and horse liver ADH1 efficiently oxidized 20-HETE, and ADH4 and ADH3 were detected in MSMC and MEC by Western blotting. N-Heptoflumamide inhibited the oxidation of 20-HETE by mouse and human ADH4 but not by ADH3. These results demonstrated that cerebral microvessels convert 20-HETE to 20-COOH-AA and that ADH catalyzes the reaction. Although ADH4 and ADH3 are expressed in MSMC and MEC, the inhibition produced by N-Heptoflumamide suggests that ADH4 is primarily responsible for 20-COOH-AA formation in the cerebral microvasculature.

20-Hydroxyeicosatetraenoic acid (20-HETE) is a lipid mediator synthesized from arachidonic acid by cytochrome P450 (CYP) ω-oxidases (1–6). Vascular smooth muscle and kidney tubular epithelial cells produce 20-HETE (5–9), and there is increasing evidence that 20-HETE is involved in the pathophysiology of some animal models of hypertension (5, 10, 11). This is thought to occur through two mechanisms. One is an effect on ion transport mediated by the Na+/K+-ATPase and the Na+/K+-/2Cl−-cotransporter in the kidney (4, 12, 13). The other is a direct effect on vascular tone that produces vasoconstriction in the renal and cerebral circulation (14–17).

A major metabolite of 20-HETE, 20-carboxyeicosatetraenoic acid (20-COOH-AA), was detected initially in rabbit kidney cells isolated from the thick ascending loop of Henle and in human polymorphonuclear leukocytes (7, 8, 18). The ω-oxidation of 20-HETE to 20-COOH-AA also was observed recently in porcine coronary artery endothelial cultures (19), and it may have been overlooked in other tissues because the difference in the retention times of 20-HETE and 20-COOH-AA in the commonly used reverse-phase high performance liquid chromatography (HPLC) separation systems is less than 2 min (7, 19). There is increasing evidence that 20-COOH-AA is a bioactive metabolite rather than an inactivation product of 20-HETE. In the rabbit kidney, the potency of 20-COOH-AA in inhibiting the medullary Na+/K+−ATPase activity, the Na+/K+−2Cl− cotransporter, and the uptake of 86Rb was similar to that of 20-HETE (7, 8, 20, 21), and it was about 20% as potent as 20-HETE in inhibiting the 70-pS K+− channel in rat kidney (22). Furthermore, 20-COOH-AA at concentrations between 0.1 and 1 μM produces vasodilation of porcine coronary arteries preconstricted with endothelin, indicating that it also may have functional activity in the microvasculature (19). However, the metabolic pathways responsible for the conversion of 20-HETE to 20-COOH-AA in vascular cells are unknown.

20-HETE is synthesized by cerebral microvascular smooth muscle cells and produces vasoconstriction in the cerebral circulation (5, 9, 15, 16, 23). To investigate the metabolism of 20-HETE in the cerebral vasculature, we have examined the conversion of 20-HETE to 20-COOH-AA in smooth muscle cells (MSMC) and endothelial cells (MEC) cultured from mouse brain microvessels, as well as in the isolated microvessels, and we characterized the subsequent metabolism of 20-COOH-AA in the cell culture preparations. Because rat and human class IV alcohol dehydrogenases (ADH4) effectively oxidize 10–16-carbon saturated ω-hydroxy fatty acids (24, 25) and are expressed in rat and human blood vessels and rat microvascular endothelium (26, 27), we tested the hypothesis that this form of ADH might be involved in 20-HETE metabolism in cerebrovascular tissue.

EXPERIMENTAL PROCEDURES

Synthesis of [3H]20-HETE and [3H]20-COOH-AA—[3H]20-HETE and [3H]20-COOH-AA were synthesized from [5,6,8,9,11,12,14,15,16]-arachidonic acid (65.9 Ci/mmol, PerkinElmer Life Sciences) by incubation of 20-HETE or 20-COOH-AA with purified rat liver ADH4 in the presence of [3H]NAD+ and [3H]NADH. The radioactivity was purified by high performance liquid chromatography and mass spectrometry. To estimate 20-HETE, 20-COOH-AA, and [3H]NAD+, the microsomal proteins were precipitated with 10% TCA, and the radioactivity was determined by Liquid Scintillation counting. The [3H]20-HETE and [3H]20-COOH-AA were counted in the presence of a [3H]TCA and a [3H]TCA with the addition of 10 μg/ml of unlabeled 20-HETE and 20-COOH-AA to the reaction mixture. The radioactivity was dissolved in 1 ml of scintillation fluid (Ultima Gold; Packard), and the radioactivity was counted in a scintillation counter. The results were obtained in triplicate, and the error bars represent the standard deviation. The radioactivity was normalized to the microsomal protein content. The radioactivity was normalized to the microsomal protein content. The radioactivity was normalized to the microsomal protein content.
bution with a microsomal preparation containing recombinant human CYP4F3B (19). Briefly, [3H]arachidonic acid (250 μCi; 15 nmol) was incubated with 50 nM recombinant human CYP4F3B, CYP reductase, cytochrome b5, and an NADPH-regenerating system (Gentest, Woburn, MA) in 200 mM phosphate-buffered saline (PBS), pH 7.4, at 37 °C for 1 h. The reaction mixture was acidified to pH 4.0 with formic acid and extracted with water-saturated ethyl acetate, and the radiolabeled products were purified by HPLC.

Cell Culture and Incubation—Microvessels were isolated from mouse brains (28), and MSMC and MEC cultured from the microvessels were isolated and grown in modified high glucose Dulbecco’s minimum essential medium (DMEM) containing 10% fetal bovine serum (FBS), as described previously (29–31). The cultures were maintained until confluent at 37 °C in a humidified atmosphere containing 5% CO2.

Analysis of the Medium and Cell Lipids—After the medium was acidified to pH 4 with formic acid, the lipids were extracted with 4 volumes of ice-cold water-saturated ethyl acetate. The lipid-soluble radioactivity was measured by liquid scintillation counting using a liquid scintillation solution at a 3:1 ratio and then passing the mixture through an in-line flow scintillation counter. In additional studies, mouse ADH4 was incubated with 20-HETE and NADPH and the products were analyzed by LC/MS.
described previously (19). The data were processed with the Hewlett-Packard Chemstation™ software program.

Statistical Analysis—Data were expressed as mean ± S.E. Analysis of variance, followed by the Student-Newman-Keuls method, was used to compare the conversion of 20-HETE to 20-COOH-AA under control and treatment conditions. Differences with p < 0.05 are considered significant.

RESULTS

20-HETE Conversion to 20-COOH-AA by Cultured Cells—We first determined whether 20-HETE contained in MSMC can be converted to 20-COOH-AA. It was necessary to load the MSMC with [3H]20-HETE to obtain enough intracellular radioactivity for metabolic studies. In preliminary experiments, we found that MSMC incubated with [3H]20-HETE took up increasing amounts of radioactivity during a 4-h incubation. The distribution of the radioactivity in the cell lipid extract as determined by thin layer chromatography was 75% in phospholipids, 16% in neutral lipid esters, and 9% as fatty acid after 1 h of incubation. This distribution did not change appreciably as the incubation continued. HPLC analysis of the hydrolyzed cell lipid extract showed that 99% of the radioactivity incorporated into the cells after 1 h remained as 20-HETE. After 2 h, 94% of the radioactivity still was present as 20-HETE. This distribution did not change appreciably when the incubation was extended to 4 h. The retention and metabolism of the incorporated [3H]20-HETE were determined during subsequent incubation of the MSMC.

Fig. 1 shows the changes in distribution of lipid radioactivity in the cells and medium during incubation of mouse cerebral microvascular smooth muscle cultures labeled with [3H]20-HETE. MSMC cultures in 6-well plates were maintained in a medium consisting of DMEM containing 10% FBS at 37 °C with 5% CO2 as the gas phase. When the cultures were 70% confluent, they were incubated for 2.5 h with 1 μM [3H]20-HETE in DMEM containing 0.1 μM BSA. This medium was removed, and after washing with 2 ml of DMEM containing BSA, the radiolabeled cells were incubated at 37 °C for up to 8 h in fresh medium containing 0.1 μM BSA but no 20-HETE or radioactivity. After incubation, the cells and medium were separated, and the lipids were extracted and assayed for radioactivity by liquid scintillation counting. The picomole values were calculated based on the specific radioactivity of the [3H]20-HETE used in the initial incubation to load the cells. Each point is the mean ± S.E. of values obtained from three separate cultures.

Fig. 1 shows the changes in distribution of lipid radioactivity in the cells and medium during incubation of mouse cerebral microvascular smooth muscle cultures labeled with [3H]20-HETE. The methods and incubation conditions are the same as described in Fig. 1, except that the radiolabeled lipids released into the medium were separated and assayed by HPLC with an in-line flow scintillation counter. The solvent system contained H2O adjusted to pH 4.0 with formic acid and an acetonitrile gradient that increased from 30% to 100% over 70 min at a flow rate of 0.7 ml/min. In addition to the main radiolabeled product, 20-COOH-AA, and 20-HETE, radiolabeled metabolites designated as X and Y were detected. Representative chromatograms from a single culture are shown from the incubations lasting 1 (A), 4 (B), and 8 h (C). Similar results were obtained from two additional cultures at each of these times.

Identification of the 20-HETE Metabolites—MSMC cultures were incubated with either 20-HETE or [2H6]20-HETE, and the products contained in the lipid extract of the media were separated by liquid chromatography and identified by negative ion electrospray mass spectrometry at 110 V. Fig. 3, A–D, shows the mass spectra obtained from the incubation with 20-HETE, and Fig. 3, E–H, shows the corresponding spectra from the incubation with [2H6]20-HETE. The most abundant product obtained from the incubation with 20-HETE, which had a retention time of 36.9 min in this liquid chromatography gradient system, contained an ion (M – H)⁻1 m/z 333 (Fig. 3A) and fragmented with loss of H2O (−18), CO2 (−44), and H2O plus CO2 (−62). The corresponding product in the incubation with [2H6]20-HETE contained (M − H)⁻1 m/z 339 and was fragmented as indicated above (Fig. 3F). These data are consistent with the structure of 20-COOH-AA and its hexa-deutero analogue (19). Na⁺ adduct ions (m/z 355 and m/z 361) are present in these and the other spectra shown in Fig. 3.

The material designated as X in Fig. 2 separated into two components in this liquid chromatography gradient. One of the components from the 20-HETE incubation (M − H)⁻1 m/z 305 (Fig. 3B) had a retention
ADH4 and 20-HETE ω-Oxidation

FIGURE 3. Mass spectra of compounds detected in the medium following incubation of the mouse brain microvascular smooth muscle cultures with 20-HETE or [16,16,17,17,18,18-2H]20-HETE. MSMC cultures were incubated for 5 h with either 20-HETE (A–D) or [16,16,17,17,18,18-2H]20-HETE and the lipids were extracted from the medium and analyzed by LC/MS. Negative ion electrospray mass spectra are shown. The spectra shown in A and E are consistent with the structure of 20-COOH-AA, and the other spectra are consistent with the structures of chain-shortened dicarboxylic acid products: B and F, 18-COOH-18:4; C and G, 18-COOH-18:3; D and H, 16-COOH-16:3.

FIGURE 4. HPLC analysis of radiolabeled lipids contained in the medium following incubation of mouse brain microvascular cultures with [3H]20-COOH-AA. MSMC and MEC cultures seeded in 6-well plates were incubated for up to 24 h in modified DMEM containing 2 μM [3H]20-COOH-AA and 0.1 μM BSA. The radioactive lipids contained in extracts of the media were assayed by HPLC as described in Fig. 2. Incubations were done in triplicate cultures for 0.5, 1, 2, 4, 8, 16, and 24 h, but only HPLC data for the 24 h incubations are shown. The chromatograms were obtained from single cultures, MSMC (A) and MEC (B), but similar results were obtained from two additional cultures in each case.

Inhibition of 20-COOH-AA Production—Several enzymes present in vascular cells, including cytochrome P450 ω-oxidases, alcohol and alde-
ADH4 and 20-HETE ω-Oxidation

Detection of ADH in Cerebral Microvascular Cells—The inhibition of 20-HETE metabolism by N-heptylformamide suggested that an ADH might be involved in the conversion of 20-HETE to 20-COOH-AA. ADH4 is the principal ADH isofrom present in rodent blood vessels (26, 27). Moreover, the effects produced by the retinoids in the MEC, and the fact that the concentrations of ethanol (a good substrate for ADH1) and 4-methylpyrazole (a potent inhibitor of ADH1) tested did not decrease radiolabeled 20-COOH-AA production, suggested that ADH4 was involved in the conversion of 20-HETE to 20-COOH-AA in the microvascular cells. Therefore, the expression of ADH4 was investigated in MEC and MEC cell extracts. The Western blots shown in Fig. 7 demonstrate that ADH4 is expressed in both cell types. Blots obtained from homogenates of stomach from normal mice and mice with deletion of the gene (33) are included to demonstrate the specificity of this polyclonal antibody for ADH4. Additional blots demonstrated that ADH4 is also expressed in the MEC and MEC extracts (Fig. 7). However, a distinct ADH1 band was not detected in these cell extracts (data not shown).

Studies with Purified Alcohol Dehydrogenases—Purified recombinant mouse ADH4, human ADH4, and human ADH3 were tested to determine whether 20-HETE was a substrate for these enzymes. The steady-state kinetic constants that were obtained are shown in TABLE ONE. Each of the enzymes oxidized 20-HETE, and the highest catalytic efficiency was obtained with human ADH4. Although N-heptylformamide

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**FIGURE 5.** Mass spectra of compounds detected in the medium following incubation of the mouse brain microvascular smooth muscle cultures with 20-COOH-AA. MSMC cultures were incubated for 8 h with 20-COOH-AA, and the lipids were extracted from the medium and analyzed by LC/MS. Negative ion electrospray mass spectra are as follows: 18-COOH-18:4 (A), 18-COOH-18:3 (B), and 16-COOH-16:3 (C).

hyde dehydrogenases, can potentially metabolize 20-HETE. In addition, 20-HETE may undergo free radical-mediated peroxidation. Therefore, we incubated the MEC with several pharmacological agents in an attempt to gain some insight into the pathways responsible for 20-COOH-AA formation. There was no appreciable effect on 20-COOH-AA formation. There was no appreciable effect on 20-COOH-AA formation.

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**FIGURE 6.** Effect of N-heptylformamide on the conversion of [3H]20-HETE to [3H]20-COOH-AA in mouse cerebral microvascular cultures and isolated microvessels. Cultures were grown in modified DMEM with 10% FBS until confluent and were then incubated with the indicated concentrations of N-heptylformamide in modified DMEM containing 0.1 μM BSA. All incubations were done at 37 °C with 5% CO2 as the gas phase. Additional microvessel preparations and cultures were similarly incubated without N-heptylformamide as a control. After 20 min, 2 μM [3H]20-HETE and 0.1 μM BSA were added, and the incubation was continued for 4 h. The medium was removed, acetylated to pH 4.0 with formic acid, extracted with ethyl acetate, and analyzed by HPLC as described in Fig. 2 with in-line scintillation counting to determine the amount of radiolabeled 20-COOH-AA formed. Data from incubations with MEC are shown in A, and data from incubations with MEC are shown in B. The values in picomoles were calculated based on the specific activity of the [3H]20-HETE substrate. The HPLC data are from mouse brain microvessels incubated with 2 μM [3H]20-HETE for 18 h at 37 °C in modified DMEM containing 0.1 μM BSA and no inhibitor (C) or 20 μM N-heptylformamide (D). Results from one experiment are shown, but similar data were obtained from two additional cerebral microvessel preparations. A and B, * indicates p < 0.05 compared with 0 μM points; # indicates p < 0.05 compared with 20 μM (A) or 10 μM (B) points, respectively. ▲ indicates p < 0.05 compared with 20 μM point (B).
inhibited mouse and human ADH4, it did not inhibit the oxidation by human ADH3.

Fig. 8 shows an analysis of the time-dependent changes in radiolabeled lipids present during incubation of purified recombinant mouse ADH4 with [3H]20-HETE. Two radiolabeled compounds were detected by HPLC, 20-HETE and a product with a retention time of 32 min. 20-HETE also was incubated in this medium without enzyme for 90 min (Fig. 8B). The fragmentation pattern of this substance was identical to that shown in Fig. 3A, consistent with the structure of 20-COOH-AA. Fig. 9C demonstrates that the selective ion scan for (M − H)− m/z 317 had a retention time of 18.5 min, and the negative ion electrospray mass spectrum of this fragment was identical to that obtained previously for 20-HETE (19). As shown in Fig. 9D, the selective ion scan for (M − H)− m/z 317 had a retention time of 20.8 min. This component fragmented with loss of H2O (−18) and CO2 (−44), consistent with the structure of the ω-aldehyde derivative of 20-HETE.

Fig. 10 shows that human ADH4 and human ADH3 also converted [3H]20-HETE to 20-COOH-AA. With the specified enzyme concentration and time, ADH3 converted about 50% of the 20-HETE to 20-COOH-AA. With a much lower concentration of enzyme, ADH4 converted 85% of the [3H]20-HETE to 20-COOH-AA.

Horse liver ADH1 also converted [3H]20-HETE to 20-COOH-AA in a sodium glycine buffer at pH 10. The reaction went to completion under these conditions, and HPLC analysis demonstrated that 20-COOH-AA was the only distinct radiolabeled product (data not shown).

DISCUSSION

These findings demonstrate that cerebral microvessels, as well as smooth muscle and endothelial cells cultured from the microvessels, can oxidize 20-HETE to 20-COOH-AA. Although the cells also converted 20-COOH-AA to chain-shortened dicarboxylic acids, most likely through β-oxidation (19), this was a relatively slow process. As a result, much of the 20-COOH-AA accumulated in the extracellular fluid where it presumably would modulate endothelial and smooth muscle vasoregulatory signaling pathways. Previous work demonstrated that 20-COOH-AA and 20-HETE have similar effects on ion

Fig. 7. Detection of ADH in mouse cerebral microvascular cultures by Western blotting. MSMC and MEC extracts containing 50 µg of protein were loaded on 10% SDS-PAGE, and the blots were probed with rabbit antiserum raised against mouse ADH3 (A) or ADH4 (1:500) (B). To verify the specificity of the antibody for ADH4, stomach homogenates obtained from normal and Adh4 gene-deleted mice were included in the assay. β-Actin was used as the control for protein loading and transfer.

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FIGURE 8. HPLC analysis demonstrating conversion of [3H]20-HETE to 20-COOH-AA by purified recombinant mouse ADH4. Mouse ADH4 (3 µg/ml) was incubated at 37 °C with 2 µM [3H]20-HETE in a reaction containing 1 mM NAD+ , 83 mM potassium phosphate, 40 mM KCl, and 0.25 mM EDTA buffer, pH 7.3. The reactions were initiated by dissolving the 20-HETE in acetonitrile, and re-evaporation was used, and the buffer contained 0.02% acetone and 0.02% Tween 80. The ethanol contained in the commercial 20-HETE was removed by evaporation under nitrogen, dissolving the 20-HETE in acetonitrile, and re-evaporation under nitrogen. Initial velocities were fitted with the HYPER program (55).

These findings demonstrate that cerebral microvessels, as well as smooth muscle and endothelial cells cultured from the microvessels, can oxidize 20-HETE to 20-COOH-AA. Although the cells also converted 20-COOH-AA to chain-shortened dicarboxylic acids, most likely through β-oxidation (19), this was a relatively slow process. As a result, much of the 20-COOH-AA accumulated in the extracellular fluid where it presumably would modulate endothelial and smooth muscle vasoregulatory signaling pathways. Previous work demonstrated that 20-COOH-AA and 20-HETE have similar effects on ion
ADH4 and 20-HETE ω-Oxidation

FIGURE 9. LC/MS-selective ion scans and negative ion electrospray mass spectra of products from incubation of purified recombinant mouse ADH4 with 20-HETE. Purified mouse recombinant ADH4 was incubated for 6 h at 37 °C with 2 μM 20-HETE as described in Fig. 8, and the lipid extract was analyzed by LC/MS. The liquid chromatography gradient began with 0% acetonitrile and was increased to 95% acetonitrile over 25 min at a flow rate of 0.7 ml/min. The total ion chromatogram is shown in A, and the selective ion scans and negative ion electrospray mass spectra (insets) are shown for (M – H)⁻ m/z 333 (B), (M – H)⁻ m/z 319 (C), and (M – H)⁻ m/z 317 (D).

Because of the potential role of 20-COOH-AA in vascular regulation, it is important to determine the enzymatic pathway that converts 20-HETE to 20-COOH-AA. Several of our findings suggest that ADH4 may play a major role in this process in the cerebral microvasculature, in agreement with the observation that ADH4 oxidizes medium and long chain ω-hydroxy fatty acids (24, 25). ADH4 was detected in the MSMC and MEC in the cerebral circulation (5, 23). An alternative possibility, suggested by the fact that 20-COOH-AA produces relaxation of porcine coronary microvessels (19), is that its function in the cerebral circulation is to modulate or terminate the vasoconstriction produced by 20-HETE.

The finding that 20-HETE is similar to that of mouse ADH4 but less than that of human ADH4. Because the sequences of human and mouse ADH3 are 92.8% identical and the substrate-binding sites are almost identical (49), these enzymes should have similar activities on 20-HETE. The oxidation of 20-HETE by ADH3 was not inhibited by N-heptylformamide. This finding is opposite to that obtained with N-heptylformamide in the cultured cells, suggesting that ADH4 rather than ADH3 probably is primarily responsible for 20-HETE oxidation in the cerebral microvasculature.

The finding that 20-HETE is also a substrate for horse liver ADH1 (a class I enzyme closely similar to mouse ADH1) indicates that 20-HETE can be oxidized in the liver, which has very high levels of ADH1. However, ADH1 accounts for only 4% of total extrahepatic ADH activity in rats (50), and it seems unlikely that it would have a major role in 20-HETE metabolism in the vasculature. In support of this conclusion, 2 mM 4-methylpyrazole, a potent inhibitor of mouse ADH1 (Kᵢ = 0.15 μM, Ref. 41), did not decrease the conversion of 20-HETE to 20-COOH-AA in the MSMC. Because the Kᵢ for 4-methylpyrazole is 10 mM for rat ADH4 (50), little inhibition of the homologous mouse ADH4 transport in the rabbit kidney loop of Henle (7), implying that 20-COOH-AA might augment some of the functional effects of 20-HETE. In this context, the 20-COOH-AA formed in cerebral microvascular tissue might potentiate the vasoconstriction initiated by 20-HETE in the cerebral circulation (5, 23). An alternative possibility, suggested by the fact that 20-COOH-AA produces relaxation of porcine coronary microvessels (19), is that its function in the cerebral circulation is to modulate or terminate the vasoconstriction produced by 20-HETE.
would be expected. Furthermore, the formation of 20-COOH-AA by the MSMC was also not decreased by 50 mM ethanol, an excellent substrate for ADH1 ($K_m = 1.4$ mM, $k_{cat} = 0.65$ s$^{-1}$, Ref. 24) but a very poor substrate for mouse ADH4 ($K_m = 1.1$ M, $k_{cat} = 22$ s$^{-1}$, Ref. 36). This difference arises in part because of the substitution of Val-294 in ADH1 with Ala in the rat and mouse ADH4 (51). Finally, ADH1 was not detected by Western blotting in either the MASM or MEC.

A physiologically relevant concentration of ethanol has been shown to inhibit the oxidation of 20-hydroxyeicosatetraenoic (LT) $B_4$ to 20-carboxy-LTB$4$, and $\omega$-hydroxy-N-acetyl-LTE$_4$ by ADH in rat liver and isolated rat hepatocytes (52, 53). These findings establish a precedent for an ADH-dependent interaction between ethanol and eicosanoid metabolism, and the present observation that 20-HETE is a substrate for ADH1 suggests the possibility of a similar interaction between ethanol and 20-HETE oxidation in the liver. Ethanol, a poor substrate for mouse ADH4 (36), did not inhibit the conversion of 20-HETE to 20-COOH-AA in the MASM. However, ethanol is a reasonably good substrate for human ADH4 (39, 48, 51), and low concentrations of ethanol could inhibit 20-HETE oxidation and facilitate the reduction of the aldehyde intermediate back to 20-HETE, as has been demonstrated with human ADH1 acting on retinol or retinal (39, 54). This suggests the possibility that ethanol may decrease catabolism of 20-HETE in the human vasculature and thereby perturb vascular reactivity. In view of these possibilities, the role of ADH4 and ethanol in the metabolism of 20-HETE in human blood vessels should be assessed.

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