11

Cytochrome P450 and the Metabolism and Bioactivation of Arachidonic Acid and Eicosanoids

Jorge H. Capdevila, Vijaykumar R. Holla, and John R. Falck

1. Introduction

The convergence of important advances in the identification of several lipid-derived mediators as inter- and intracellular signaling molecules, and in the biochemistry of oxidative lipid metabolism, has focused interest in the functional roles of pathways responsible for their formation, and the physiological significance of their products. Among these, the studies of the enzymes of the arachidonic acid (AA) cascade, consisting of prostaglandin H_2 synthase[1, 2], lipoxygenases[3], and cytochrome P450[4, 5], constitute a premier example of the biological importance of these reactions and of their products (eicosanoids). Studies of the last two decades, have implicated the enzymes of the AA cascade in the pathophysiology of diseases such as hypertension, diabetes, and cancer, and some of these enzymes serve as molecular targets for drugs of extensive use in clinical medicine, including many nonsteroidal anti-inflammatory, antipyretic, and anti-asthmatic drugs[1–3]. The biological and signaling properties of eicosanoids are derived from the enzymatic, regio-, and stereoselective oxygenation of AA, a rather simple molecular template. While the informational content stored in the AA metabolites, is limited compared to that contained in complex informational molecules such as proteins or nucleic acids, low energy cost, versatility, and rapid turnover, makes them efficient on/off molecular switches for rapid and efficient intra- or intercellular signaling. Metabolism by prostaglandin H_2 synthase generates a cyclic endoperoxide, prostaglandin H_2 (PGH_2) that serves as the precursor for the formation of prostaglandins, prostacyclin, and thromboxanes[1, 2]. Metabolism by lipoxygenases leads to the formation of several regioisomeric hydroperoxides, the precursors of leukotrienes, regioisomeric *cis/trans* conjugated hydroxyeicosatetraenoic acids (HETEs), lipoxins, and hepoxilins[3]. Metabolism by microsomal cytochrome P450s (P450s) generates several hydroxy- and epoxy-AA derivatives[4, 5]. The reactions catalyzed by prostaglandin H_2 synthase and lipoxygenases are mechanistically similar to those of the free-radical-mediated autooxidation of polyunsaturated fatty acids in that they are initiated by hydrogen atom abstraction from a bis-allylic methylene carbon, followed by coupling of the resulting carbon radical to ground state molecular oxygen. The kinetics, regiochemistry, and chirality of these

Jorge H. Capdevila • Departments of Medicine and Biochemistry, Vanderbilt University Medical School, Nashville, TN. **Vijaykumar R. Holla** • Department of Medicine, Vanderbilt University Medical School, Nashville, TN. **John R. Falck** • Department of Biochemistry, Southwestern Medical Center, Dallas, TX.

Cytochrome P450: Structure, Mechanism, and Biochemistry, 3e, edited by Paul R. Ortiz de Montellano
Kluwer Academic / Plenum Publishers, New York, 2005.

reactions are under strict enzymatic control. In contradistinction to the cytochrome P450-catalyzed, redox coupled, activation of molecular oxygen and delivery to ground state carbon, prostaglandin H_2 synthase and the lipoxygenases are typical dioxygenases that catalyze substrate carbon activation instead of oxygen activation.

It is apparent from the literature that the P450 gene superfamily of hemoproteins is, as a group, one of the most intensively studied enzyme systems and yet our knowledge of their endogenous metabolic or physiological roles remains limited. This is partly due to the complexity of mammalian P450 isoforms, and the wide structural diversity of substrates known to be metabolized by these proteins. This catalytic versatility pointed to, and served as the basis for, many of the documented roles for P450 in the metabolism of foreign chemicals, and has contributed to establish its toxicological and pharmacological importance. In the last few years, there has been an increasing interest in the understanding of the physiological significance of the P450 enzyme system, and its role(s) in the metabolism of endogenous substrates. In this regard, the studies of the P450 branch of the AA cascade have provided a new focus to these efforts, and far-reaching results from several laboratories are generating new paradigms in fatty acid metabolism, as well as in cell and organ physiology[4–10].

The studies of the role of P450 in the metabolism and bioactivation of AA were initiated in 1981 with the demonstration that liver and kidney microsomal fractions, as well as purified P450 isoforms[11–13] actively catalyzed the NADPH-dependent, oxidative metabolism of AA to products that were different from prostanoids and leukotrienes[11–13]. The widely documented physiological importance of AA suggested that these observations were unique and likely to be functionally significant, and led to the rapid structural characterization of most P450-eicosanoids, their chemical synthesis, and subsequent biological evaluation[4–10]. Interest in these novel P450 reactions was stimulated by: (a) the initial demonstration that some of the products displayed potent biological activities, including the inhibition of Na^+ reabsorption in the distal nephron[14], (b) the documentation of P450 participation in the *in vivo* metabolism of endogenous AA pools[15], and (c) the proposal of a role for these enzymes in the pathophysiology of genetically controlled experimental hypertension[6, 7]. These earlier studies established P450 AA monooxygenation as a formal metabolic pathway, P450 as an endogenous member of the AA metabolic cascade, and more importantly, suggested functional roles for this enzyme in the bioactivation of the fatty acid and thus, in cell and organ physiology. Many of the biological activities attributed to the P450-derived eicosanoids, as well as the potential physiological importance of these reactions, have been reviewed[6–10].

Prior to the demonstration of AA metabolism by P450, several groups demonstrated the role of microsomal P450s in the ω/ω-1 hydroxylation of prostanoids[16–20] and, more recently, leukotrienes[20]. Most of these reactions are considered to be involved in eicosanoid catabolism and excretion, but their potential relevance in eicosanoid bioactivation or inactivation, and/or in the control of organ/cell eicosanoid levels has only begun to be explored. We will first discuss the role of P450 in the metabolism of eicosanoids, and then concentrate on the studies of its role in AA metabolism and bioactivation.

2. Metabolism of Eicosanoids

During the metabolism of eicosanoids, depending on the nature of the oxygenated substrate, P450 catalyzes both NADPH-dependent and -independent reactions. This differential requirement for NADPH-mediated changes in the redox state of the heme-iron illustrates the marked differences in oxygen chemistries for these reactions, that is, the isomerization of AA peroxides, vs the more demanding activation and delivery of a reactive form of atomic oxygen to ground state carbon–hydrogen bonds.

2.1. NADPH-Independent Reactions

P450 catalyzes the isomerization of a variety of fatty acid hydroperoxides, including 15-hydroperoxyeicosatetraenoic acid (15-HPETE)[21, 22], and of the prostaglandin H_2 (PGH_2) endoperoxide[23]. A distinctive feature of some P450 fatty acid peroxide isomerases is their inability to accept electrons from NADPH and to activate molecular

oxygen[21–23]. Moreover, while all these enzymes possess a heme-thiolate prosthetic group, their overall homology to other members of the P450 gene superfamily is limited and suggests an early evolutionary functional specialization[23–26]. The mechanism by which the hemoprotein cleaves the peroxide oxygen–oxygen bond, that is, homolytic or heterolytic scission, plays a decisive role in determining the catalytic outcome of these reactions and is highly dependent on the nature of the P450 isoform, the chemical properties of the organic peroxide, and the nature of the oxygen acceptor[21–23, 27]. A homolytic pathway was proposed for the formation of 11- and 13-hydroxy-14, 15-epoxyeicosatrienoic acids (EETs) from 15-HPETE by rat liver microsomes[22]. Prostacyclin and thromboxane synthases are P450-like proteins containing a heme-thiolate prosthetic group[23–26]. The heterolytic cleavage of PGH_2 and an oxygen atom transfer or oxenoid mechanism has been proposed to account for the P450-catalyzed formation of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2)[23]. The participation of P450s in the biosynthesis of these important mediators of endothelial cell and platelet function was one of the first demonstrations of a role for this enzyme system in vascular biology. However, its pharmacological and clinical implications remain to be fully explored.

2.2. NADPH-Dependent Reactions

P450 plays an important role in the NADPH-dependent metabolism of several bioactive oxygenated eicosanoids[16–20]. These reactions are of importance in that they: (a) increase eicosanoid structural diversity and, hence, modify informational content, (b) may alter the pharmacological profile of the substrate, and (c) may participate in the regulation of steady state and/or stimulated levels of physiologically relevant molecules. While these oxidations were generally viewed as catabolic, that is, yielding an attenuated biological activity, recent studies indicate that some ω-oxidized prostanoids show unique and potent biological properties[6–10]. However, in most cases the sequence of steps leading to ω/ω-1 oxidized prostanoids from endogenous AA pools remains to be clarified.

Typically, the P450-dependent metabolism of eicosanoids results in the hydroxylation of their terminal (C_{20} or ω-carbon) or penultimate carbon atoms (C_{19} or ω-1 carbon). However, the epoxidation of infused PGI_2 by a perfused kidney preparation[28], and the metabolism of 5,6- and 8,9-EET by prostaglandin H_2 synthase, were described several years ago[29, 30]. The former leads to a variety of 5,6-oxygenated prostanoids[29]. Oxidation of the latter was stereodependent, that is, 8(*S*),9(*R*)-EET formed 11(*R*)-hydroxy-8(*S*),9(*R*)-epoxyeicosatrienoic acid exclusively, whereas the 8(*R*),9(*S*)-enantiomer formed both C_{11} and C_{15} hydroxylated metabolites[30]. A detailed study of the secondary metabolism of 12(*R*)-HETE and 14,15-EET by P450 has been reported[31, 32]. The efficient ω/ω-1 oxidation of EETs by rat CYP4A isoforms to the corresponding regioisomeric epoxy-alcohols at rates comparable to those observed with lauric acid, a prototype substrate for these enzymes, was published recently[33]. One of these metabolites, the ω-alcohol of 14,15-EET, was shown to bind and activate the peroxisomal proliferator activated receptor alpha ($PPAR_\alpha$) type of nuclear receptor[33]. It is important to note that, with the exception of the ω/ω-1 hydroxylation of prostanoids and leukotrienes, none of the transformations described above has been shown to occur *in vivo* from endogenous precursors.

2.2.1. ω/ω-1 Oxidation of Prostanoids

Since the initial report of *in vivo* ω-1 hydroxylation of prostanoids in 1966[34, 35], ω- and ω-1 hydroxylation has become a recognized route of prostanoid metabolism. Early studies of prostanoid ω/ω-1 hydroxylation demonstrated these reactions were NADPH-dependent, localized to the endoplasmic reticulum[16, 36], and catalyzed by microsomal P450 (ref. [36]). Incubations with purified enzymes or recombinant P450s showed that most of these reactions were catalyzed by members of the 4 gene family of P450s[20, 36–43]. In general, while CYP4F isoforms are more active in the metabolism of eicosanoids than fatty acids, the opposite appears to be true for most CYP4A isoforms[20, 44]. Approximately 18 CYP4F isoforms have been identified in rats (4F1, 4F4, 4F5, and 4F6)[45], mice (4f13, 4f14, 4f15, 4f16, 4f17, 4f37, 4f39, and 4f40)[45], and humans (4F2, 4F3, 4F8, 4F11, 4F12,

Table 11.1. Metabolism of Fatty Acids and Prostanoids by Cytochrome P450 4A Isoforms

4A isoform	Species	Enzymatic activities[a]
4A1	Rat	ω-oxidation of laurate and arachidonate
4A2	Rat	ω/ω-1 oxidation of laurate and arachidonate
4A3	Rat	ω/ω-1 oxidation of laurate and arachidonate
4A8	Rat	ω/ω-1 oxidation of laurate and arachidonate
4A4	Rabbit	ω-oxidation of palmitate, arachidonate, and of prostaglandins A, E, D, and $F_{2\alpha}$
4A5	Rabbit	ω/ω-1 oxidation of laurate and palmitate, some ω-oxidation of PGA_1 and arachidonate
4A6	Rabbit	ω-oxidation of laurate, palmitate, and arachidonate. Low PGA_1 ω-oxidation
4A7	Rabbit	ω-oxidation of laurate, palmitate, arachidonate, and PGA_1, inactive toward PGE_2
4a10	Mouse	ω-oxidation of laurate
4a12	Mouse	ω/ω-1 oxidation of laurate and arachidonate
4a14	Mouse	ω/ω-1 oxidation of laurate
4A11	Human	ω/ω-1 oxidation of laurate and arachidonate
4a22	Human	Unknown

[a]Compiled from references [4]–[10], [17]–[20], [36]–[44], [46]–[52].

and 4F22)[45]. On the other hand, approximately 11 CYP4A isoforms have been cloned and/or isolated and purified from rats (4A1, 4A2, 4A3, and 4A8)[45], mouse (4a10, 4a12, and 4a14)[45], and rabbits (4A4, 4A5, 4A6, and 4A7)[45]. In stark contrast with the known multiplicity of rodent CYP4A and of rodent and human CYP4F isoforms, only two highly homologous CYP4A genes, *CYP4A11* and *CYP4A22*, have been identified in humans[45–48]. Most CYP4A enzymes that have been characterized enzymatically, are either inactive toward prostanoids or catalyze their ω- or ω/ω-1 hydroxylation at rates that are generally substantially lower than fatty acid hydroxylation (Table 11.1)[20, 36–44, 49–51]. A special case is that of rabbit lung CYP4A4, an isoform induced during pregnancy[20, 37, 42, 52] and active in the ω-hydroxylation of several prostanoids, including PGE_2 (Table 11.1)[20, 52]. As with AA, none of the CYP4A isoforms characterized to date is selective for the ω-1 carbon of prostanoids (Table 11.1)[20, 36–42, 49–52]. A more specific role for CYP4F isoforms as predominantly prostanoid and eicosanoid ω/ω-1 hydroxylases has emerged during the last few years[20]. The cDNA coding for CYP4F8 was cloned from human seminal vesicles, and the recombinant protein was shown to catalyze the ω-1 hydroxylation of PGH_2, the precursor of all prostanoids[43]. Based on its catalytic activity, and its high levels of expression in the seminal vesicles it was proposed that CYP4F8 is involved in the formation of 19-hydroxy-PGE_2, present at high concentrations in human seminal fluid[34, 43]. CYP4F12 is a regioselective AA ω-3 hydroxylase[53] but, it is also active in the hydroxylation of prostanoids and several prostanoid analogs[53].

2.2.2. ω/ω-1 Oxidation of Leukotrienes and Other Eicosanoids

The ω-oxidation of leukotriene B_4 (LTB_4), an important proinflammatory mediator[54, 55], has been documented in whole animals, isolated cells, and subcellular fractions[20, 56–60], and shown to be catalyzed by a unique P450 isoform, distinct from those involved in fatty acid and prostanoid metabolism[20, 56–60]. Soon after, the cDNA coding for CYP4F3 was cloned and expressed, and recombinant CYP4F3 shown to catalyze the ω-oxidation of LTB_4 with a K_m of 0.71 μM[61, 62]. A role for

CYP4F3 as an endogenous LTB_4 hydroxylase is supported by its selective expression in human polymorphonuclear leukocytes, and its lack of activity toward fatty acids such lauric, palmitic, and AAs[20, 61, 62]. CYP4F3 also supports the ω-hydroxylation of lipoxygenase metabolites such as lipoxins A and B, and of 5- and 12-HETE[62]. A splice variant of CYP4F3, CYP4F3B, is expressed in liver and kidney, and shows significant structural and functional similarities to CYP4F2[63]. Human CYP4F2 and rat 4F1 are active LTB_4 ω-hydroxylases capable of HETE ω-hydroxylation[20, 62, 64]. CYP4F2 is expressed in human liver and kidney, and responsible for most of the hepatic hydroxylation of LTB_4[65]. Four members of the rat 4F gene subfamily (CYPs 4F1, 4F4, 4F5, and 4F6) have been cloned[45]. Recombinant CYPs 4F1, 4F4, and 4F5 catalyze the ω-hydroxylation of LTB_4, and CYP4F1 also metabolizes lipoxins and HETEs[64, 66]. The ω-oxidation of 12(*S*)-HETE by polymorphonuclear leukocytes was demonstrated in 1984 by Wong *et al.*[67] and Marcus *et al.*[68]. Moreover, the latter authors further showed that endogenous AA pools are converted to 12,20-dihydroxyeicosatetraenoic acid by a co-incubated mixture of human platelets and polymorphonuclear leukocytes, thus providing one of the first examples of intercellular eicosanoid metabolism[68]. Both 5- and 15-HETE are known to undergo ω-oxidation by P450[69, 70].

3. Metabolism of Arachidonic Acid: The Arachidonic Acid Monooxygenase

As with the other enzymes of the AA metabolic cascade, P450 metabolizes only free, nonesterified forms of AA and thus, *in vivo* metabolism requires the release of the fatty acid from selected glycerophospholipid pools. CYP P450, prostaglandin H_2 synthase, and lipoxygenases are capable of metabolizing polyunsaturated fatty acids other than AA, however, it is the unique nature of the AA containing phospholipids, and the control of its release by hormonally sensitive phospholipases that makes the oxidative metabolism of AA distinctive, and functionally important. Under conditions favoring primary metabolism, the P450 AA monooxygenase oxidizes AA by one or more of the following of reactions: (a) *bis-allylic oxidation* (*lipoxygenase-like reaction*) to generate any of six regioisomeric HETEs containing a *cis,trans*-conjugated dienol functionality (5-, 8-, 9-, 11-, 12-, and 15-HETEs) (Figure 11.1), (b) *Hydroxylations at or near the terminal* sp^3 *carbon (AA ω/ω-1 hydroxylase)* affording 16-, 17-, 18-, 19-, and 20-HETEs (16-, 17-, 18-, 19-, and 20-HETE) (ω, ω-1, ω-2, ω-3, and ω-4 alcohols) (Figure 11.1), and (c) *Olefin epoxidation (AA epoxygenase)* furnishing four regioisomeric EETs

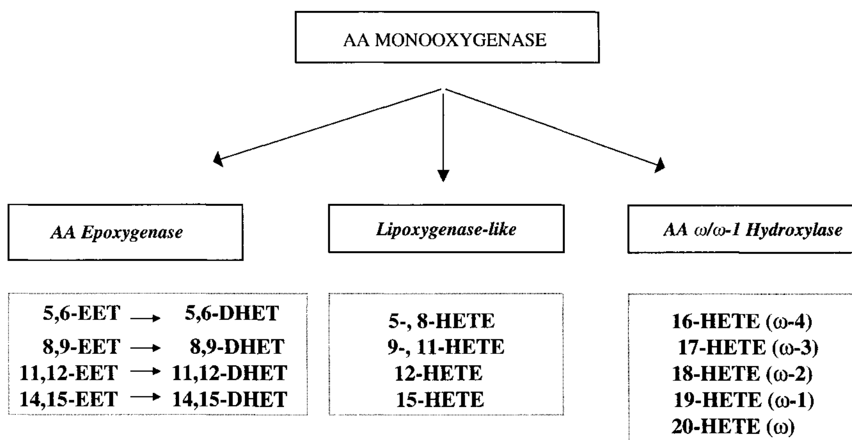

Figure 11.1. The cytochrome P450 arachidonic acid monooxygenases.

(5,6-, 8,9-, 11,12-, and 14,15-EETs) (Figure 11.1). The classification of P450-derived eicosanoids in Figure 11.1 continues to provide a rational and useful framework for most of the studies of this branch of AA metabolic cascade[4, 5].

The chemistry of the P450-derived eicosanoids is highly dependent on the tissue source of enzymes, animal species, sex, age, hormonal status, diet, and exposure to xenobiotics[4, 5]. For example, EETs are the predominant products generated by most liver microsomal fractions ($\geq$70% of total products), while most kidney microsomal fractions generate mainly a mixture of 19- and 20-HETE (77% of total products)[5]. Studies with microsomal, purified, and/or recombinant forms of rat, rabbit, and human P450s[4–10] showed that the hemoprotein controls, in an isoform-specific fashion, oxygen insertion into the fatty acid template at three levels: (a) *type of reaction*, that is, olefin epoxidation, bis-allylic oxidation, or hydroxylations at the C_{16}–C_{20} sp^3 carbons, (b) *regioselectivity of oxygen insertion*, that is, epoxidation at either of the four olefin bonds, allylic oxidation initiated at any of the three bis-allylic methylene, or hydroxylation at C_{16}–C_{20} (ref. [37]), and (c) the *enantiofacial selectivity* of oxygenation leading to chiral products. The broad structural, functional, regulatory, and catalytic redundancy displayed by many P450 isoforms, as well as their often overlapping patterns of tissue expression, continues to complicate the task of assigning catalytic roles to a P450 or to a group of P450 isoforms. This is of current interest because many of the P450 eicosanoids are biologically active[6–10], and the identification and characterization of P450 isoforms involved in the *in vivo* metabolism of AA is needed for accurate molecular descriptions of their mechanism of action, regulatory control, and ultimately, physiological significance. For example, several CYP 2B, 2C, 2D, 2E, and 2J proteins have been shown to catalyze the *in vitro* oxidation of AA to hydroxy- and/or epoxy-acids[71–87]; however, their participation in metabolism of the endogenous fatty acid pools remains unclear[5, 86]. In a few cases, a role for individual 2 gene family isoforms in endogenous AA bioactivation has been suggested based on enzymatic and/or immunological evidence[4–7, 86, 87]. Nevertheless, the identities of the P450 isoforms responsible for organ-specific AA metabolism remains preliminary (and many cases speculative) and these unresolved issues continue to offer a major challenge for this area of research.

3.1. bis-Allylic Oxidation (Lipoxygenase-Like Reactions)

The products of these reactions are structurally similar to those of plant and mammalian lipoxygenases, and yet there is no evidence that hydroperoxide intermediates are formed during the P450-catalyzed reactions[4, 5, 88, 89]. A mechanism for P450-dependent HETE formation involving bis-allylic oxidation at either C7, C10, or C13, followed by acid-catalyzed rearrangement to the corresponding *cis-trans* dienols was proposed, and the intermediate 7-, 10-, and 13-HETEs isolated[88, 89]. Since 12(*R*)-HETE is the predominant enantiomer generated by a P450-catalyzed reaction[90], it was thought that all the mammalian 12(*R*)-HETE was a product of the P450 enzyme system[91]. However, the cloning and characterization of mammalian 12(*R*)-lipoxygenases has led to a reevaluation of the role of P450s in 12(*R*)-HETE biosynthesis[92, 93]. The formation of 12(*S*)- and 12(*R*)-HETE by P450-independent and -dependent pathways in bovine cornea epithelium has been reported[94, 95]. Importantly, *in vitro* studies showed that 12(*R*)-HETE is a powerful and enantioselective inhibitor of Na^+/K^+ ATPase[94]. Finally, the enzymatic formation of 12(*R*)-hydroxy-5,8,14-eicosatrienoic acid (12(*R*)-HETrE), an ocular proinflammatory and vasodilatory substance in rabbits, has been described[96]. Areas in need of clarification are: (a) the role of P450 in the biosynthesis of endogenous HETE and HETrE pools, (b) the identity and molecular properties of the P450 isoforms responsible for these reactions, and (c) the contributions of P450 and 12-lipoxygenases to organ-specific 12(*R*)-HETE and 12-HETrE biosynthesis.

3.2. Hydroxylation at C_{16}–C_{20} (ω/ω-1 Hydroxylase Reactions)

3.2.1. Introduction

The hydroxylation of saturated medium-chain fatty acids at their ultimate and penultimate carbons

was one of the first enzymatic activities attributed to microsomal P450s[97]. In general, medium-chain saturated fatty acids (C_{12}–C_{16}) are far better substrates for the microsomal ω/ω-1 hydroxylases than AA[16, 17, 20, 97], and reaction rates decrease as the substrate carbon-chain length increases from C12 to C18. For example, lauric acid, a fatty acid absent from most mammalian tissues, is metabolized by the microsomal ω/ω-1 hydroxylases or by purified CYP4A isoforms at rates significantly higher than AA[33, 50, 51]. Common oxygen chemistries and reaction mechanisms for these reactions are suggested by the fact that, regardless of the carbon length of the fatty acid or its degree of saturation, the ω/ω-1 hydroxylases deliver a reactive form of oxygen to ground state, sp^3 carbons. However, the unequal chemical reactivities of the carbon atoms in the AA molecular template impose additional steric requirements on the P450 catalyst. Hydroxylation at the thermodynamically less reactive C_{16} through C_{20} rather than at the chemically comparable C_2 through C_4 indicates a rigid and highly structured binding site for the AA molecule. This binding site must position the acceptor carbon atom(s) in optimal proximity to the heme-bound active oxygen, with complete segregation of the AA-reactive olefins and bis-allylic methylene carbons. Studies with CYP102 (P450BM3), a high turnover bacterial AA hydroxylase of known atomic structure[98, 99], suggested a rigid active-site binding geometry for AA, and indicated that the regiochemistry of P450 oxygen insertion was determined by the fatty acid binding coordinates, and not by chemical properties of the acceptor carbon or the heme-bound active oxygen species[98, 99]. Thus, X-ray crystallography, molecular modeling, site-specific mutational analysis, as well as enzymatic studies indicate that the "substrate access channel" in CYP BM-3, holds the AA molecule in a rigid orientation that: (a) precludes significant rotation and/or displacement along the channel's longitudinal axis, and (b) shields the heme-bound oxidant from non-acceptor carbons[98, 99].

3.2.2. Enzymology, Isoform Specificity

AA ω/ω-1 hydroxylation has been observed in microsomal fractions from several organs, including liver, kidney, brain, lung, intestine, olfactory epithelium, and anterior pituitaries[4–10]. However, it is in renal tissues that these reactions are best characterized, most prevalent, and have been assigned their most important functional roles[4–10]. Extensive biological, enzymatic, and molecular evidence shows that the CYP4A isoforms are the predominant, and functionally relevant, AA ω/ω-1 hydroxylases in the mammalian kidney[4–10, 100]. The *CYP4A* gene subfamily encodes a group of structurally and functionally conserved proteins that are specialized for fatty acid oxidation and that show little or no activity toward xenobiotics[16, 20]. The expression of the CYP4A fatty acid hydroxylases is regulated by a variety of physiological and pathophysiological effectors such as age, sex hormones, dietary lipids, fasting, starvation, mineralocorticoids, insulin, diabetes, and hypertension[4–10, 100–108]. Moreover, the sexual dimorphic, androgen sensitive, expression of rat kidney CYPs 4A2 and 4A8, and of mouse kidney Cyp4a12 have been demonstrated[100, 104, 109, 110].

In rats and rabbits, the 4A gene subfamily is composed of four highly homologous genes[45]. Amino acid sequence analysis showed that the rat 4A proteins could be divided into two groups that share ≥71% overall homology (Figure 11.2)[45]. CYPs 4A1 and 4A8 (76% sequence identity) constitute one group, and the other is composed of the highly homologous CYPs 4A2 and 4A3 (98% sequence identity) (Figure 11.2)[45]. The high level of nucleotide sequence identity shared by the CYPs 4A2 and 4A3 genes extends into their intronic areas, suggesting that they arose from a relatively recent gene duplication event[45]. The three characterized murine Cyp4a genes are localized in a ~200 kb segment of chromosome 4

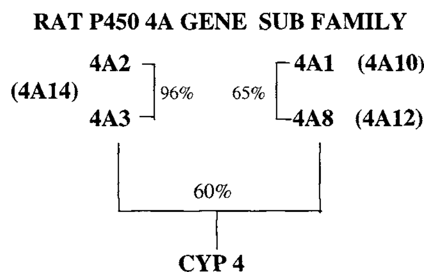

Figure 11.2. Nucleotide sequence identity between rat and murine CYP4A isoforms.

(ref. [110]). Cyps 4a10 and 4a12 are the murine homologs of rat CYPs 4A10 and 4A8, respectively (Figure 11.2)[45]. The presence of a single murine gene (CYP4a14) highly homologous to both rat CYPs 4A2 and 4A3 indicates that the 4A2/4A3 gene duplication event occurred after the evolutionary separation of rat and mouse (Figure 11.2). Southern analysis and the human genome database show that *CYPs 4A11* and *4A22* are likely to be the only members of the *CYP4A* gene subfamily in humans[46–48, 108]. These two genes share 96% nucleotide sequence identity, contain 12 exons, and have similar intron/exon distributions[48]. The cDNA coding for CYP4A11 has been cloned, expressed, and characterized as an active renal fatty acid ω-hydroxylase[44, 46, 47, 51]. On the other hand, the enzymatic activity of CYP4A22 is unknown, and its mRNA is expressed in kidney at levels that can be detected only after RT-PCR amplification[48].

Table 11.1 summarizes the published fatty acid metabolic properties for several purified and recombinant CYP4A isoforms. All enzymatically characterized CYP4A proteins (either purified or recombinant proteins) catalyze saturated fatty acid ω-oxidation and most also hydroxylate AA at either the C_{20}, or the C_{19} and C_{20} carbon atoms[16–19, 38–42, 44, 46, 47, 49 52]. To date, none of them have been shown to be selective for fatty acid ω-1 hydroxylation. Despite their high structural homology, CYP4A2 metabolizes AA while CYP4A3 is either inactive[50], or reacts at very low rates[49, 51]. Both CYP 4A2 and 4A3 are, on the other hand, active lauric acid ω/ω-1 hydroxylases[50, 51]. A microsomal form of recombinant rat CYP4A2 was shown to oxidize AA to 20-HETE and 11,12-EET[49]. In contrast, two different laboratories showed that purified recombinant CYP4A2 oxidizes AA to only 19- and 20-HETE[50, 51]. All three murine Cyp4a proteins are active lauric acid hydroxylases, but only Cyp4a12 catalyzes AA ω/ω-1 hydroxylation, providing an explanation for the low levels of 20-HETE synthase activity present in microsomes isolated from the kidneys of female *129SvJ* mice[100]. An unresolved issue is that of the relative roles played by CYPs 4A11 and 4F2 in the biosynthesis of 20-HETE by human kidney. As discussed, recombinant CYP4F2 is an active LTB_4 hydroxylase[20, 65], and it has been reported to be active toward AA ω-hydroxylation[20, 44]. On the other hand, kinetic and immunological evidence suggested that both CYPs 4A11 and 4F2 may contribute to the biosynthesis of 20-HETE by human kidney microsomes and nephron segments[44].

The liver microsomal P450 hydroxylation of AA at C_{16}, C_{17}, C_{18}, and C_{19}, but not at C_{20}, was induced after treatment of the animals with α-naphthoflavone or dioxin[111, 112]. Reconstitution experiments using purified liver CYPs 1A1 and 1A2, the major liver P450 isoforms induced by these chemicals, demonstrated that CYPs 1A1 and 1A2 were more or less regioselective for oxidations at the AA C_{16}–C_{19} carbons (87% and 44% of total products for CYP1A1 and 1A2, respectively)[111]. Furthermore, while CYP1A1 oxidized AA preferentially at C_{19}, oxygenation by CYP1A2 occurred predominantly at C_{16} (ref. [111]). It is of interest that despite a very limited sequence homology, CYPs 1A and 4A show distinct regioselectivities for the adjacent C_{19} and C_{20} carbons of AA. Purified CYP2E1, an isoform induced in rat liver by diabetes, fasting, and alcohol, converts AA stereoselectively to 19(*S*)- and 18(*R*)-HETE as its major reaction products[85]. Finally, members of the 2J gene subfamily are also active AA ω-1 hydroxylases[86, 87] and, recently, Cyp 2j9, an isoform expressed in mouse brain was cloned, expressed and shown to be a regioselective AA ω-1 hydroxylase[113].

The potent biological activities attributed to the products of the AA ω/ω-1 hydroxylases have stimulated an intense search for the physiological and/or pathophysiological roles of these reactions[4–10]. Among these, 20-HETE[4–10] has been characterized as: (a) a powerful vasoconstrictor of the renal and cerebral microcirculations, (b) an inhibitor of vascular calcium-dependent K channels, (c) a regulator of Na^+/K^+ ATPase activity, and (d) a modulator of Ca^{++} and Cl^- fluxes. Furthermore, analysis of the segmental distribution of CYP 4A isoforms along the rat nephron is consistent with many of the proposed renal actions of 19- and 20-HETE[114]. A role for 20-HETE as a powerful mitogen in cultured kidney epithelial cells, as well as in vasopressin, parathyroid hormone and norepinephrine signaling has been described[6–10]. Of importance during analyses of the functional significance of the AA ω/ω-1 hydroxylases is the recognition that, as discussed, many P450 fatty acid ω/ω-1 hydroxylases also play important roles in the metabolism of

bioactive eicosanoids such as prostanoids and leukotrienes.

The expression of several CYP4A isoforms is under transcriptional control by the nuclear $PPAR_\alpha$[105]. The coordinated, $PPAR_\alpha$-controlled, induction of peroxisomal fatty acid β-oxidation and microsomal ω/ω-1 hydroxylation, has suggested a role for CYP4A isoforms in hepatic lipolysis and fatty acid homeostasis, and a role for these isoforms in PPARα-signaling has been advanced based on gene knockout studies[115]. The potential for an involvement of CYP4A isoforms in fatty acid and lipid homeostasis is opening new opportunities for an understanding of the physiological roles of these enzymes, *vis-a-vis* their recognized functional roles as AA hydroxylases.

3.3. Olefin Epoxidation (Epoxygenase Reactions)

3.3.1. Introduction

The demonstration of NADPH-dependent metabolism of AA to 11,12- and 14,15-dihydroxyeicosatrienoic acids (DHETs) by microsomal incubates indicated a role for P450 in AA epoxidation[13]. Soon after, 5,6-, 8,9-, 11,12-, and 14, 15-EET were isolated and shown to be products of the P450-dependent metabolism of AA[116]. In mammals, the epoxidation of polyunsaturated fatty acids to nonallylic, *cis*-epoxides is unique to the P450 enzyme system and, in contrast with fatty acid ω/ω-1 hydroxylation, is more or less selective for AA[4, 5]. Thus, while the enzymatic or nonenzymatic reduction and/or isomerization of polyunsaturated fatty acid hydroperoxides can yield epoxides or epoxy-alcohol derivatives, these products are structurally different from those generated by the P450 enzymes[3, 22]. The EETs are bis-allylic epoxides and as such, remarkably resistant to attack by nucleophiles such as water and glutathione (GSH). However, in most cells and organ tissues, the EETs are metabolically unstable and are rapidly esterified to glycerophospholipids, degraded by fatty acid β-oxidation pathways[117, 118], conjugated to GSH[120], and/or hydrated and excreted[119]. Cytosolic epoxide hydrolase and GSH-transferases catalyze the enzymatic conversion of EETs to the corresponding *vic*-DHETs (Figure 11.2)[119], and GSH-conjugates[120], respectively. The biological role(s) and *in vivo* significance of EET hydration or GSH conjugation remain mostly unexplored, although recently a role for cytosolic epoxide hydrolase in the regulation of antihypertensive EETs levels has been proposed[121, 122]. The catalysis of AA epoxidation, or the presence of endogenous EET pools has been demonstrated using microsomal fractions or samples obtained from numerous tissues, including liver, kidney, lung, skin, pituitary, brain, adrenal, endothelium, and ovaries[4–10].

3.3.2. Enzymology, Isoform Specificity

The isoform multiplicity of the AA epoxygenase was first suggested by changes in the regio- and stereoselectivity of the microsomal enzymes, resulting from animal treatment with known P450 inducers[71]. For example, animal treatment with phenobarbital inverted the overall enantiofacial selectivity of the rat liver microsomal AA epoxygenases (Table 11.2), and caused marked increases in the organ levels of endogenous of 8(*S*),9(*R*)-EET, an effect likely due to the induction of CYPs 2B1 and 2B2, both of which are highly stereoselective 8(*S*),9(*R*)-epoxygenases[71]. These studies showed that the regio- and stereochemical selectivity of the microsomal AA epoxygenase was under regulatory control and could be altered, *in vivo*, by animal manipulation[4, 5, 71]. Subsequently, it was demonstrated that arachidonate epoxidation was highly asymmetric and that P450s control, in an

Table 11.2. Effect of Phenobarbital Treatment on the Enantioselectivity of the Liver Microsomal Arachidonic Acid Epoxygenase

EET enantiomer (% distribution)	Liver microsomes	
	Control	Phenobarbital
8(*S*),9(*R*)-EET	32 ± 2	78 ± 2
8(*R*),9(*S*)-EET	68 ± 2	22 ± 2
11(*S*),12(*R*)-EET	19 ± 1	83 ± 2
11(*R*),12(*S*)-EET	81 ± 1	17 ± 2
14(*S*),15(*R*)-EET	67 ± 2	25 ± 3
14(*R*),9(*S*)-EET	33 ± 2	75 ± 3

Microsomes were isolated from the livers of control- and phenobarbital-treated rats (10 days; 0.05% w/v phenobarbital in the drinking water). Values are averages ± SE calculated seven different experiments. See ref. [71] for experimental details.

isoform-specific fashion, the regio- and enantioselectivities of the reaction[4, 5, 71, 73–77, 79]. These properties of the AA epoxygenase are in contrast with those of prostaglandin H_2 synthases, where the known isoforms of the enzyme oxidize AA to the same single product[1, 2].

Another distinctive feature of the AA epoxygenase pathway is the ability of a single P450 isoform to epoxidize, stereoselectively, multiple olefins of the AA template. For example, purified recombinant rat kidney CYP2C23 generates 11,12-EET as its major reaction product (58% of total) but, it is also an efficient AA 8,9-, and 14,15-epoxygenase[77]. Despite this limited regioselectivity, CYP2C23 is highly stereoselective and forms the corresponding 8(*R*),9(*S*)-, 11(*R*),12(*S*)-, and 14(*S*), 15(*R*)-EETs enantiomers with optical purities of 94%, 89%, and 75%, respectively[77]. On the other hand, the other two 2C AA epoxygenases expressed in rat kidney, CYPs 2C11 and 2C24, show moderate regioselectivity for the 11,12- and 14,15-olefins[123], and epoxidized the 8,9- and 14,15-olefins with opposing enantiofacial selectivities[123]. Extensive studies with a variety of organ purified and/or recombinant epoxygenases, including several CYP 2B and 2C isoforms, showed that: (a) with the exception of rat CYP2B12, which generates 11,12-EET as nearly the only reaction product[74], most do not catalyze the selective epoxidation of a single AA olefin to the exclusion of the other three[4, 5], and (b) most mammalian P450 isoforms preferentially epoxidize the 11,12- and 14,15-double bonds[4, 5]. The role that single amino acid residues play in the regio- and stereochemical selectivity of AA epoxidation was revealed by replacements introduced into rat and bacterial P450s 2B1 and BM3, respectively[73, 99]. Recombinant CYP2B1 metabolizes AA to predominantly 11,12- and 14,15-EET[73]. Replacement of isoleucine 114 for alanine in CYP2B1, changed its regioselectivity toward the preferential epoxidation of the AA 5,6- and 8,9-olefins[73]. On the other hand, a single active-site replacement, phenylalanine 78 for valine, changed P450 BM3 from a predominantly AA 18(*R*)-hydroxylase into a regio- and enantioselective 14(*R*),15(*S*)-epoxygenase (14(*R*), 15(*S*)-EET, ≥98% of total products)[99]. These studies indicate that the outcome of the reactions catalyzed by many of these highly homologous P450 isoforms is determined by a few amino acid residues, strategically located within the confinements of what, in most other cases, is known to be a rather promiscuous active-site cavity.

Reconstitution experiments using purified P450 isoforms and/or recombinant proteins show that most AA epoxygenases belong to the CYP 2 gene family[4–10]. The CYP2B and 2C subfamily isoforms identified so far as epoxygenases include rat 2B1, 2B2, 2B12, 2C11, 2C23, and 2C24; rabbit 2B4, 2C1, and 2C2; mouse 2b19, 2c37, 2c38, 2c39, and 2c40; and human 2C8, 2C9/2C10, 2C18, and 2C19 (refs [4]–[10], [71]–[83], [123]). On the other hand, CYPs 2J2 and 2J4 have also been identified as organ-specific epoxygenases and ω-1 hydroxylases[86, 87]. CYPs 1A1, 1A2, and 2E1 are active AA ω/ω-1 oxygenases, that also produce low and variable amounts of EETs (≤20% of total products)[71, 80, 85]. A P450 purified from the livers of dioxin-treated chick embryos has structural features typical of proteins of the 1A gene subfamily, but metabolizes AA to EETs as the major reaction products[124]. Recent studies characterized 11,12-EET as an "endothelium-derived hyperpolarizing factor" (EDHF)[125] and CYP2C34, the porcine homolog of human CYPs 2C8 and 2C9, as a coronary artery EDHF synthase[126]. While members of the CYP2C gene subfamily share extensive sequence homology, this structural homology is often accompanied by significant catalytic heterogeneity[4, 5]. For example, CYPs 2C8 and 2C9 proteins are ~90% homologous in their amino acid sequences, yet recombinant CYPs 2C8 and 2C9 epoxidize AA with distinct regio- and stereochemical selectivities[81].

Comparisons of the regio- and enantioselectivity of the microsomal epoxygenases with that of purified recombinant P450 isoforms, as well as antibody inhibition experiments indicate that CYPs 2C11 and 2C9, and 2C23 and 2C8 are the major AA epoxygenases in rat and human liver and kidney, respectively[5, 77, 81, 123]. Thus, for example, of the three major 2C epoxygenases expressed in the rat kidney, CYPs 2C11, 2C23, and 2C24 (ref. [123]), only CYP2C23 mimics the regio- and stereochemical selectivity of the microsomal enzymes[77, 123]. CYP2C23 was shown to be abundantly expressed in rat kidney, and anti-P450 2C23 antibodies were selective inhibitors of the renal microsomal epoxygenase[5, 123]. Furthermore, with the exception of CYPs 2C23 and 2C11, none of the members of the CYP2 gene family expressed in kidney, including 2A, 2B, 2C, 2E, and 2J isoforms, can account for the degree of regio- and stereoselectivity displayed by the rat

renal microsomal epoxygenase[77–123]. Sequence comparisons show that the degree of homology between CYP2C23 and the remaining 2C rat proteins is limited, indicating its early evolutionary divergence from the other CYP2C proteins. The regulation of renal CYP2C23 levels by dietary salt intake[123], and its hormonally controlled expression in the renal microcirculation[127, 128] has suggested important roles for this enzyme in kidney physiology. The application of recombinant DNA methods and heterologous protein expression should continue to facilitate unequivocal assignments of regio- and enantioselectivities as well as epoxygenase activities to individual P450 isoforms. As more of these recombinant isoforms become available, they will be useful in defining their: (a) contribution to the epoxidation of endogenous AA pools, (b) tissue and/or organ-specific distribution, and (c) regulation by physiologically meaningful stimuli.

Several powerful *in vitro* biological activities have been described for the products of the AA epoxygenases. The EETs[4–10] have been described as: (a) mediators for the release of several peptide hormones, (b) inhibitors of Na^+ reabsorption in the distal nephron, (c) vasodilators in several microvascular beds and activators of Ca^{++}-dependent vascular K^+ channels, mediators of Ca^{++} influx in several isolated cell systems, powerful mitogens, and mediators of EGF and Angiotensin II signaling.

3.3.3. P450 Arachidonic Acid Epoxygenase: A Member of the Endogenous Arachidonic Acid Metabolic Cascade

In view of their known catalytic versatility, the *in vitro* catalysis of AA epoxidation by microsomal P450s was not completely unexpected. It was therefore apparent that the uniqueness and significance of the P450 AA epoxygenase reaction was going to be defined by whether or not the enzyme system participated in the *in vivo* metabolism of the fatty acid. Since asymmetric synthesis is an accepted requirement for the biosynthetic origin of most eicosanoids, the demonstration of chiral EET pools in several rat and human organs and plasma proved their enzymatic origin and established the AA epoxygenase as a formal metabolic pathway and a member of the AA cascade[4, 5, 15]. Moreover, the analysis of the effects of known P450 inducers on the levels and stereochemistries of endogenous EETs confirmed the role of P450 in the *in vivo* epoxidation of AA[15]. These experiments documented a new metabolic function for the P450 enzyme system in the oxidation and bioactivation of endogenous fatty acids such as AA, and demonstrated that the tissue levels and chemical properties of the endogenous EETs reflect the organ biosynthetic capacity, as well the contribution of tissue-specific regio- and stereoselective EET metabolism[4, 5, 117–119]. The presence of endogenous chiral EETs has been shown in rat liver, lung, kidney, brain, plasma, and urine; in rabbit lung, kidney, and urine; and in human liver, kidney, lung, brain, plasma, and urine[4–10].

A distinctive feature of endogenous EET pools in rat liver and kidney is their presence as esters of several glycerophospholipids (~99% of the total liver EETs)[117] with 55% of the total liver EETs in phosphatidylcholine, 32% in phosphatidyl-ethanolamine, and 12% in phosphatidylinositols[117]. Chiral analysis of the fatty acids at *sn-2* revealed an enantioselective preference for 8(*S*),9(*R*)-, 11(*S*), 12(*R*)-, and 14(*R*), 15(*S*)-epoxyeicosatrienoates in all three classes of phospholipids[117]. EET-phospholipid formation involves a multistep process, initiated by the P450 enantioselective epoxidation of AA, ATP-dependent activation, and enantiomer-selective lysophospholipid acylation[117]. This EET *in vivo* esterification process is unique since most endogenously formed eicosanoids are either secreted, excreted, or further oxidized. Furthermore, these studies also show, in contrast to other classes of eicosanoids, the potential for the rapid, hormonally controlled, generation of preformed bioactive EETs via hydrolytic reactions, thus obviating the need for AA oxidative metabolism. The asymmetric nature of the esterified EETs established the existence of novel oxidized glycerolipid pools and demonstrated their enzymatic synthesis from endogenous precursors and under normal physiological conditions[117]. Greater than 90% of the circulating EETs in rat and human plasma were also found esterified to the phospholipids present in the VLDL, LDL, and HDL lipoprotein fractions[129].

The biosynthesis of endogenous phospholipids containing esterified EET moieties in several human, rat, and rabbit organs suggested a new and potentially important functional role for

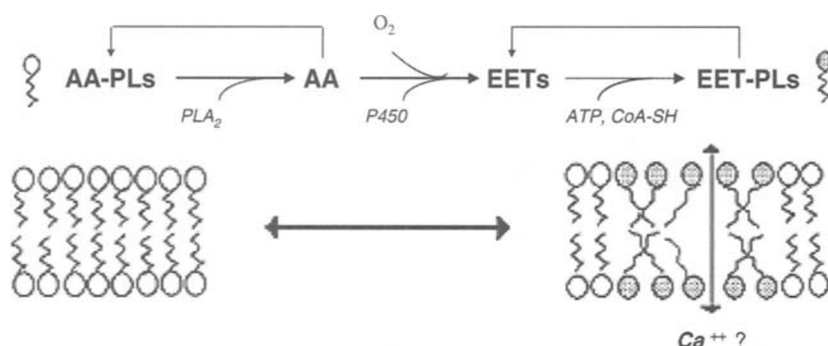

Figure 11.3. Regulation of membrane microenvironments by P450 epoxygenase metabolites.

P450 in membrane biology. A few years ago, based on studies of the capacity of synthetic epoxyeicosatrienoyl-phosphocholines to alter the Ca^{++} permeability of synthetic liposomes, we proposed that microsomal P450s could participate in the real-time control of cellular membrane microenvironments and, hence their functional properties (Figure 11.3)[5, 130]. This proposal envisioned as an initial step, the phospholipase-catalyzed release of AA from membrane phospholipids, followed by sequential P450-dependent epoxidation, EET activation to the corresponding acyl-CoA thioesters, and enzymatic lysophospholipid acylation to generate the EET-containing phospholipid pools present in many mammalian organs. The process could then be terminated by phospholipase-A_2-mediated EET release and enzymatic hydration to the corresponding DHETs. Under conditions favorable for EET acylation we were unable to show the acylation of lysolipids by DHETs[117]. Many of the enzymatic steps described above have been characterized *in vitro* using either purified proteins or microsomal membranes[5]. Inasmuch as it is well documented that oxidation of the fatty acid moieties present in membrane-bound phospholipids has profound effects on membrane structural properties, fluidity, and permeability, the formation and incorporation of EETs into cellular lipids may provide the molecular basis underlying some of the EET biological properties, many of which can be attributed to their ability to alter the physicochemical properties of cellular membranes[6–10].

3.4. Functional Roles of the P450 Arachidonic Acid Monooxygenase

Recent reviews provide excellent and detailed descriptions of the biological roles attributed to the products and enzymes of the AA monooxygenase[6–10]. We address here what we considered to be the two most relevant issues, and for which a more or less generalized consensus exists regarding their potential physiological importance.

3.4.1. Vascular Reactivity; Ion channel regulation

Because of their physiological and pathophysiological implications, the vasoactive properties of EETs and 20-HETE are under extensive scrutiny. The consensus is that EETs (5,6-and 11,12-EET in particular) and 20-HETE are powerful vasodilators and vasoconstrictors of small diameter vascular beds, respectively[6–10], and that these actions are associated with their ability to inactivate (20-HETE) or activate (EETs) Ca^{++}-activated vascular smooth muscle K^+ channels[6–10, 125, 131]. It has been proposed that an EET-mediated hyperpolarization event is required to complete the vasodilatory response of vascular smooth muscle cells to hormones such as, for example, bradykinin[8, 9, 127, 128]. The identification of 11,12-EET as an EDHF[9, 125, 126], and of 20-HETE as an anti-EDHF molecule[7, 8, 10, 131] are supported by the demonstration of hormonally controlled 20-HETE and EET biosynthesis by isolated vascular smooth

muscle and endothelial cells, respectively[7–10, 125, 131]. By facilitating the introduction of molecular and mechanistic approaches to the characterization of the renal and vascular roles of the P450 eicosanoids, these studies are generating conceptually a coherent and mechanistic understanding of their ion transport and vasoactive properties[4–10]. The accumulating evidence for a role of microsomal P450s in vascular biology is creating new and important avenues for research, and generating new concepts and experimental approaches for the study of cardiovascular and renal physiology.

3.4.2. Blood Pressure Control and Hypertension

An important contribution to the studies of the functional significance of the AA monooxygenase was the proposal of a role for renal P450s in the pathophysiology of experimental hypertension[6, 7]. In addition to its potential clinical relevance, animals models of genetically controlled hypertension afforded the opportunity to associate functional phenotypes with alterations in gene structure, function, and/or regulation. From integrations of the renal responses to P450-eicosanoids, and correlations between their biosynthesis and the development of hypertension, pro- and antihypertensive roles were identified for the AAω/ω-1 hydroxylases and epoxygenases[6, 7]. For example, the developmental phase of hypertension, in the Spontaneously Hypertensive Rat model (SHR model), was shown to be accompanied by increases in renal CYP4A2 expression and 20-HETE synthase activity[6, 7]; and chemical[132] or antisense nucleotide[133] inhibition of renal 20-HETE biosynthesis or CYP4A expression, lowered the blood pressure of hypertensive SHR rats. Importantly, 20-HETE is a powerful vasoconstrictor of the renal microcirculation[6–10], may mediate the autoregulatory responses of renal afferent arterioles[6–10], is formed *in situ* by CYP4A isoforms[6–10, 114], and its vasoactive properties are consistent with its proposed prohypertensive role[6–10]. Dahl Salt Sensitive (DS) rats fed high salt diets become hypertensive while comparable Dahl Salt Resistant (DR) animals remain normotensive. An antihypertensive role for CYP4A2 and 20-HETE was proposed based on: (a) P450 inhibitor studies, and their effects on tubular Na^+ transport[10], (b) differences between DS and DR rats in CYP4A expression and 20-HETE synthase activity[10, 134], and (c) normalization of Cl^- transport in DS rats by 20-HETE[10, 135].

The inhibition of distal nephron Na^+ reabsorption by 5,6-EET[6–8, 14], the induction of kidney CYP2C23 and EET biosynthesis by excess dietary salt[76, 123], and EET-induced dilation of microcirculatory beds[6–10] suggested antihypertensive functions for the EETs[6, 7]. In agreement with this: (a) clotrimazole inhibition of the rat kidney epoxygenases caused reductions in renal EET biosynthesis[136], and the development of clotrimazole-dependent, salt-sensitive hypertension[136], (b) high salt diets failed to induce the activity of the kidney AA epoxygenase in hypertensive DS rats[136], and (c) the EETs contribute to the prostanoid- and NO-independent dilation of renal afferent arterioles[6–10, 127, 128]. In summary, the pro- and antihypertensive roles attributed to the ω-hydroxylase and epoxygenase eicosanoids can be rationalized in terms of their biological properties and their site of action[10]. In the renal tubule, EETs and 20-HETE block Na^+ transport and function as antihypertensive molecules[6–10]. In the renal vasculature, the EETs and 20-HETE have opposing activities as either powerful vasodilators (EETs) or a vasoconstrictor (20-HETE), and can act, therefore, as anti- or prohypertensive mediators, respectively[6–10].

Conclusive evidence for a role of P450s in renal and cardiovascular physiology was provided by the demonstration that the disruption of the murine Cyp4a14 gene caused a type of hypertension that, like most human hypertension, was sexually dimorphic, and more severe in males[100]. As shown in Figure 11.4, lack of a Cyp4a14 gene product(s) raises the mean arterial blood pressure of male Cyp4a14 (−/−) mice by 38 mm of Hg, respectively[100]. Hypertensive Cyp4a14 (−/−) mice show increases in plasma androgen levels[100], and in renal 20-HETE synthase activity (Figure 11.4)[100]. Castration reduced kidney microsomal 20-HETE biosynthesis, and normalized the blood pressure of hypertensive Cyp4a14 (−/−) mice (Figure 11.4), while, on the other hand, androgen administration raised systemic blood pressures and microsomal 20-HETE biosynthesis, regardless of the animal's genotype[100]. Northern

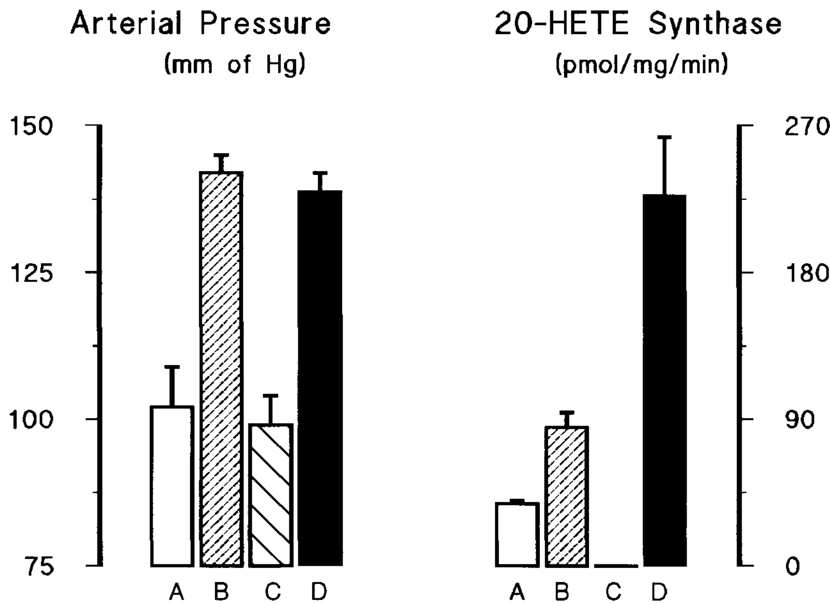

Figure 11.4. Genetically controlled or experimentally-induced alterations in the levels of plasma androgens levels are associated with changes in systemic blood pressure and renal 20-HETE synthase activity. The mean arterial blood pressure of conscious, wild-type, and Cyp4a14 (−/−) adult male mouse, was determined and described[100]. Androgens were administered by means of implanted 5α-dehydrotestosterone containing pellets (21-day release pellets; 5 mg/day; Innovative Research of America, Sarasota, FL). After 10 days of treatment, groups of animals were utilized for either pressure measurements, or the determination of kidney microsomal 20-HETE synthase activity. For more details see ref. [100]. A, wild-type; B, Cyp4a14 (−/−); C, castrated Cyp4a14 (−/−); D, castrated Cyp4a14 (−/−) mice, treated for 10 days with 5-α-dehydrotestosterone. Values show are averages ± SE, calculated from groups of at least 10 (Arterial Pressure) or 7 (20-HETE synthase) mice.

blot analyses showed that either disruption of the Cyp4a14 gene, or androgen administration caused the upregulation of Cyp4a12, an active, androgen sensitive, renal 20-HETE synthase[100], and provided a convenient explanation to paradoxical effects of Cyp4a14 disruption in renal AA ω-hydroxylase activity. Based on: (a) the known hemodynamic effects of 20-HETE[6–10], and (b) the increased renovascular resistance and impaired afferent arteriole autoregulatory capacity of Cyp4a14 (−/−) mice[100], it was proposed that androgen-mediated increases in the renal biosynthesis of vasoconstrictor 20-HETE were responsible for the hypertensive phenotype of Cyp4a14 (−/−) mice[100]. The prohypertensive effects of androgens were confirmed by administering 5-α-dehydrotestosterone to male or female Sprague–Dawley rats. In these animals, chronic treatment with 5-α-dehydrotestosterone raised the systolic blood pressure of male and female rats by 29 and 57 mm of Hg, respectively, and caused parallel increases in 20-HETE biosynthesis and in the kidney levels of CYP4A8 mRNAs[137].

The characterization of hypertensive Cyp4a14 knockout mice showed that the pressure effects of this gene were apparently independent of the intrinsic AA monooxygenase activity of its encoded protein but, rather were associated with changes in the regulation of alternate AA ω/ω-1 hydroxylases (Cyp4a12)[100]. Based on the known prohypertensive properties of 20-HETE[6–10], and the described gene-dependent, androgen-mediated, control of 20-HETE renal expression and activity, we concluded that blood pressure regulation by kidney CYP4A proteins involves a combination of transcriptional and hemodynamic mechanisms that determine the levels and site of expression of CYP 4A ω-hydroxylases and, ultimately, the level and site(s) of 20-HETE biosynthesis. Support for this conclusion was provided by the demonstration that

dissected rat renal microvessels, the target organ for most of the prohypertensive effects of 20-HETE, possess an androgen-regulated CYP4A8 20-HETE synthase[137].

The mechanism(s) by which Cyp4a14 gene product(s) control plasma androgen levels are yet to be defined; however, ample precedent supports a role for the kidney androgen receptor in regulating renal Cyp 4A and 2C expression[101–104]. Furthermore, in male and female rats, the androgen-mediated increases in systemic blood pressure, and in the levels of kidney CYP4A8 transcripts[137], are accompanied by a marked decrease in the levels of renal CYP2C23 epoxygenase protein and diminished microsomal EET biosynthesis[137]. The androgen-mediated counter-regulation of renal CYP4A ω-hydroxylases and 2C epoxygenases[101], suggests that its effects on blood pressure results from coordinated, nephron site-specific, increases in the biosynthesis of prohypertensive 20-HETE, and decreases in antihypertensive EET formation. Finally, in mice, activation of PPARα upregulates the expression of Cyps 4a10 and 4a14 (but not 4a12)[138], and the counter-regulation of rat Cyps 4A and 2C isoforms by PPARα ligands is published[139]. Nevertheless, the pressure effects of PPAR ligands in mice have yet to be fully defined, although, they have been characterized as antihypertensive in rats[10, 140, 141]. The studies summarized suggest that blood pressure regulation by renal P450s involves combinations of regulatory (transcriptional) and functional (tubular and hemodynamic) components, and that the organ balance of pro- and antihypertensive mediators and thus, its functional status, is dictated by: (a) the nephron segment-specific expression and regulation of the corresponding genes, (b) the enzymatic properties of the encoded proteins, and (c) the expression and activities of ancillary enzymes responsible for EET and/or HETE metabolism, disposition or activation.

4. Conclusion

The studies of the P450 AA monooxygenase have uncovered new and important roles for P450 in the metabolism of endogenous substrates, and added P450 to the list of enzymes that participate in the metabolism of AA, a fatty acid that serves as the precursor for the biosynthesis of several physiologically important lipid mediators. The functional relevance of this metabolic pathway is suggested by the many important biological activities attributed to its products. These studies, as well as the documented endogenous roles of P450s in cholesterol, steroid, and vitamin metabolism are contributing to establish this enzyme system as a major participant in the regulation of cell, organ, and body physiology. Among these, the phenotypic analysis of mice carrying disrupted copies of the CYP4a14 gene unveiled new and important roles for the P450 enzymes in cardiovascular physiology and the control of systemic blood pressures, and suggested the human homologs of the rodent CYP 2C and 4A AA epoxygenases and ω-hydroxylases as candidate genes for the study of their role in the pathophysiology of hypertension, and cardiovascular disease.

Acknowledgments

None of the studies carried out in the authors' laboratory could have been possible without the continuous and generous grant support from NIGMS and NIDDK.

References

1. Smith, W.L., D.L. DeWitt, and R.M. Garavito (2000). Cyclooxygenases: Structural, cellular, and molecular biology. *Ann. Rev. Biochem.* **69**, 145–182, and cited references.
2. Prescott, S.M. and C. Yang (2002). Many actions of cyclooxygenase-2 in cellular dynamics and in Cancer. *J. Cell. Biol.* **190**, 279–286.
3. Brash, A.R. (1999). Lipoxygenases: Occurrence, function, catalysis, and acquisition substrate. *J. Biol. Chem.* **274**, 23679–23680, and cited references.
4. Capdevila, J.H. and J.R. Falck (2001). The CYP P450 arachidonic acid monooxygenases: From cell signaling to blood pressure regulation. *Biochem. Biophys. Res. Comm.* **285**, 571–576, and cited references.
5. Capdevila, J.H. and J.R. Falck (2002). Biochemical and molecular properties of the cytochrome P450 arachidonic acid monooxygenases. *Prostaglandins other Lipid Mediat.* **68–69**, 325–344, and cited references.
6. McGiff, J.C. and J. Quilley (1999). 20-HETE and the kidney: Resolution of old problems and new

beginnings. *Am. J. Physiol.* **277**, R607–R623, and cited references.
7. McGiff, J.C. and J. Quilley (2001). 20-Hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids and blood pressure. *Curr. Opin. Nephrol. Hypertens.* **10**, 31–237, and cited references.
8. Imig, J.D. (2000). Eicosanoid regulation of renal vasculature. *Am. J. Physiol.* **279**, F965–F981, and cited references.
9. Campbell, W.B. and D.R. Harder (1999). Endothelium-derived hyperpolarizing factors and vascular cytochrome P450 metabolites of arachidonic acid in the regulation of tone. *Circ. Res.* **84**, 484–488, and cited references.
10. Roman, R.J. (2002). P450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.* **82**, 131–185, and cited references.
11. Capdevila, J.H., N. Chacos, J. Werringloer, R.A. Prough, and R.W. Estabrook (1981). Liver microsomal cytochrome P-450 and the oxidative metabolism of AA. *Proc. Natl. Acad. Sci. USA* **78**, 5362–5366.
12. Morrison, A.R. and N. Pascoe (1981). Metabolism of AA through NADPH-dependent oxygenase of renal cortex. *Proc. Natl. Acad. Sci. USA* **78**, 7375–7378.
13. Oliw, E.H. and J.A. Oates (1981). Oxygenation of AA by hepatic microsomes of the rabbit. Mechanism of biosynthesis of two vicinal diols. *Biochim. Biophys. Acta* **666**, 327–340.
14. Jacobson, H.R., S. Corona, J.H. Capdevila, N. Chacos, S. Manna, A. Womack *et al.* (1984). Effects of epoxyeicosatrienoic acids on ion transport in the rabbit cortical collecting tubule. In P. Braquet, J.C. Frolich, S. Nicosia, and R. Garay (eds), *Prostaglandins and Membrane Ion Transport.* Raven Press, New York, p. 311.
15. Karara, A., E. Dishman, I. Blair, J.R. Falck, and J.H. Capdevila (1989). Endogenous epoxyeicosatrienoic acids. Cytochrome P-450 controlled stereoselectivity of the hepatic arachidonic acid epoxygenase. *J. Biol. Chem.* **264**, 19822–19827.
16. Kupfer, D. (1982). Endogenous substrates of monooxygenases: Fatty acids and prostaglandins. In J.B. Schenkman and D. Kupfer (eds), *Hepatic Cytochrome P-450 Monooxygenase System.* Pergamon Press, New York, pp. 157–187.
17. Sharma, R.K., M.V. Doig, D.F.V. Lewis, and G. Gibson (1989). Role of hepatic and renal cytochrome P450 IVA1 in the metabolism of lipid substrates. *Biochem. Pharmacol.* **38**, 3621–3629.
18. Imaoka, S., S. Tanaka, and Y. Funae (1989). (ω/ω-1) hydroxylation of lauric acid and arachidonic acid by rat renal cytochrome P-450. *Biochem. Int.* **18**, 731–740.
19. Johnson, E.F., D.L. Walker, and K.J. Griffin (1990). Cloning and expression of three rabbit kidney cDNAs encoding lauric acid-hydroxylases. *Biochemistry* **29**, 873–879.
20. Kikuta, Y., E. Kusunose, and M. Kusunose (2002). Prostaglandin and leukotriene ω-hydroxylases. *Prostaglandins other Lipid Mediat.* **68–69**, 325–344, and cited references.
21. Rahimtula, A.D. and P.J. O'Brien (1974). Hydroperoxide catalyzed liver microsomal aromatic hydroxylation reactions involving cytochrome P-450. *Biochem. Biophys. Res. Commun.* **60**, 440–447.
22. Weiss, R.H, J.L. Arnold, and R.W. Estabrook (1987). Transformation of an arachidonic acid hydroperoxide into epoxyhydroxy and trihydroxy fatty acids by liver microsomal cytochrome P-450. *Arch. Biochem. Biophys.* **252**, 334–338.
23. Hecker, M. and V. Ullrich (1989). On the mechanism of prostacyclin and thromboxane A_2 biosynthesis. *J. Biol. Chem.* **264**, 141–150.
24. Hara, S., A. Miyata, C. Yokoyama, R. Brugger, F. Lottspeich, V. Ullrich *et al.* (1995). Molecular cloning and expression of prostacyclin synthase from endothelial cells. *Adv. Prostaglandin Thromboxane Leukot. Res.* **23**, 121–123.
25. Yokoyama, C., A. Miyata, H. Ihara, V. Ullrich, and T. Tanabe (1991). Molecular cloning of the human platelet thromboxane A synthase. *Biochem. Biophys. Res. Commun.* **178**, 1479–1484.
26. Ohashi, K., K.H. Ruan, R.J. Kulmacz, K.K. Wu, and L.H. Wang (1992). Primary structure of human thromboxane synthase determined from the cDNA sequence. *J. Biol. Chem.* **267**, 789–793
27. White, R.E. and M.G. Coon (1980). Oxygen activation by cytochrome P-450. *Ann. Rev. Biochem.* **49**, 315–356, and references therein.
28. Wong, P.Y.K., K.U. Malik, B.M. Taylor, W.P. Schneider, J.C. McGiff, and F.F. Sun (1985). Epoxidation of prostacyclin in the rabbit kidney. *J. Biol. Chem.* **260**, 9150–9153.
29. Oliw, E.H. (1984). Metabolism of 5(6)-epoxyeicosatrienoic acid by ram seminal vesicles formation of novel prostaglandin E_1 metabolites. *Biochim. Biophys. Acta.* **793**, 408–415.
30. Zhang, J.Y., C. Prakash, K. Yamashita, and I.A. Blair (1992). Regiospecific and enantioselective metabolism of 8,9-epoxyeicosatrienoic acids by cyclooxygenase. *Biochem. Biophys. Res. Commun.* **183**, 138–143.
31. Jajoo, H.K., J.H. Capdevila, J.R. Falck, R.K. Bhatt, and I.A. Blair (1992). Metabolism of 12(R)-hydroxyeicosatetraenoic acid by rat liver microsomes. *Biochim. Biophys. Acta* **1123**, 110–116.
32. Capdevila, J., P. Mosset, P. Yadagiri, Sun Lumin, and J.R. Falck (1988). NADPH-Dependent microsomal metabolism of 14,15-epoxyeicosatrienoic acid to diepoxides and epoxyalcohols. *Arch. Biochem. Biophys.* **261**, 122–132.

33. Cowart, L.A., S. Wei, M.-S. Hsu, E.F. Johnson, M.U. Krishna, J.R. Falck *et al.* (2002). The CYP 4A isoforms hydroxylate epoxyeicosatrienoic acids to form high affinity peroxisome proliferator-activated receptor ligands. *J. Biol. Chem.* **277**, 35105–35112.
34. Hamberg, M. and B. Samuelsson (1966). Prostaglandins in human seminal plasma. Prostaglandins and related factors. *J. Biol. Chem.* **241**, 257–263.
35. Israelson, U., M. Hamberg, and B. Samuelsson (1969). Biosynthesis of 19-hydroxy-prostaglandin A_1. *Eur. J. Biochem.* **11**, 390–394.
36. Kupfer, D., I. Jansson, L.V. Favreau, A.D. Theoharides, and J.B. Schenkman (1988). Regioselective hydroxylation of prostaglandins by constitutive forms of cytochrome P-450 from rat liver: Formation of a novel metabolite by a female-specific P-450. *Arch. Biochem. Biophys.* **262**, 186–195.
37. Matsubara, S., S. Yamamoto, K. Sogawa, N. Yokotani, Y. Fujii-Kuriyama, M. Haniu *et al.* (1987). cDNA cloning and inducible expression during pregnancy of the MRNA for rabbit pulmonary prostaglandin ω-hydroxylase (cytochrome P-450_p-2). *J. Biol. Chem.* **62**, 13366–13371.
38. Sharma, R.K., M.V. Doig, D.F.V. Lewis, and G. Gibson (1989). Role of hepatic and renal cytochrome P450 IVA1 in the metabolism of lipid substrates. *Biochem. Pharmacol.* **38**, 3621–3629.
39. Yokotani, N., R. Bernhardt, K. Sogawa, E. Kusunose, O. Gotoh, M. Kusunose *et al.* (1989). Two forms of ω-hydroxylase toward prostaglandin A and laurate. cDNA cloning and their expression. *J. Biol. Chem.* **264**, 21665–21669.
40. Kusonuse, E., A. Sawamura, H. Kawashima, I. Kubota, and M. Kusunose (1989). Isolation of a new form of cytochrome P450 with prostaglandin A and fatty acid ω-hydroxylase activities from rabbit kidney cortex microsomes. *J. Biochem.* **106**, 194–206.
41. Kikuta, Y., E. Kusunose, T. Okumoto, I. Kubota, and M. Kusunose (1990). Purification and partial characterization of two forms of cytochrome P450 with ω-hydroxylase activity towards prostaglandin A and fatty acids from rat liver microsomes. *J. Biochem.* **107**, 280–286
42. Roman, L.J., C.A.N. Palmer, J.E. Clark, A.S. Muerhoff, K.J. Griffin, E.F. Johnson *et al.* (1993). Expression of rabbit cytochrome P4504A which catalyzes the ω-hydroxylation of fatty acids, and prostaglandins. *Arch. Biochem. Biophys.* **307**, 57–67.
43. Bylund, J., M. Hidestrand, M. Ingelman-Sundberg, and E.H. Oliw (2000). Identification of CYP4F8 in human seminal vesicles as a prominent 19-hydroxylase of prostaglandin endoperoxides. *J. Biol. Chem.* **275**, 21844–21849.
44. Lasker, J.M., B.W. Chen, I. Wolf, B.P. Blswck, P.D. Wilson, and P.K. Powell (2000). Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid in human kidney. *J. Biol. Chem.* **264**, 17845–17853.
45. An updated list of P450 genes and sequences is found at: http://drnelson.utmem.edu/CytochromeP450.html
46. Imaoka, S., H. Ogawa, S. Kimura, and F.J. Gonzalez (1993). Complete cDNA sequence and cDNA-directed expression of CYP4A11, a fatty acid omega-hydroxylase expressed in human kidney. *DNA Cell Biol.* **12**, 893–899.
47. Kawashima, H., T. Naganuma, E. Kusunose, T. Kono, R. Yasumoto, K. Sugimura *et al.* (2000). Human fatty acid ω-hydroxylase, CYP 4A11: Determination of complete genomic sequence and characterization of purified recombinant protein. *Arch. Biochem. Biophys.* **378**, 333–339.
48. Bellamine, A., Y. Wang, M.R. Waterman, J.V. Gainer, E. Dawson, N.J. Brown *et al.* (2003). Characterization of the CYP4A11 gene, a second CYP4A gene in humans. *Arch. Biochem. Biophys.* **409**, 221–227.
49. Wang, M.H., D.E. Stec, M. Balazy, V. Mastyugin, C.S. Yang, R.J. Roman *et al.* (1997). Cloning, sequencing, and cDNA-directed expression of rat renal CYP4A2: AA ω-hydroxylation and 11,12-epoxidation by CYP4A2 protein. *Arch. Biochem. Biophys.* **336**, 240–250.
50. Helvig, C., E. Dishman, and J.H. Capdevila (1998). Molecular, enzymatic, and regulatory characterization of rat kidney cytochromes P450 4A2 and 4A3. *Biochemistry* **37**, 12546–12558.
51. Hoch, U., Z. Zhang, D.L. Kroetz, and P.R. Ortiz de Montellano (2000). Structural determination of the substrate specificities and regioselectivities of the rat and human fatty acid omega hydroxylases. *Arch. Biochem. Biophys.* **373**, 63–71.
52. Nishimoto, M., J.E. Clark, and B.S.S. Masters (1993). Cytochrome P450 4A4: Expression in *Escherichia coli*, purification, and characterization of catalytic properties. *Biochemistry* **32**, 8863–8870.
53. Bylund, J., M. Bylund, and E.H. Oliw (2001). cDNA cloning and expression of CYP4F12, a novel human cytochrome P450. *Biochem. Biophys. Res. Commun.* **280**, 892–897.
54. Yokomizo, T., T. Izumi, and T. Shimizu (2001). Leukotriene B_4: Metabolism and signal transduction. *Arch. Biochem. Biophys.* **385**, 231–241, and cited references.
55. Haeggstrom, J.Z. and A. Wetterholm (2002). Enzymes and receptors in the leukotriene cascade. *Cell. Mol. Life Sci.* **59**, 742–753, and cited references.
56. Powell, W.S. (1984). Properties of leukotriene B_4 20-hydroxylase from polymorphonuclear leukocytes. *J. Biol. Chem.* **259**, 3082–3089.

57. Shak, S. and I.M. Goldstein (1984). Oxidation is the major pathway for the catabolism of leukotriene B_4 in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259**, 10181–10187.
58. Romano, M.C., R.D. Eckardt, P.E. Bender, T.B. Leonard, K.M. Straub, and J.F. Newton (1987). Biochemical characterization of hepatic microsomal leukotriene B_4 hydroxylases. *J. Biol. Chem.* **262**, 1590–1595.
59. Soberman, R.J., R.T. Okita, B. Fitzsimmons, J. Rokach, B. Spur, and K.F. Austen (1987). Stereochemical requirements for substrate specificity of LTB_4 20-hydroxylase. *J. Biol. Chem.* **262**, 12421.
60. Sumimoto, J., K. Takeshige, and S. Minakami (1988). Characterization of human neutrophil leukotriene B4 omega-hydroxylase, a system involving a unique cytochrome P-450 and NADPH-cytochrome P-450 reductase. *Eur. J. Biochem.* **172**, 315–324.
61. Kikuta, Y., E. Kusunose, K. Endo, S. Yamamoto, K. Sogawa, Y. Fujii-Kuriyama *et al.* (1993). A novel form of cytochrome P450 family 4 in human polymorphonuclear leukocytes. cDNA cloning and expression of leukotriene B_4 ω-hydroxylase. *J. Biol. Chem.* **268**, 9376–9380.
62. Kikuta, Y., E. Kusunose, H. Sumimoto, Y. Mizukami, K. Takeshige, T. Sakai *et al.* (1998). Purification and characterization of recombinant human neutrophil leukotriene B_4 ω-hydroxylase (Cytochrome 4F3). *Arch. Biochem. Biophys.* **355**, 201–205.
63. Christmas, P., S.R. Ursino, J.W. Fox, and R.J. Soberman (1999). Expression of the *CYP4F3* gene: Tissue-specific splicing and alternative promoters generate high and low Km forms of leukotriene B_4 ω-hydroxylase. *J. Biol. Chem.* **274**, 21191–21199.
64. Kikuta, Y., E. Kusunose, M. Ito, and M. Kusunose (1999). Purification and characterization of recombinant rat hepatic 4F1. *Arch. Biochem. Biophys.* **369**, 193–196.
65. Jin, R., D.R. Koop, J.L. Raucy, and J.M. Lasker (1998). Role of human CYP4F2 in hepatic catabolism of the proinflammatory agent, leukotriene B_4. *Arch. Biochem. Biophys.* **359**, 89–98.
66. Kawashima, H., E. Kusunose, C.M. Thompson, and H.W. Strobel (1997). Protein expression, characterization, and regulation of *CYP4F4* and *CYP4F5* cloned from rat brain. *Arch. Biochem. Biophys.* **347**, 148–154.
67. Wong, P.Y.K., P. Westlund, M. Hamberg, E. Granstrom, P.W.H. Chao, and B. Samuelsson (1984). ω-Hydroxylation of 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259**, 2683–2686.
68. Marcus, A.J., L.B. Safier, H.L. Ullman, N. Broekman, T. Islam, D. Oglesby *et al.* (1984). 12*S*, 20-Dihydroxyeicosatetraenoic acid: A new icosanoid synthesized by neutrophils from 12*S*-hydroxyeicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. *Proc. Natl. Acad. Sci. USA* **81**, 903–907.
69. Flaherty, J.T. and J. Nishihira (1987). 5-Hydroxyeicosatetraenoate promotes Ca^{2+} and protein kinase C mobilization in neutrophils. *Biochem. Biophys. Res. Commun.* **148**, 575–579.
70. Okita, R.T., R.J. Soberman, J.M. Bergholte, B.S.S. Masters, R. Hayes, and R.C. Murphy (1987). ω-Hydroxylation of 15-hydroxyeicosatetraenoic acid by lung microsomes from pregnant rabbits. *Mol. Pharmacol.* **32**, 706–709.
71. Capdevila, J.H., A. Karara, D.J. Waxman, M.V. Martin, J.R. Falck, and F.P. Guengerich (1990). Cytochrome P-450 enzyme-specific control of the regio- and enantiofacial selectivity of the microsomal AA epoxygenase. *J. Biol. Chem.* **265**, 10865–10871.
72. Knickle, L.C. and J.R. Bend (1994). Bioactivation of arachidonic acid by the cytochrome P450 monooxygense of guinea pig lung: The orthologue of cytochrome P450 2B4 is solely responsible for the formation of epoxyeicosatrienoic acids. *Mol. Pharmacol.* **45**, 1273–1280.
73. Laethem, R.M., J.R. Halpert, and D.R. Koop (1994). Epoxidation of arachidonic acid as an active site probe of cytochrome P450 2B isoforms. *Biochim. Biophys. Acta* **1206**, 42–48.
74. Keeney, D.S., C. Skinner, S. Wei, T. Friedberg, and M.R. Waterman (1998). A keratinocyte-specific epoxygenase, CYP2B12, metabolizes arachidonic acid with unusual selectivity, producing a single major epoxyeicosatrienoic acid. *J. Biol. Chem.* **273**, 9279–9284.
75. Keeney, D.S., C. Skinner, J.B. Travers, J.H. Capdevila, J.B. Nanney, L.E. King *et al.* (1998). Differentiating keratinocytes express a novel cytochrome P450 enzyme, CYP2B19, having arachidonate monooxygenase activity. *J. Biol. Chem.* **273**, 32071–32079.
76. Capdevila, J.H., S. Wei, Y. Yan, A. Karara, H.R. Jacobson, J.R. Falck *et al.* (1992). Cytochrome P-450 arachidonic acid epoxygenase. Regulatory control of the renal epoxygenase by dietary salt loading. *J. Biol. Chem.* **267**, 21720–21726.
77. Karara, A., K. Makita, H.R. Jacobson, J.R. Falck, F.P. Guengerich, R.N. DuBois *et al.* (1993). Molecular cloning, expression, and enzymatic characterization of the rat kidney cytochrome P-450 arachidonic acid epoxygenase. *J. Biol. Chem.* **268**, 13565–13570.
78. Imaoka, S., P.J. Zwedlung, H. Ogawa, S. Kimura, F.J. Gonzalez, and H.Y. Kim (1993). Identification of CYP2C23 expressed in rat kidney as an arachidonic acid epoxygenase. *J. Pharmacol. Exp. Ther.* **267**, 1012–1016.

79. Daikh, B.E., R.M. Laethem, and D. Koop (1994). Stereoselective epoxidation of arachidonic acid by cytochrome P-450s 2CAA and 2C2. *J. Pharmacol. Exp. Ther.* **269**, 1130–1135.
80. Rifkind, A.B., C. Lee, T.K. Chang, and D.J. Waxman (1995). Arachidonic acid metabolisms by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: Regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch. Biochem. Biophys.* **320**, 380–389.
81. Zeldin, D.C., R.N. DuBois, J.R. Falck, and J.H. Capdevila (1995). Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform. *Arch. Biochem. Biophys.* **322**, 76–86.
82. Luo, G., D.C. Zeldin, J.A. Blaisdell, E. Hodgson, and J.A. Goldstein (1998). Cloning, and expression of murine CYP2Cs and their ability to metabolize arachidonic acid. *Arch. Biochem. Biophys.* **357**, 45–57.
83. Tsao, C.C., S.J. Coulter, A. Chien, G. Luo, N.P. Clayton, R. Maronpot *et al.* (2001). Identification and localization of five CYP2Cs in murine extrahepatic tissues and their metabolism of arachidonic acid to regio- and stereoselective products. *J. Pharmacol. Exp. Ther.* **299**, 39–47.
84. Thompson, C.M., J.H. Capdevila, and H.W. Strobel (2000). Recombinant cytochrome P450 2D18 metabolism of dopamine and arachidonic acid. *J. Pharmacol. Exp. Ther.* **294**, 1120–1130.
85. Laethem, R.M., M. Balazy, J.R. Falck, C.L. Laethem, and D.R. Koop (1993). Formation of 19(S)-, 19(R)-, and 18(R)-hydroxyeicosatetraenoic acids by alcohol-inducible cytochrome P450 2E1. *J. Biol. Chem.* **268**, 12912–12918.
86. Scarborough, P.E., J. Ma, W. Qy, and D.C. Zeldin (1999). P450 subfamily CYP2J and their role in the bioactivation of arachidonic acid in extrahepatic tissues. *Drug Metab. Rev.* **31**, 205–234, and cited references.
87. Zeldin, D.C. (2001). Epoxygenase pathways of arachidonic acid metabolism. *J. Biol. Chem.* **276**, 36059–36062, and cited references.
88. Oliw, E.H. (1993). *bis*-Allylic hydroxylation of linoleic acid and AA by human hepatic monooxygenases. *Biochim. Biophys. Acta* **1166**, 258–263.
89. Brash, A.R., W.E. Boeglin, J.H. Capdevila, S. Yeola, and I.A. Blair (1995). 7-HETE, 10-HETE, and 13-HETE are major products of NADPH-dependent AA metabolism in rat liver microsomes: Analysis of their stereochemistry, and the stereochemistry of their acid-catalyzed rearrangement. *Arch. Biochem. Biophys.* **321**, 485–492.
90. Capdevila, J.H., P. Yadagiri, S. Manna, and J.R. Falck (1986). Absolute configuration of the hydroxyeicosatetraenoic acids (HETEs) formed during catalytic oxygenation of AA by microsomal cytochrome P-450. *Biochem. Biophys. Res. Commun.* **141**, 1007–1011.
91. Woollard, P.M. (1986). Stereochemical differences between 12-hydroxy-5,8,10,14-eicosatetraenoic acid in platelets and psoriatic lesions. *Biochem. Biophys. Res. Commun.* **136**, 169–176.
92. Boeglin, W.E., R.B. Kim, and A.R. Brash (1998). A 12R-lipoxygenase in human skin: Mechanistic evidence, molecular cloning, and expression. *Proc. Natl. Acad. Sci. USA* **95**, 6744–6749.
93. Sun D., M. McDonnel, X.S. Chen, M.M. Lakkis, S.N. Isaacs, S.H. Elsea *et al.* (1998). Human 12(R)-lipoxygenase and the mouse ortholog. Molecular cloning, expression, and gene chromosomal assignment. *J. Biol. Chem.* **273**, 33540–33547.
94. Schwartzman, M.L., M. Balazy, J. Masferrer, N.G. Abraham, J.C. McGiff, and R.C. Murphy (1987). 12(*R*)-Hydroxyeicosatetraenoic acid: A cytochrome P450-dependent arachidonate metabolite that inhibits Na^+,K^+-ATPase in the cornea. *Proc. Natl. Acad. Sci. USA* **84**, 8125–8129.
95. Oliw, E.H. (1993). Biosynthesis of 12(S)-hydroxyeicosatetraenoic acid by bovine corneal epithelium. *Acta Physiol. Scand.* **147**, 117–121.
96. Murphy, R.C., J.R. Falck, S. Lumin, P. Yadagiri, J.A. Zirrolli, M. Balazy *et al.* (1988). 12(*R*)-Hydroxyeicosatrienoic acid: A vasodilator cytochrome P-450-dependent arachidonate metabolite from bovine corneal epithelium. *J. Biol. Chem.* **263**, 17197–17202.
97. Lu, A.Y., H.K. Junk, and M.J. Coon (1969). Resolution of the cytochrome P-450 containing ω-hydroxylation system of liver microsomes into three components. *J. Biol. Chem.* **244**, 3714–3721.
98. Capdevila, J.H., S. Wei, C. Helvig, J.R. Falck, Y. Belosludtsev, G. Truan *et al.* (1996). The highly stereoselective oxidation of polyunsaturated fatty acids by cytochrome P450BM-3. *J. Biol. Chem.* **271**, 22663–22671
99. Graham-Lorence, S., G. Truan, J.A. Peterson, J.R. Falck, S. Wei, and C. Helvig *et al.* (1997). An active site substitution, F87V, converts cytochrome P450 BM3 into a regio- and stereoselective *(14)S,15(R)*-AA epoxygenase. *J. Biol. Chem.* **272**, 1127–1135.
100. Holla, V.R., F. Adas, J.D. Imig, X. Zhao, E. Price, N. Olsen *et al.* (2001). Alterations in the regulation of androgen-sensitive Cyp 4a monooxygenases cause hypertension. *Proc. Natl. Acad. Sci. USA* **98**, 5211–5216.
101. Henderson, C.J., A.R. Scott, C.S. Yang, and C.R. Wolf (1990). Testosterone-mediated regulation of mouse renal cytochrome P-450 isoenzymes. *Biochem. J.* **266**, 675–681.

102. Lund, J., P.G. Zaphiropoulos, A. Mode, M. Warner, and J.A. Gustafsson (1991). Hormonal regulation of cytochrome P450 gene expression. *Adv. in Pharmacol.* **22**, 325–354, and cited references.
103. Henderson, C.J. and C.R. Wolf (1991). Evidence that the androgen receptor mediates sexual differentiation of mouse renal cytochrome P450 expression. *Biochem. J.* **278**, 499–503.
104. Sundseth, S.S. and D.J. Waxman (1992). Sex-dependent expression and clofibrate inducibility of cytochrome P450 AA fatty acid ω-hydroxylases. *J. Biol. Chem.* **267**(6), 3915–3921.
105. Lee, S.S., T. Pineau, J. Frago, E.J. Lee, J.W. Owens, D.L. Kroetz *et al.* (1995). Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisomal proliferators. *Mol. Cell. Biol.* **15**, 3012–3022.
106. Waxman, D.J. and T.K.H. Chang (1995). Hormonal regulation of liver cytochrome P450 enzymes. In P.R. Ortiz de Montellano (ed.), *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd edn. Plenum Press, New York, pp. 391–418.
107. Waxman, D.J. (1999). P450 gene induction by structurally diverse xenochemicals: Central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.* **369**, 11–23.
108. Savas, U., M-H. Hsu, and E.F. Johnson (2003). Differential regulation of human CYP4A genes by peroxisome proliferators and dexamethasone. *Arch. Biochem. Biophys.* **409**, 212–220.
109. Stromsteadt, M., S. Hayashi, P.G. Zaphiropoulos, and J.A. Gustafsson (1990). Cloning and characterization of a novel member of the cytochrome P450 subfamily IVA in rat prostate. *DNA Cell Biol.* **8**, 569–577.
110. Heng, Y.M., C.S. Kuo, P.S. Jones, R. Savory, R.M. Schultz, S.R. Tomlinson *et al.* (1997). A novel murine P-450 gene, Cyp4a14, is part of a cluster of Cyp4a and Cyp4b, but not of CYP4F, genes in mouse and humans. *Biochem. J.* **325**, 741–749.
111. Falck, J.R., S. Lumin, I.A. Blair, E. Dishman, M.V. Martin, D.J. Waxman *et al.* (1990). Cytochrome P-450-dependent oxidation of arachidonic acid to 16-, 17-, and 18-hydroxyeicosatetraenoic acids. *J. Biol. Chem.* **265**, 10244–10249.
112. Rifkind, A.B., A. Kanetoshi, J. Orlinick, J.H. Capdevila, and C. Lee (1994). Purification and biochemical characterization of two major cytochrome P-450 isoforms induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in chick embryo liver. *J. Biol. Chem.* **269**, 3387–3396.
113. Qu, W., J.A. Bradbury, C-C. Tsao, R. Maronpot, G.J. Harry, C.E. Parker *et al.* (2001). Cytochrome P450 CYP2J9, a new mouse arachidonic acid ω-1 hydroxylase predominantly expressed in brain. *J. Biol. Chem.* **276**(27), 25467–25479.
114. Marji, J.S., M.H. Wang, and M. Laniado-Schwartzman (2002). Cytochrome *P*-450 4A isoform expression and 20-HETE synthesis in renal preglomerular arteries. *Am. J. Physiol.* **283**, F60–F67.
115. Hashimoto, T., T. Fujita, N. Usuda, W. Cook, C. Qi, J.M. Peters *et al.* (1999). Peroxisomal and mitochondrial fatty acid oxidation in mice nullizygous for both peroxisome proliferator-activated receptor and peroxisomal fatty acyl-CoA oxidase. Genotype correlate with fatty liver phenotype. *J. Biol. Chem.* **274**, 19228–19236.
116. Chacos, N., J.F. Falck, C. Wixtrom, and J. Capdevila (1982). Novel epoxides formed during the liver cytochrome P-450 oxidation of AA. *Biochem. Biophys. Res. Commun.* **104**, 916–922.
117. Karara, A., E. Dishman, J.R. Falck, and J.H. Capdevila (1991). Endogenous epoxyeicosatrienoyl-phospholipids. A novel class of cellular glycerolipids containing epoxidized arachidonate moieties. *J. Biol. Chem.* **266**, 7561–7569.
118. Fang, X., T.L. Kaduce, M. VanRollins, N.L. Weintraub, and A.A. Spector (2000). Conversion of epoxyeicosatrienoic acids (EETs) to chain-shortened epoxy fatty acids by human skin fibroblasts. *J. Lipid Res.* **41**, 66–74.
119. Zeldin, D.C., J. Kobayashi, J.R. Falck, B.S. Winder, B.D. Hammock, J.R. Snapper *et al.* (1993). Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *J. Biol. Chem.* **268**, 6402–6407.
120. Spearman, M.E., R.A. Prough, R.W. Estabrook, J.R. Falck, S. Manna, K.C. Leibman *et al.* (1985). Novel glutathione conjugates formed from epoxyeicosatrienoic acids (EETs). *Arch. Biochem. Biophys.* **242**, 225–230.
121. Yu, Z., F. Xu, L.M. Huse, C. Morisseau, A.J. Draper, J.W. Newman *et al.* (2000). Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* **87**, 992–998.
122. Sinal, C.J., M. Miyata, M. Tohkin, K. Nagata, J.R. Bend, and F.J. Gonazalez (2000). Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J. Biol. Chem.* **275**, 40504–40510.
123. Holla, V., K. Makita, P.G. Zaphiropoulos, and J.H. Capdevila (1999). The kidney cytochrome P450 2C23 AA epoxygenase is upregulated during dietary salt loading. *J. Clin. Invest.* **104**, 751–760
124. Rifkind, A.B., A. Kanetoshi, J. Orlinick, J.H. Capdevila, and C. Lee (1994). Purification and biochemical characterization of two major cytochrome P-450 isoforms induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in chick embryo liver. *J. Biol. Chem.* **269**, 3387–3396.
125. Campbell, W.B., D. Gebremedhin, P.F. Pratt, and D.R. Harder (1996). Identification of epoxye-

icosatrienoic acids as endothelium-derived hyperpolarizing factors. *Cir. Res.* **78**, 415–423.
126. Fisslthaler, B., R. Popp, L. Kiss, M. Potente, D.R. Harder, I. Fleming *et al.* (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **410**, 493–497.
127. Imig, J.D., J.R. Falck, S. Wei, and J.H. Capdevila (2001). Epoxygenase metabolites contribute to nitric oxide-independent afferent arteriolar vasodilation in response to bradykinin. *J. Vasc. Res.* **38**, 247–255.
128. Imig, J.D., X. Zhao, J.R. Falck, S. Wei, and J.H. Capdevila (2001). Enhanced renal microvascular reactivity to angiotensin II in hypertension is ameliorated by the sulfonimide analog of 11,12-epoxyeicosatrienoic acid. *J. Hypertension* **19**, 983–992.
129. Karara, A., S. Wei, S.D. Spady, L. Swift, J.H. Capdevila, and J.R. Falck (1992). Arachidonic acid epoxygenase: Structural characterization and quantification of epoxyeicosatrienoates in plasma. *Biochem. Biophys. Res. Commun.* **182**, 1320–1325.
130. Capdevila, J.H., Y. Jin, A. Karara, and J.R. Falck (1993). Cytochrome P450 epoxygenase dependent formation of novel endogenous epoxyeicosatrienoyl-phospholipids. In S. Nigam, L.J. Marnett, K.V. Honn, and T.L. Walden Jr. (eds), *Eicosanoids and Other Bioactive Lipids in Cancer, Inflamation and Radiation Injury*. Kluwer Academic Publishers, Boston, MA, pp. 11–15.
131. Zou, A.P., J.T. Fleming, J.R. Falck, E.R. Jacobs, D. Gebremedhin, and D.R. Harder *et al.* (1996). 20-HETE is an endogenous inhibitor of the large-conductance Ca^{+2}-activated K^{+} channel in renal arterioles. *Am. J. Physiol.* **270**, R228–R237.
132. Su, P., K.M. Kaushal, and D.L. Kroetz (1998). Inhibition of renal arachidonic acid omega-hydroxylase activity with ABT reduces blood pressure in the SHR. *Am. J. Physiol.* **275**, R426–R438.
133. Wang, M.H., F. Zhang, J. Marji, B.A. Zand, A. Nasjletti, and M. Laniado-Schwartzman (2001). CYP4A1 antisense oligonucleotide reduces mesenteric vascular reactivity and blood pressure in SHR. *Am. J. Physiol.* **280**, 255–261.
134. Ma, Y.H., M.L. Schwartzman, and R.J. Roman (1994). Altered renal P-450 metabolism of arachidonic acid in dahl salt-sensitive rats. *Am. J. Physiol.* **267**, R579–R589.
135. Zhou, A.P., H.A. Drummond, and R.J. Roman (1996). Role of 20-HETE in elevating loop chloride reabsorption in Dahl SS/Jr. rats. *Hypertension* **27**, 631–635.
136. Makita, K., K. Takahashi, A. Karara, H.R. Jacobson, J.R. Falck, and J.H. Capdevila (1994). Experimental and/or genetically controlled alterations of the renal microsomal cytochrome P450 epoxygenase induce hypertension in rats fed a high salt diet. *J. Clin. Invest.* **94**, 2414–2420.
137. Nakagawa, K., J.S. Marji, M.L. Schwartzman, M.R. Waterman, and J.H. Capdevila (2003). The androgen-mediated induction of kidney arachidonate hydroxylases is associated with the development of hypertension. *Am. J. Physiol.* **284**, R1055–R1062.
138. Kroetz, D.L., P. Yook, P. Costet, P. Bianchi, and T. Pineau (1998). Peroxisome proliferator-activated receptor α controls the hepatic CYP4A induction adaptative response to starvation and diabetes. *J. Biol. Chem.* **273**, 31581–31589.
139. Corton, J.C., L-Q. Fan, S. Brown, S.P. Anderson, C. Bocos, and R.C. Cattley *et al.* (1998). Down-regulation of cytochrome P450 2C family members and positive acute-phase response gene expression by peroxisomal proliferator chemicals. *Mol. Pharmacol.* **54**, 463–473.
140. Shatara, R.K., D.W. Quest, and T.W. Wilson (2000). Fenofibrate lowers blood pressure in two genetic models of hypertension. *Can. J. Physiol. Pharmacol.* **78**, 367–371.
141. Roman, R.J., Y.H. Ma, B. Frohlich, and B. Markham (1993). Clofibrate prevents the development of hypertension in Dahl salt-sensitive rats. *Hypertension* **21**, 985–988.