Induction of Renal Cytochrome P450 Arachidonic Acid Epoxygenase Activity by Dietary \(\gamma\)-Linolenic Acid

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ABSTRACT

Dietary \(\gamma\)-linolenic acid (GLA), a \(\omega-6\) polyunsaturated fatty acid found in borage oil (BOR), lowers systolic blood pressure in spontaneously hypertensive rats (SHRs). GLA is converted into arachidonic acid (AA) by elongation and desaturation steps. Epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) are cytochrome P450 (P450)-derived AA eicosanoids with important roles in regulating blood pressure. This study tested the hypothesis that the blood pressure-lowering effect of a GLA-enriched diet involves alteration of P450-catalyzed AA metabolism. Microsomes and RNA were isolated from the renal cortex of male SHRs fed a basal fat-free diet for 5 weeks to which 11% by weight of sesame oil (SES) or BOR were added. There was a 2.6- to 3.5-fold increase in P450 epoxygenase activity in renal microsomes isolated from the BOR-fed SHRs compared with the SES-fed rats. Epoxygenase activity accounted for 58% of the total AA metabolism in the BOR-treated kidney microsomes compared with 33% in the SES-treated rats. More importantly, renal 14,15- and 8,9-EET levels increased 1.6- to 2.5-fold after dietary BOR treatment. The increase in EET formation is consistent with increases in CYP2C23, CYP2C11, and CYP2J protein levels. There were no differences in the level of renal P450 epoxygenase mRNA between the SES- and BOR-treated rats. Enhanced synthesis of the vasodilatory EETs and decreased formation of the vasoconstrictive 20-HETE suggests that changes in P450-mediated AA metabolism may contribute, at least in part, to the blood pressure-lowering effect of a BOR-enriched diet.

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ABBREVIATIONS: SHR, spontaneously hypertensive rat; PUFA, polyunsaturated fatty acid; GLA, \(\gamma\)-linolenic acid; BOR, borage oil; SES, sesame oil; P450, cytochrome P450; EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; sEH, soluble epoxide hydrolase; DHET, dihydroxyeicosatrienoic acid; TGF, tubuloglomerular feedback; HPLC, high-performance liquid chromatography; LC/MS/MS, liquid chromatography-tandem mass spectrometry; mEH, microsomal epoxide hydrolase; EH, epoxide hydrolase.
England Nuclear (Boston, MA) and radiolabeled arachidonic acid from the essential fatty acid linoleic acid by a physiological effects of dietary GLA supplementation, little is known about the effect of this diet on the synthesis of biologically active metabolites derived from GLA.

GLA is either taken up directly from the diet or converted from the essential fatty acid linoleic acid by a Δ6-desaturase-catalyzed desaturation step and can be converted through chain elongation and desaturation steps into arachidonic acid (Mathews et al., 2000). Metabolites of arachidonic acid are highly potent regulatory molecules with important roles in inflammation, blood pressure control, and platelet aggregation.

The major cytochrome P450 (P450)-derived eicosanoids are regio- and stereoisomeric epoxide eicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE). EETs are further converted by soluble epoxide hydrolase (sEH) into corresponding dihydroyeicosatrienoic acids (DHETs) (Kroetz and Zeldin, 2002). P450 eicosanoids function as autocrine and paracrine factors and mediate local cellular responses, including regulation of renal vascular tone, tubuloglomerular feedback (TGF), and sodium reabsorption (Kroetz and Zeldin, 2002). The formation of 20-HETE is mediated mainly by enzymes of the CYP4A and CYP4F family, and 20-HETE has vasoconstrictive, natriuretic, and diuretic properties (Kroetz and Zeldin, 2002). Epoxidation of arachidonic acid is catalyzed largely by members of the CYP2C (Karara et al., 1993) and CYP2J (Wu et al., 1997) families. Once formed, EETs can be either incorporated into membrane phospholipid pools or efficiently hydrolyzed to less active DHETs and secreted into the extracellular space (Zeldin et al., 1993). EETs have vasodilatory and natriuretic properties and are putative endothelium-derived hyperpolarizing factors (Hecker et al., 1994; Campbell et al., 1996). EETs also have anti-inflammatory (Node et al., 1999) and fibrinolytic properties (Node et al., 2001). The critical role of EETs and 20-HETE in the control of renal arteriole diameter and sodium reabsorption suggests an important contribution to integrated renal function and regulation of blood pressure.

In light of the vasoactive and renal regulatory roles of P450 eicosanoids, it is reasonable to postulate that dietary GLA could affect P450 eicosanoid levels and alter blood pressure. This hypothesis is supported by studies showing altered P450-catalyzed arachidonic acid metabolism in multiple models of hypertension (Sarkis and Roman, 2004). Although the beneficial effect of dietary PUFAs on cardiovascular disease is well recognized, there is a paucity of data on the molecular and/or genetic basis for the effect of GLA on blood pressure. In this study, we determined the effect of a GLA-enriched diet on renal arachidonic acid metabolism and renal P450 and sEH expression. The novel finding of induction of vasoprotective EET formation by GLA treatment provides a mechanism for the antihypertensive effects of a GLA-rich diet.

Materials and Methods

Materials. Radiolabeled nucleotides were purchased from New England Nuclear (Boston, MA) and radiolabeled arachidonic acid from Amersham Life Science (Arlington Heights, IL). Restriction enzymes were obtained from New England Biolabs (Beverly, MA), modifying enzymes from Gibco/BRl (Gaithersburg, MD), and RNase from Ambion (Austin, TX). All molecular biology grade chemicals, HPLC solvents, and ScintiVerse LC were from Fisher Scientific (Pittsburgh, PA). Nitrocellulose membranes were from Micron Separations (Westborough, MA). 15(S)-HETE-d8 and all regioisomeric EETs and DHETs were purchased from Cayman Chemicals (Ann Arbor, MI). All other reagents were of the highest grade available and were purchased from Fisher Scientific or Sigma Chemical (St. Louis, MO).

Animals and Dietary Preparation. Details of the GLA diet and effects on blood pressure have been described previously (Engler and Engler, 1998). Male SHR (14–15 weeks old) were obtained from Harlan (Indianapolis, IN) and housed two to three per cage at constant temperature (26°C) with 12-h light/12-h dark cycles. The rats were randomly assigned to two groups and fed a diet of a fat-free basal mix for 5 weeks (Teklad, Madison, WI) with the addition of 11% of weight of either sesame oil (SES) (Amend Drug and Chemical Co., Irvington, NJ) or borago oil (BOR) (Traco Labs, Seymour, IL). The oils were used to provide similar proportions of total 18-carbon monounsaturated and polyunsaturated fatty acids in the diet yet control for GLA (18:3n-6). The content of GLA in BOR was 24.4%. The control SES-based diet was rich in oleic acid (18:1n-9, 35%) and devoid of GLA. At the end of the 5-week dietary treatment, anesthesia was administered with a mixture of oxygen (70%), nitrous oxide (20%), and halothane (5%). The abdominal cavities were opened, and the kidneys were perfused with ice-cold saline. Perfused kidneys were rapidly removed, and the cortex was dissected out and immersed in liquid nitrogen. All tissue was stored at −80°C until preparation of RNA or microsomes. Animal use was approved by the University of California San Francisco Committee on Animal Research and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

Renal Arachidonic Acid Metabolism. Microsomes were prepared from frozen cortex using differential centrifugation and stored at −80°C. Renal cortical arachidonic acid metabolism was measured in incubations containing [1-14C]arachidonic acid (85 µM, 0.2 µCi) using reverse-phase HPLC with radiometric detection as described previously (Kroetz et al., 1997; Su et al., 1998; Yu et al., 2000a,b; Xu et al., 2004).

Extraction Surrogates and Internal Standard. 10(11)-Epoyxheptadecanoic acid and 10,11-dihydroxyx-nonadecanoic acid, kindly provided by Dr. John W. Newman (University of California, Davis), served as EET and DHET extraction surrogates for liquid chromatography-tandem mass spectrometry (LC/MS/MS) quantification of renal eicosanoids. The validation and synthesis of these standards have been previously described (Newman et al., 2002). Surrogate recoveries were calculated relative to the internal standard, 15(S)-HETE-d8. Calibration solutions comprising eicosanoid standards (5–1500 µM) were prepared in acetone, sealed, and stored under nitrogen at −20°C.

Lipid Extraction and Hydrolysis. Frozen tissues were thawed on ice and approximately 100 mg of kidney cortex was used for lipid extraction. Butylated hydroxytoluene (0.05 g/l) was added during the extraction step to prevent peroxyl radical-propagated transformation of PUFAs. An equal volume of methanol and 2 volumes of chloroform and extraction surrogates were added to the tissue, followed by homogenization and vortex mixing. The mixture was extracted twice with chloroform, and the combined organic phase was washed once with distilled water. After evaporation of the organic solvent under nitrogen, the dry residue was dissolved in 1 N sodium hydroxide (1 ml) and incubated at room temperature for at least 3 h to hydrolyze the fatty acids from the phospholipid backbone. Lipids were extracted twice with ethyl acetate, evaporated to dryness under a stream of nitrogen, and stored at −80°C until LC/MS/MS analysis. Immediately before analysis, the lipid residue was dissolved in 90 µl of acetone/trimethylamine and supplemented with 10 µl of 15(S)-HETE-d8 (10
ng) as an internal control. A 10-μl sample aliquot was injected for analysis.

**HPLC and Mass Spectrometry Instrumentation.** Analysis of lipids by HPLC was performed using a 2.0 × 150 mm, 5-μm Luna C18(2) column (Phenomenex, Torrance, CA) held at room temperature. Lipids were eluted from the reverse-phase HPLC column with acetonitrile-methanol-water-acetic acid, (55:10:35:0.01) at 0.8 ml/min. The effluent was introduced onto a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK) and subjected to negative mode electrospray ionization with a capillary voltage at −3.0 kV. Collision voltage ranged from −13 to −28 eV, and cone voltage ranged from −50 to −60 V for EETs, DHETs, and 20-HETE, optimized for sensitivity and interexperiment consistency. Multireaction monitoring was used to simultaneously detect the four regioisomeric EETs and corresponding DHETs, 20-HETE, and internal controls. Characteristic mass transition monitoring was adopted from the assay developed by the Hammock laboratory (Newman et al., 2002) with the exception of 15(S)-HETE-d8, for which the [M-H]⁻ → [fragment]⁻ transition was monitored from m/z 327.2 to 182.3.

**Ribonuclease Protection Assays.** Total RNA was isolated from frozen renal cortex tissue by acid-phenol extraction. Construction of specific CYP4A, CYP2C23, CYP2E1, CYP2J2, CYP2J4, mEH, and sEH riboprobes and details of the ribonuclease protection assays were described previously (Kroetz et al., 1997; Yu et al., 2000a,b). The CYP4A3 probe is used to detect both the CYP4A3 and CYP4A2 mRNA transcripts. A rat GAPDH ribonuclease protection probe was included as a control in all hybridizations. RNAse protection assays were carried out as described earlier with the exception that [α-32P]UTP was used in place of [α-32P]CTP for the CYP2C23 and CYP2E1 probes. Gels were dried, and protected fragments were visualized with a PhosphorImager and analyzed using ImageQuant Software (Molecular Dynamics, Sunnyvale, CA).

**Western Immunoblotting.** Renal cortical microsomes (10 μg) were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to nitrocellulose in 25 mM Tris-192 mM glycine-20% methanol using a semidry transfer system (BioRad, Hercules, CA). Primary antibodies used in these studies were a rabbit anti-rat CYP2C23 antisera that was a gift from Dr. Jorge H. Capdevila (Vanderbilt University, Nashville, TN) and a rabbit anti-human CYP2J2 IgG kindly provided by Dr. Darryl C. Zeldin (National Institute of Environmental Health Sciences). Goat anti-rat CYP4A1 and anti-rat CYP2C11 antisera were obtained from Gentest (Woburn, MA). A rabbit anti-mouse sEH antisera was kindly provided by Dr. Bruce Hammock (University of California, Davis, CA), and a goat actin antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Western blots were incubated with a 1:5000 (CYP2C23), 1:1000 (CYP2J2), 1:3000 (CYP2J4), 1:1000 (CYP4A1), or 1:2000 (sEH)-fold dilution of the primary antibody followed by a 1:10,000-fold dilution of Alexa Fluor 680 donkey anti-goat IgG or IRDye 800 conjugated affinity-purified anti-rabbit IgG. Immunoreactive proteins were visualized and quantitated using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). P450 and sEH immunoreactive protein levels were expressed relative to the level of actin quantitated on the same blots.

**Statistics.** Arachidonic acid metabolism and endogenous fatty acid quantification were performed in duplicate samples from individual animals and the results are expressed as means ± S.E.M. of five animals per treatment group. Statistical significance of differences between treatment groups was evaluated by an analysis of variance with multiple comparison testing with a modified t test. A value of p < 0.05 was considered to be statistically significant.

**Results**

**Renal Arachidonic Acid Metabolism in Vitro.** Arachidonic acid metabolism was measured in renal cortical microsomes from SES- and BOR-fed rats. Epoxygenase activities are expressed as the sum of EET and DHET formation, because epoxygenase activity is a prerequisite for DHET formation. All reactions were performed under conditions that were linear with respect to protein and time, and the formation of all metabolites was NADPH-dependent (data not shown). The formation of 19-HETE increased 1.5-fold, and epoxide formation increased 3.6-fold in the kidneys of BOR-fed rats compared with the SES group, whereas 20-HETE formation was unchanged (Fig. 1A). The increase in EET formation in BOR-treated rats was similar for the 8,9-, 11,12- and 14,15-EET regioisomers (Fig. 1B). In both SES- and BOR-treated rats, renal epoxygenase activity is highest for the 11,12-EET regioisomer. Interestingly, the contribution of 19- and 20-HETE to total renal P450 eicosanoid formation decreased in the BOR-treated rats (15 versus 11% for 19-HETE and 51 versus 31% for 20-HETE) (Table 1). In contrast, EET formation was increased from 34% in SES-fed rats to 58% in BOR-fed rats, indicating that arachidonic acid is shunted toward the P450 epoxygenase pathway during BOR diets. Total renal arachidonic acid metabolism increased 2-fold in BOR-fed rats relative to SES-fed rats.

**Renal EET Levels.** Whereas in vitro studies are important to characterize metabolic pathways of arachidonic acid metabolism, they cannot address many critical steps related to arachidonic acid metabolism in vivo. Endogenous P450 eicosanoid levels in the kidney cortex were quantified by LC-tandem mass spectrometry to determine the effect of SES and BOR diets on cellular levels of EETs within the kidney. Because the 5,6-EET metabolite is extremely labile and suffers extensive decomposition during sample preparation, its levels were not quantified in this study (Capdevila et al., 2002).
1981). The amount of each regiosomer of DHET is only approximately 10% of its corresponding EETs. The cortical 11,12- and 8,9-DHET levels are 1.7-fold higher in the BOR-fed rats compared with rats fed the control diet (Fig. 2A). Endogenous 14,15-EET levels increased 1.6-fold, and 8,9-EET content increased 2.5-fold in the renal cortex of BOR-fed relative to SES-fed SHRs (Fig. 2B) \( (p < 0.01) \). Although the renal 11,12-EET levels remain unchanged between the two groups, increased formation of 11,12-EET is evident from the higher renal 11,12-DHET content in BOR-fed rats. The extent of increase in the EET cellular content is consistent with the increase in P450 epoxygenase activity.

**Renal P450 Arachidonic Acid Epoxygenase and EH Protein Expression.** The expression of P450 epoxygenases was characterized to explore the mechanistic basis for the increased epoxygenase activity in the BOR-fed SHR kidney. CYP2C and CYP2J protein levels were examined in cortical microsomes from SES- and BOR-fed SHRs by Western blotting, and the expression level of these P450s was quantitated using the Odyssey Infrared Imaging System. Actin was also detected on each blot and representative results from actin staining are shown. The P450 and sEH protein levels were quantitated using the Odyssey Infrared Imaging System and expressed relative to the level of actin. Protein levels in samples from the SES-fed SHRs are set to 100%. *p*, significantly different from SES group, \( p < 0.05 \).

**Renal P450 Arachidonic Acid Epoxygenase and EH mRNA Levels.** RNase protection assays were used to quantitate the corresponding levels of CYP2C23, CYP2C11, and CYP2J immunoreactive protein in kidneys from the BOR-fed SHRs compared with the SES-fed rats. Increased levels of CYP2C23, CYP2C11, and CYP2J immunoreactive protein were consistent with the increased arachidonic acid epoxygenase activity and endogenous EET levels in the kidney cortex of BOR-fed SHRs. Peptide-based antisera against CYP2J4 and CYP2J9 detect rat CYP2J4 and CYP2J3 proteins, respectively, the two rat CYP2J isoforms currently identified. However, neither of these antisera detected differences in expression of CYP2J4 immunoreactive proteins between SES- and BOR-fed SHRs (data not shown). Thus, the identity of the CYP2J immunoreactive protein induced by a BOR diet remains unknown. The BOR diet had no effect on the levels of CYP4A1, mouse sEH, or actin. Immunoreactive proteins were visualized using the Odyssey Infrared Imaging System.

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**Table 1**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Metabolite Formation*</th>
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<tr>
<td></td>
<td>% total</td>
</tr>
<tr>
<td>19-HETE</td>
<td>15.0 ± 0.61</td>
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<tr>
<td>20-HETE</td>
<td>50.8 ± 3.66</td>
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<tr>
<td>EETs</td>
<td>34.2 ± 3.94</td>
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<tr>
<td></td>
<td>10.9 ± 0.26*</td>
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<td></td>
<td>31.4 ± 0.90*</td>
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<td>57.7 ± 1.12*</td>
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* Significantly different from sesame oil group, \( p < 0.01 \).

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**Fig. 2.** Endogenous DHET (A) and EET (B) levels in kidneys of SES- (solid bars) and BOR- (hatched bars) fed SHRs. Lipids were extracted from kidney cortex and quantified by LC/MS/MS as described under Materials and Methods. The values shown are the means ± S.E.M. for five animals per group. **, significantly different from SES group, \( p < 0.01 \).

**Fig. 3.** Western immunoblots of P450 arachidonic acid epoxygenases and soluble epoxide hydrolase in kidney cortex from BOR- and SES-fed SHRs. A, renal cortical microsomal proteins (10 μg) were separated on 10% SDS-polyacrylamide gels, transferred to nitrocellulose, and blotted with antisera against human CYP2J2, rat CYP2C11, rat CYP2C23, rat CYP4A1, mouse sEH, or actin. Immunoreactive proteins were visualized using the Odyssey Infrared Imaging System. The blots shown are representative of results from four to six animals per experimental group. Actin was also detected on each blot and representative results from actin staining are shown. B, the P450 and sEH protein levels were quantitated using the Odyssey Infrared Imaging System and expressed relative to the level of actin. Protein levels in samples from the SES-fed SHRs are set to 100%. *p*, significantly different from SES group, \( p < 0.05 \).
kidneys of SES- and BOR-fed SHRs (data not shown). These data suggest that the increased level of the CYP2C23, CYP2C11, and CYP2J2 immunoreactive proteins in the kidneys of BOR-fed SHRs is not a consequence of increased transcription and/or stabilization of the corresponding P450 epoxygenase mRNAs. Consistent with no change in CYP4A protein levels, the renal mRNA levels of CYP4A1, CYP4A2, CYP4A3, and CYP4A8 are comparable between rats receiving different dietary treatments (data not shown).

Discussion

We have previously reported that blood pressure is decreased in BOR-fed SHRs (Engler et al., 1998). The current analysis demonstrates that renal P450 epoxygenase-mediated arachidonic acid metabolism is elevated following a diet rich in GLA, suggesting that an increase in vasoprotective EETs might contribute to the blood pressure-lowering effects of this diet. The rat kidney is deficient in Δ6-desaturase (Huang et al., 1994), the enzyme necessary to convert linoleic acid to GLA, but it can catalyze the conversion of GLA to arachidonic acid. It is possible that the antihypertensive effect of GLA resides in its ability to bypass the defective Δ6-desaturase pathway in the kidney. Thus, dietary supplementation with GLA increases arachidonic acid levels in the kidney, thereby providing increased substrate for P450-catalyzed metabolism. Indeed, we observed a significant increase in endogenous EET levels in the kidney and in renal cortical P450 epoxygenase activity. This is consistent with the increase in arachidonic acid and prostaglandin levels in a rat renal failure model fed a BOR diet (Ingram et al., 1996).

Extensive investigation in recent years has established a role for cytochrome P450 eicosanoids in the regulation of blood pressure (Kroetz and Zeldin, 2002). P450 eicosanoids are synthesized throughout the body but act in a paracrine and autocrine fashion to regulate cellular function. The kidney produces significant levels of P450 eicosanoids with effects on renal tubular transport function and vascular reactivity, and altered levels of renal EETs and HETEs are associated with changes in blood pressure (Su et al., 1998; Yu et al., 2000b; Xu et al., 2002). For these reasons, the current study focused on renal mechanisms that might contribute to the blood pressure-lowering effects of a GLA-enriched diet. Essential fatty acid deficiency exacerbates hypertension in the SHR (Church and Schoene, 1977), and there is a lower percentage of arachidonic acid in the SHR kidney relative to the Wistar Kyoto rat (Delachambre et al., 1998). Phospholipid acyl transferase activity and Δ6- and Δ5-desaturase activity are also decreased in the SHR relative to the Wistar Kyoto rat (Narce and Poisson, 1995). This low arachidonic acid content in the SHR membrane phospholipid pool might limit the synthesis of antihypertensive EETs in the kidneys and vasculature. A BOR diet is associated with increased levels of renal arachidonic acid (Ingram et al., 1996). However, numerous eicosanoids can be synthesized from arachidonic acid, often with opposing physiological effects. It is therefore difficult to predict the effect of increasing arachidonic acid pools on eicosanoid levels. Our data indicate an increase in all regioisomeric EETs in BOR-fed SHRs, consistent with the broad inducibility of multiple P450 epoxygenases.

It is of interest to note that the renal microsomal epoxygenases catalyzed the highly asymmetric epoxidation of arachidonic acid to generate EETs, with a preference for 11,12-EET and then 8,9- and 14,15-EET. This finding is consistent with data in the literature (Holla et al., 1999; Yu et al., 2000a) and demonstrates the characteristic renal epoxygenase regioselectivity in rats. In contrast, the regiochemical composition of renal endogenous EETs was different from the regioselectivity observed in vitro. The major endogenous regioisomeric EET in kidneys is 14,15-EET, followed by lower amounts of 8,9-EET and 11,12-EET in the SES-fed rats. The fact that the endogenous 14,15-EET content in rat kidney is the highest among all regioisomers is consistent with similar measurements in SHR urine (Yu et al., 2000a) and human liver (Zeldin et al., 1996), heart (Wu et al., 1996), and kidney (Karara et al., 1990). Furthermore, in BOR-fed SHRs, renal endogenous 8,9-EET levels increased more than the levels of other regioisomers, and 8,9-EET becomes the major endogenous EET. This is in contrast with the parallel increase in the formation of all regioisomeric EETs in renal microsomes. The apparent discrepancy between in vitro activity and in vivo quantification might be due to the heterogeneous distribution of P450 epoxygenases in the kidney and/or unequal availability of free arachidonic acid throughout the kidney compared with kidney microsomal incubations, in which enzyme kinetic properties are the only determinant of metabolite formation from exogenously added arachidonic acid. In addition, P450 arachidonic acid metabolism is shunted from a predominant production of the prohypertensive 20-HETE to the antihypertensive EETs, consistent with the decrease in blood pressure associated with GLA treatment.

Several lines of evidence suggest a role for P450 epoxygenase activity in the regulation of blood pressure. P450 epoxy-
genase activity is increased in animal models of hypertension, including the SHR (Yu et al., 2000a) and a high-salt diet (Makita et al., 1994). The elevation in EET biosynthesis could be a consequence of the elevated blood pressure and might represent a compensatory response of the animal to deleterious increases in blood pressure. Moreover, inhibition of epoxygenases by clotrimazole leads to salt-dependent hypertension (Holla et al., 1999) and an inability to increase P450 epoxygenase activity with excess dietary salt intake in Dahl salt-sensitive rats might contribute to the hypertensive phenotype of these animals (Makita et al., 1994). Down-regulation of CYP2C23 and CYP4A4 expression is associated with obesity-induced hypertension (Wang et al., 2003). The induction of CYP2C and CYP2J expression by a BOR diet in parallel with a decrease in blood pressure in BOR-fed SHRs supports a protective role for P450 epoxygenase-mediated arachidonic acid metabolism in cardiovascular disease. Future studies using P450 epoxygenase inhibitors and antisense oligonucleotides could provide additional evidence for an antihypertensive effect of a BOR-mediated induction of renal EET formation. It is also of interest to examine whether the inductive effects of the BOR diet on renal epoxygenase activity are consistent throughout the development of hypertension in the SHR and whether early feeding of this diet can prevent the hypertensive phenotype. It is exciting to speculate that pharmacologic modulation of P450 epoxygenase activity might be a novel therapeutic target for blood pressure control.

In addition to influencing blood pressure, dietary GLA can suppress smooth muscle cell proliferation in vivo and retard the development of diet-induced atherosclerosis in apolipoprotein E knockout mice (Fan et al., 2001). As recently demonstrated, EETs possess anti-inflammatory properties and decrease leukocyte adhesion to the vascular wall (Node et al., 1999), inhibit smooth muscle cell migration (Sun et al., 2002), and reduce the formation of thrombi (Node et al., 2001). The anti-inflammatory and fibrinolytic activity of EETs may lower the risk of cardiovascular disease and partially explain the beneficial effect of dietary PUFAs in preventing atherosclerosis.

In this study, we detected elevations in the protein levels of CYP2C23, CYP2C11, and CYP2J2 immunoreactive proteins. Changes in P450 epoxygenase protein levels have also been documented in other studies, including an increase in response to a high-salt diet (Holla et al., 1999) and a decrease in response to a high-fat diet (Wang et al., 2003). The mechanism underlying these protein expression changes has not been addressed. Many P450 enzymes are transcriptionally induced by activation of nuclear receptors. However, this mechanism does not account for the increased P450 epoxygenase levels with a BOR diet because mRNA levels were unaltered. Likewise, mRNA stability changes cannot be responsible for the changes in P450 epoxygenase levels. Post-transcriptional modifications of protein translation or stabilization are known to be important in regulating P450 expression (Song et al., 1989). In particular, CYP2C23 protein levels were up-regulated during dietary salt loading, whereas the mRNA levels remain unchanged (Holla et al., 1999). Similar observations published recently also demonstrated an increase in CYP2C23 protein levels with no change in mRNA levels in transgenic rats overexpressing renin and angiotensinogen genes (Muller et al., 2004). It is reasonable to hypothesize that a GLA-rich diet induces P450 epoxygenase protein levels by stabilizing the protein and/or decreasing proteolysis.

In summary, these studies demonstrate that a GLA-enriched diet induces CYP2C and CYP2J arachidonic acid epoxygenases and endogenous EET levels in the SHR renal cortex. The antihypertensive properties of EETs suggest that GLA-induced changes in arachidonic acid metabolism contribute to the reduction of blood pressure in BOR-fed SHRs. A similar association between arachidonic acid metabolism and blood pressure regulation should be explored in humans consuming a GLA-enriched diet.


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