Cytochrome P450 Enzymes in the Bioactivation of Polyunsaturated Fatty Acids and Their Role in Cardiovascular Disease

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Abstract

Various members of the cytochrome P450 (CYP) superfamily have the capacity of metabolizing omega-6 and omega-3 polyunsaturated fatty acids (n-6 and n-3 PUFAs). In most mammalian tissues, CYP2C and CYP2J enzymes are the major PUFA epoxygenases, whereas CYP4A and CYP4F subfamily members function as PUFA hydroxylases. The individual CYP enzymes differ in their substrate specificities as well as regio- and stereoselectivities and thus produce distinct sets of epoxy and/or hydroxy metabolites, collectively termed CYP eicosanoids. Nutrition has a major impact on the endogenous CYP-eicosanoid profile. "Western diets" rich in n-6 PUFAs result in a predominance of arachidonic acid-derived metabolites, whereas marine foodstuffs rich in n-3 PUFAs shift the profile to eicosapentaenoic and docosahexaenoic acid-derived metabolites. In general, CYP eicosanoids are formed as second messengers of numerous hormones, growth factors and cytokines regulating cardiovascular and renal function, and a variety of other physiological processes. Imbalances in the formation of individual CYP eicosanoids are linked to the development of hypertension, myocardial infarction, maladaptive cardiac hypertrophy, acute kidney injury, stroke

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Abbreviations: *AA* arachidonic acid, *ALA* alpha-linolenic acid, *COX* cyclooxygenase, *CYP* cytochrome P450, *DHA* docosahexaenoic acid, *EDHF* endothelium-derived hyperpolarizing factors, *EDP* epoxydocosapentaenoic acid, *EEQ* epoxyeicosatetraenoic acid, *EET* epoxyeicosatrienoic acid, *EPA* ecosapentaenoic acid, *HDoHE* hydroxydocosahexaenoic acid, *HEPE* hydroxyeicosapentaenoic acid, *HETE* hydroxyeicosatetraenoic acid, *HETrE* hydroxyeicosatrienoic acid, *I/R* ischemia-reperfusion, *KO* knockout, *LA* linoleic acid, *LOX* lipoxygenase, *PLA2* phospholipase A2, *PUFA* polyunsaturated fatty acid, *ROS* reactive oxygen species, *sEH* soluble epoxide hydrolase, *SHR* spontaneously hypertensive rat, *TAC* transverse aortic constriction, *TG* transgene, WT wild-type.

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and inflammatory disorders. The underlying mechanisms are increasingly understood and may provide novel targets for the prevention and treatment of these disease states. Suitable pharmacological agents are under development and first proofs of concept have been obtained in animal models.

Keywords
Arachidonic acid • Eicosapentaenoic acid • Docosahexaenoic acid • Hydroxylases • Epoxygenases • Hypertension • Ischemia/reperfusion injury • Cardiac hypertrophy

6.1 Introduction: Discovery of the Third Branch of the Arachidonic Acid Cascade

The discovery chain leading to our current understanding of the pivotal role of cytochrome P450 (CYP) enzymes in the generation of biologically active metabolites of polyunsaturated fatty acids (PUFAs) was initiated with a series of seminal findings in the 1980s. In 1981, three laboratories demonstrated that liver and kidney microsomal as well as purified CYP enzymes catalyzed the oxygenation of arachidonic acid (AA; 20:4 n-6) [1–4]. Structural characterization of the metabolites indicated that CYP enzymes can metabolize AA via three reaction types [5, 6] (Fig. 6.1): (1) allylic oxidation to form cis,trans-conjugated "mid-chain" hydroxyeicosatetraenoic acids (5-, 8-, 9-, 11-, 12- and 15-HETE); (2) hydroxylation at or near the terminal methyl group (ω-/(ω−1)-hydroxylase reaction) yielding 20-, 19-, 18-, 17- and 16-HETE; and (3) olefin epoxidation (epoxygenase reaction) generating four regioisomeric epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12- and 14,15-EET), each of which can be formed as either the *R*,*S* or the *S*, *R* enantiomer.

Subsequent studies revealed the presence of EETs as endogenous constituents in rat liver, rabbit kidney, and human urine providing the first proof for an active role of CYP enzymes in AA metabolism under in vivo conditions [7–10]. The biological tissues contained unique sets of regio- and stereoisomeric EETs substantiating the enzymatic origin of these metabolites and stimulating the search for the individual CYP enzymes involved in the regio- and enantioselective epoxidation of endogenous AA pools [11] (compare Sect. 6.2). Moreover, it became clear that CYP enzymes require free AA as a substrate suggesting that phospholipase-mediated AA release from membrane phospholipids provides the starting point for the formation and action of CYP-dependent AA metabolites under in vivo conditions (compare Sect. 6.3). Phospholipase activation is a common feature of the receptor-mediated actions of numerous vasoactive hormones, growth factors and cytokines. Accordingly, CYP-dependent AA metabolites were increasingly recognized to function as "second messengers". This concept became the key for our present understanding of how PUFA-metabolizing CYP enzymes are integrated into the regulation of a wide variety of physiological and pathophysiological processes.

Early studies on the potential physiological roles of CYP-dependent AA metabolites revealed effects on renal salt reabsorption and vascular tone and led to the hypothesis that alterations in the formation of 20-HETE and EETs contribute to the pathophysiology of hypertension [12]. Actually, it was then that the results obtained with two animal models of genetic hypertension until now have shaped our thinking and research about the renal and cardiovascular functions of 20-HETE and EETs

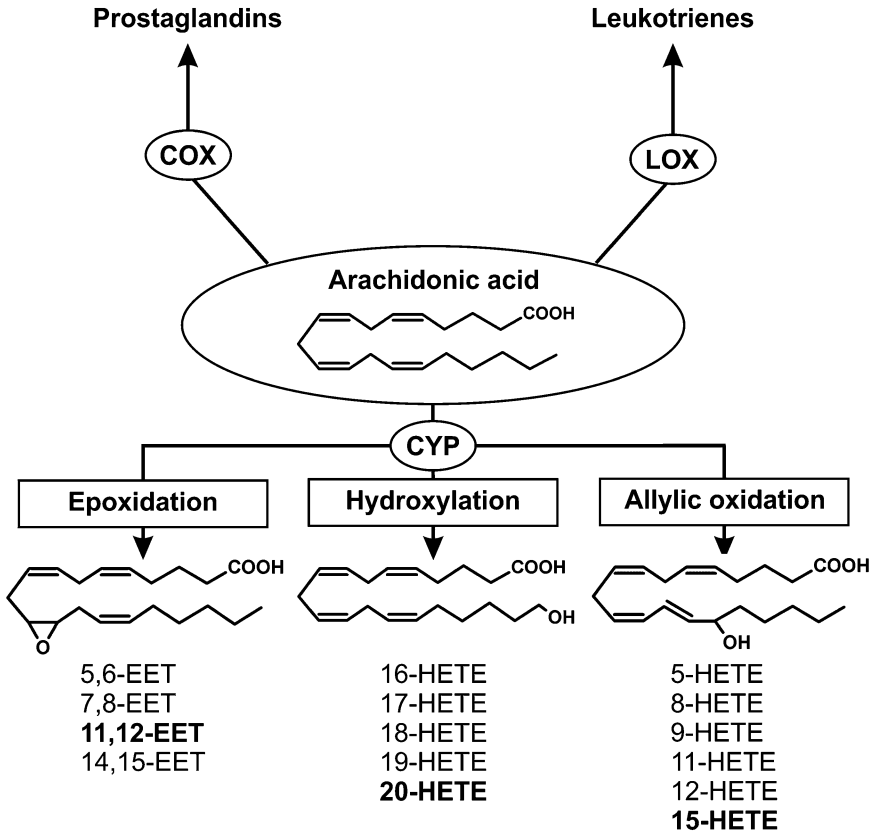

Fig. 6.1 Bioactivation of arachidonic acid (*AA*). Cyclooxygenases (*COX*), lipoxygenases (*LOX*), and CYP enzymes initiate the production of biologically active AA metabolites. CYP enzymes are able to metabolize AA by three different reaction types. Olefin epoxidation results in formation of regioisomeric epoxyeicosatrienoic acids (*EETs*). Hydroxylation at or near the terminal methyl group generates hydroxyeicosatetraenoic acids (*HETEs*) and allylic oxidation produces cis,trans-conjugated "mid-chain" HETEs (compare Sect. 6.1). The individual CYP enzymes involved in AA metabolism differ in their reaction specificities as well as regio- and stereoselectivities and thus produce enzyme specific sets of hydroxy- and epoxy metabolites (compare Sect. 6.2)

(compare Sect. 6.4). A study published in 1989 demonstrated that increased renal 20-HETE production contributes to the elevation of blood pressure in spontaneously hypertensive rats (SHR) [13]. Later on, the development of hypertension in salt-sensitive Dahl rats was attributed to the inability of this strain to upregulate renal EET biosynthesis in response to salt loading [14]. In conclusion, it appeared that hypertension can be caused by an imbalance of pro-(20-HETE) and antihypertensive (EETs) AA metabolites produced by CYP hydroxylases and CYP epoxygenases, respectively. Challenging this simplified view, a series of further studies showed that Dahl salt-sensitive rats exhibit not only a deficiency in EET formation but also in renal CYP hydroxylase expression and 20-HETE production [15]. The apparent paradox was solved after recognizing that the prohypertensive role of 20-HETE is related to its action as a potent vasoconstrictor in the vascular system of the kidney, whereas the antihypertensive role of 20-HETE is based on its capacity to inhibit sodium reabsorption in

different segments of the nephron [16]. In contrast to the dual and site-specific role of 20-HETE, the vascular and tubular actions of EETs are apparently unidirectional and antihypertensive because they promote both vasodilation and salt excretion [16, 17].

Taken together, these early biochemical and pathophysiological studies established the CYP-dependent formation of biologically active hydroxy- and epoxymetabolites of AA as the so-called "third branch of the AA cascade" complementary to the previously discovered cyclooxygenase (COX) and lipoxygenase (LOX) initiated pathways of prostanoid and leukotriene formation [18] (Fig. 6.1). Collectively, the AA metabolites produced via all three pathways as well as nonenzymatic reactions are termed eicosanoids (from Greek eicosa = twenty, reflecting that these metabolites are derivatives of a 20 carbon fatty acid). In general and also in the present review, the term eicosanoid is used more broadly to also include related metabolites derived from other PUFAs. Currently, over a hundred different eicosanoids have been identified and the analysis of their specific biological functions has remained a highly active area of research [19].

Noteworthy in the historical context, the CYP branch of eicosanoid formation was discovered almost 50 years after recognizing the essentiality of PUFAs in the mammalian diet, 20 years after elucidating the enzymatic formation and structure of prostaglandins [20], 10 years after aspirin-like antiinflammatory and analgesic drugs were shown to act by inhibiting prostaglandin formation [21], and shortly after understanding the biosynthetic pathway of leukotrienes and their roles in inflammation and asthma [22]. Moreover, at that time, microsomal CYP enzymes were investigated primarily because of their recognized roles in drug and xenobiotic metabolism and their corresponding importance in pharmacology and toxicology. Thus, the discovery of CYP eicosanoids and their potential roles in the pathophysiology of hypertension indicated that microsomal CYP enzymes may be involved in important biological actions beyond drug metabolism and raised the hope of finding novel mechanisms regulating cardiovascular and renal function.

6.2 Reaction and Substrate Specificity of PUFA-Metabolizing CYP Enzymes in Human, Rat and Mouse

6.2.1 CYP Enzymes Involved in 20-HETE Generation

20-HETE is produced by ω-hydroxylation of AA. The capacity of catalyzing this reaction type is widespread among members of the CYP4A and CYP4F subfamilies [17, 23]. In addition to 20-HETE, typically minor amounts of 19-HETE are also generated. The resulting 20-HETE/19-HETE ratio may range from more than 20:1 to 8:1 and is an inherent feature of the individual CYP4A and CYP4F enzymes.

In the **human**, CYP4A11 and CYP4F2 contribute to renal and hepatic 20-HETE formation [24, 25]. A functional variant of CYP4A11 characterized by phenylalanine-to-serine substitution at amino acid position 434 is associated with essential hypertension (T8590C polymorphism of the CYP4A11 gene, compare Sect. 6.4.1) [26]. CYP4A22, the only other member of the human CYP4A subfamily, lacks hydroxylase activity, presumably due to an amino acid substitution at position 130 that is occupied by glycine in all other CYP4A enzymes but by serine in CYP4A22 [26]. However, recently discovered genetic polymorphisms of CYP4A22 include potential gain-of-function mutations (Gly130Ser) making this gene of particular interest for understanding interindividual differences in 20-HETE production [27, 28]. CYP4F3, originally identified as leukotriene B4 (LTB4) ω-hydroxylase in human blood cells, is a further interesting candidate for the production of 20-HETE in man. Alternative splicing of the CYP4F3 pre-mRNA occurs in the liver, kidney and other tissues resulting in a shift of substrate specificity of the mature enzyme from LTB4 to AA [29, 30]. In vitro, the corresponding CYP4F3B variant displayed significantly higher

AA ω-hydroxylase activity than CYP4A11 and CYP4F2 [31].

Recently, CYP2U1, a human CYP enzyme specifically expressed in the thymus and brain, was shown to function as an ω- and (ω−1)-hydroxylase of AA and other PUFAs [32], indicating that also CYP enzymes beyond the CYP4A and CYP4F subfamily members can contribute to 20-HETE production, in particular, in less investigated tissues and physiological conditions. However, exciting novel results can also be expected identifying the endogenous substrates and reaction specificities of CYP4V2 and CYP4F12, the still "orphan" members of the human CYP4 family. Polymorphisms in CYP4V2 and CYP4F12 genes are associated with ocular (Bietti's crystalline corneoretinal dystrophy) and skin disease (lamellar ichthyosis), respectively [33].

The **rat** genome encodes four members of the CYP4A subfamily. Among them, CYP4A1 is the most active AA ω-hydroxylase followed by CYP4A2, CYP4A3 and CYP4A8 [34, 35]. Rat CYP4F enzymes shown to generate 20-HETE include CYP4F1 and CYP4F2 [36]. Based on protein expression data and immunoinhibition experiments, it has been suggested that CYP4A1 is the major AA ω-hydroxylase in the rat heart and kidney, whereas CYP4A2 and/or CYP4F1/4 are the major 20-HETE producing enzymes in the rat lung and liver [37]. In the rat kidney, CYP4A1, CYP4A2 and CYP4A3 are expressed both in different segments of the nephron and in preglomerular arterioles [17, 38]. CYP4A8 was specifically localized to the renal and cerebral vasculature, where its enhanced expression is associated with androgen-induced hypertension in the normal rat and the severity of ischemic stroke in SHR, respectively [39, 40].

Compared to human and rat, the **mouse** genome contains the most extended cluster of CYP4A genes (http://drnelson.uthsc.edu/4ABX.2005.rat.pdf). The individual genes are located within the so-called Cyp4abx cluster on chromosome 4 [41]. Among the functional Cyp4a enzymes identified, Cyp4a12a is the predominant 20-HETE generating enzyme in the kidney of male mice [42]. In comparison, Cyp4a10 that is expressed in both male and female mice displays only a weak AA ω-hydroxylase activity. The female-specific Cyp4a14 lacks the ability of hydroxylating AA but shows significant ω-hydroxylase activity with lauric acid as substrate [42]. Surprisingly, Cyp4a14 gene disruption resulted in increased renal AA ω-hydroxylase activities and caused hypertension in male mice [43]. The mechanism obviously involves increased plasma androgen levels in the Cyp4a14 gene-disrupted mice followed by androgen-induced upregulation of the 20-HETE producing Cyp4a12. Subsequent studies proved that Cyp4a12a overexpression increases 20-HETE levels in preglomerular arterioles and is alone sufficient to elevate blood pressure in mice [44]. Providing a further example of the complex regulation of CYP-eicosanoid formation in mice, deletion of the Cyp4a10 gene caused salt-sensitive hypertension, associated with impaired regulation of the EET-generating Cyp2c44 and of the kidney epithelial sodium channel [45]. The mouse kidney also expresses a series of Cyp4f enzymes. However, their ability to metabolize AA has not yet been demonstrated [46].

6.2.2 CYP Enzymes Involved in EET Generation

Studies with purified or recombinant CYP enzymes demonstrated that, in particular, various members of the CYP2C and CYP2J subfamilies can function as AA epoxygenases [17, 47]. The CYP2C (compare: http://drnelson.uthsc.edu/rat2C.pdf) and CYP2J subfamilies (compare: http://drnelson.uthsc.edu/2Jrat.pdf) evolved differently in human, rat and mouse, making it difficult to identify orthologous genes and to transfer results from animal studies directly to human cardiovascular disease [41].

In the **human**, the CYP2C subfamily consists of four members (CYP2C8, CYP2C9, CYP2C18 and CYP2C19) and there is only a single CYP2J gene (CYP2J2). All corresponding CYP enzymes are able to produce EETs but differ in their

catalytic activities, regio- and stereoselectivities as well as tissue specificities of expression. CYP2C8 and CYP2C9 have been considered as the major source of EETs in the human kidney and liver [48]. CYP2C8 generates 11,12- and 14,15-EET in a ratio of about 1.25:1 and preferentially produces the *R*,*S* enantiomers of both metabolites with a selectivity greater than 80 % [49, 50]. In porcine coronary arteries, antisense oligonucleotides downregulating a CYP2C8-related enzyme decreased bradykinin-induced EET formation and vascular relaxation [51]. This experiment provided direct evidence for the involvement of CYP2C enzymes in vascular EET formation and confirmed the concept that EETs function as endothelium-derived hyperpolarizing factors (EDHF) in various vascular beds [52–54]. Endothelial-specific overexpression of CYP2C8 lowers blood pressure and attenuates hypertension-induced renal injury in mice [55]. Surprisingly, the same CYP2C8 transgenic mice are more susceptible to myocardial infarction injury than wild-type (WT) mice [56]. This detrimental effect was explained by CYP2C8-mediated enhanced formation of reactive oxygen species (ROS) and cardiodepressive linoleic acid (LA) metabolites [56]. Compared to CYP2C8, CYP2C9 is less regio- and stereoselective and metabolizes AA to mixtures of 8(*S*),9(*R*)-, 11(*S*),12(*R*)- and 14(*R*),15(*S*)-EETs with optical purities of 66, 69 and 63 %, respectively [49, 50]. In addition to its ability of producing EETs, CYP2C9 was identified as a functionally significant source of ROS in coronary arteries [57]. In line with this finding, inhibition of CYP2C9 with sulfaphenazole improves endothelium-dependent, nitric oxide–mediated vasodilatation in patients with coronary artery disease [58]. The few studies with CYP2C18 and CYP2C19 show that these enzymes produce 8,9-, 11,12- and 14,15-EET [59, 60].

CYP2J2 has been identified as the major AA epoxygenase of the human heart [61] but is also expressed in other tissues including the vasculature, gastrointestinal tract and islets of Langerhans cells in the pancreas [62]. CYP2J2 generates all four regioisomeric EETs. The enzyme shows enantioselectivity in producing 14(*R*),15(*S*)-EET with an optical purity of 76 % but forms 8,9- and 11,12-EET as racemic mixtures [61]. Compared to CYP2C subfamily members, recombinant CYP2J2 displays rather weak enzymatic activities [60]. Nonetheless, transgenic mice with tissue-specific overexpression of CYP2J2 were developed as one of the most successful tools for studying the diverse beneficial effects of enhanced endogenous EET formation in cardiovascular disease [63, 64] (compare Sect. 6.4). Unlike CYP2C8 and CYP2C9, CYP2J2 is presumably not a relevant source of ROS [65].

The **rat** genome harbors 11 functional CYP2C genes. CYP2C23 has been identified as the predominant renal AA epoxygenase [66, 67]. This enzyme produces 8,9-, 11,12- and 14,15-EET in a ratio of 1:2:0.7. The enzyme shows a high degree of stereoselectivity and generates 8(*R*),9(*S*)-, 11(*R*),12(*S*)- and 14(*S*),15(*R*)-EET with optical purities of 95, 85, and 75 % [66]. CYP2C23 protein expression and activity is upregulated in the rat kidney upon excessive dietary salt intake [68]. A deficiency in CYP2C23-mediated renal EET formation is associated with the development of angiotensin II-induced hypertension and renal failure in the rat [69–71]. CYP2C11 was identified as the major AA epoxygenase in the liver of male rats [72]. However, CYP2C11 is also expressed in the heart, kidney and lung [37]. Moreover, CYP2C11 attracted particular interest as an EET-generating CYP enzyme in astrocytes and its potential role in the regulation of cerebral blood flow [73, 74]. Compared with CYP2C23, CYP2C11 is less regio- and stereoselective. CYP2C11 metabolizes AA to 8,9-, 11,12- and 14,15-EETs and also produces significant amounts of mid-chain HETEs [68].

The rat CYP2J gene cluster comprises five functional genes. Among them, CYP2J3 has been identified as a major AA epoxygenase in the heart [75]. Recombinant CYP2J3 metabolized AA to 14,15-, 11,12- and 8, 9-EETs and 19-HETE as the principal reaction products [75]. CYP2J4 is expressed in rat liver, intestine, olfactory mucosa, kidney, heart, and

lung and can contribute to EET and HETE formation in these organs [76, 77].

Analysis of the **mouse** genome indicated the presence of 15 functional Cyp2c genes. Among them, Cyp2c44 is the enzyme most closely related to rat CYP2C23 [78]. Cyp2c44 metabolizes AA primarily to 8,9-, 11,12- and 14,15-EETs in a ratio of about 1:3:1 and shows a high stereoselectivity in producing the *R*,*S*-enantiomers of 8,9- and 11,12-EET with optical purities of 95 and 94 %, respectively [78]. Cyp2c44 is expressed in the liver, kidney and adrenals. Recent studies on Cyp2c44 knockout mice revealed an important role of this enzyme in the regulation of renal tubular salt reabsorption via EET-mediated inhibition of the epithelial sodium channel (ENaC) [79]. This function of Cyp2c44-derived EETs is essential for dopamine-induced natriuresis/diuresis and for preventing sodium reabsorption and hypertension in response to high dietary potassium intake [80, 81]. Other members of the mouse CYP2C subfamily shown to metabolize AA primarily to EETs include Cyp2c29, Cyp2c38, Cyp2c39, Cyp2c50 and Cyp2c54 [82, 83]. Cyp2c37 metabolizes AA to 12-HETE [82]. Cyp2c55 produces both EETs and HETEs [83]. Cyp2c40, a major Cyp2c enzyme expressed in the murine gastrointestinal tract, produces 16-HETE > 14,15-EET ≫ 8,9-EET > 11,12-EET in a moderate stereoselective manner with preference for 16(*R*)-HETE (66 %), 14(*R*),15(*S*)-EET (62 %), 11(*S*),12(*R*)-EET (70 %) and 8(*S*),9(*R*)-EET (86 %) [84]. The biological functions of most of these murine Cyp2c enzymes have not been characterized. Cyp2c29 apparently resembles human CYP2C9 regarding its capacity of producing both EETs and ROS in the vasculature [85]. Moreover, Cyp2c29 is involved in hypoxic pulmonary vasoconstriction [86]. Recently, Cyp2c knockout mice were developed by deleting the whole Cyp2c gene cluster [87]. This model can become important for studying the in vivo functions of Cyp2c genes and for establishing transgenic mice expressing selected human CYP2C enzymes.

Seven functional CYP2J genes (Cyp2j5, Cyp2j6, Cyp2j8, Cyp2j9, Cyp2j11, Cyp2j12 and Cyp2j13) are predicted by the sequence of the mouse genome [41]. Among them, Cyp2j5 has been most extensively characterized. Recombinant Cyp2j5 metabolizes AA to 14,15-, 11,12- and 8,9-EETs and 11- and 15-HETE [88]. Renal expression of Cyp2j5 is upregulated by androgens and downregulated by estrogens [89]. Female Cyp2j5 knockout mice show reduced plasma 17β-estradiol levels and increased blood pressure that can be normalized by estrogen replacement [90]. Recombinant Cyp2j6 was inactive with AA as substrate but metabolized benzphetamine [91]. Cyp2j9 was identified as an AA (ω−1)-hydroxylase predominantly expressed in the mouse brain [92]. Recently, the remaining four members of the murine Cyp2j subfamily (Cyp2j8, Cyp2j11, Cyp2j12 and Cyp2j13) were also cloned and heterologously coexpressed with the human NADPH-CYP oxidoreductase in insect cells [93]. The recombinant enzymes metabolized AA as well as LA (18:2 n-6) to enzyme-specific sets of epoxy and hydroxy metabolites [93].

Based on the studies summarized above, members of the CYP2C and CYP2J subfamilies are clearly the first candidates when searching for the identity of AA epoxygenases involved in the generation of biologically active EETs. However, it is important to note that other CYP enzymes share this catalytic ability [47]. Providing an example, Cyp2b19, a CYP enzyme specifically expressed in mouse skin keratinocytes, metabolizes AA and generates 14,15- and 11,12-EETs, and 11-, 12- and 15-HETEs. Cyp2b19-catalyzed AA metabolism is highly stereoselective for 11(*S*),12(*R*)- and 14(*S*),15(*R*)-EET, and 11(*S*)-, 12(*R*)- and 15(*R*)-HETE [94]. Cyp2b19 is the major source of endogenous EETs in mouse skin [95] and its enzymatic action can contribute to the regulation of epidermal cornification [96]. CYP2B12 is presumably the rat homolog of murine Cyp2b19 [97]. The human epidermis expresses various genes of the CYP1–4 families including CYP2B6, but the functional counterpart to Cyp2b19 remains to be identified [98]. In contrast to murine Cyp2b19, CYP2B6, the single representative of the CYP2B subfamily in

humans, shows only a very weak AA epoxygenase activity [48]. Interestingly, however, CYP2B6 is remarkably active in epoxidizing the 14,15-and 11,12-double bonds of N-arachidonoylethanolamine (anandamide) [99]. Extending the uncertainties in predicting the reaction specificity of individual CYP enzymes solely based on their subfamily membership, human CYP1A2 also functions predominantly as an AA epoxygenase [48, 59]. Moreover, CYP2S1, one of the most recently discovered human CYP enzymes, is expressed in macrophages and metabolizes AA to EETs [100].

6.2.3 CYP Enzymes Involved in Subterminal AA Hydroxylation

The principle metabolites generated by subterminal hydroxylation are 16-, 17-, 18- and 19-HETE. Human CYP enzymes preferentially metabolizing AA to 19-HETE include CYP1A1 and CYP2E1. CYP1A1 generates 19-, 18-, 17- and 16-HETE in a ratio of 5:3:1:1.5, and also minor amounts of 14,15-EET [101]. CYP2E1 metabolizes AA predominantly to 19 and 18-HETE comprising 46 and 32 % of the total products formed [102]. CYP2E1 produces 19(*R*)- and 19(*S*)-HETE in a ratio of about 70:30 and 18(*R*)-HETE with an optical purity of essentially 100 % [102]. 19-HETE counteracts the vasoconstrictory and proinflammatory effects of 20-HETE [103], suggesting that changes in vascular CYP1A1 and CYP2E1 expression may contribute to the regulation of blood pressure. In line with this hypothesis, the SHR model of genetic hypertension shows reduced CYP2E1 expression [104]. As mentioned above, murine Cyp2j9 provides a unique example of an enzyme that almost exclusively metabolizes AA to 19-HETE [92], whereas other CYP2J subfamily members function predominantly as epoxygenases and produce 19-HETE only as a minor product. CYP4F8 and CYP4F12, two human CYP enzymes primarily involved in prostaglandin metabolism, metabolize AA by (ω−2)/(ω−3)-hydroxylation and produce 18-HETE as the main product [105]. Murine Cyp2c40 is currently the only CYP enzyme known to convert AA predominantly to 16-HETE [84].

6.2.4 CYP Enzymes Involved in the Generation of Mid-Chain HETEs

The enzymatic mechanism and biological significance of CYP-catalyzed AA conversion to mid-chain HETEs (5-, 8-, 9-, 11-, 12- and 15-HETE) is only partially understood. This class of CYP-dependent AA metabolites could be directly formed by hydroxylation with double bond migration or by bisallylic oxidation at C7, C10 or C13 followed by rearrangement to the corresponding dienols [106–108]. Mid-chain HETEs were identified as products of NADPH-dependent AA metabolism by liver microsomes as well as various recombinant CYP enzymes including CYP1A2, CYP2C8, CYP2C9 and CYP3A4 [109, 110]. In general, CYPs producing mid-chain HETEs function simultaneously as AA epoxygenases. A CYP enzyme exclusively catalyzing this reaction type has not yet been identified. Among the mid-chain HETEs generated by CYP enzymes, 12(*R*)-HETE attracted particular attention. 12-HETE can be further metabolized to a keto intermediate followed by a keto-reduction reaction yielding the dehydro-metabolite, 12-hydroxyeicosatrienoic acid (12-HETrE) [111]. 12-HETrE was detected in human tear film and follow-up studies in animal models implicated CYP4B1 and 12-HETrE as important components in corneal inflammation and neovascularization [112–114].

6.2.5 Long-Chain Omega-3 Fatty Acids as Alternative Substrates of AA Metabolizing CYP Enzymes

Traditionally, AA (20:4 n-6) has been considered as the main precursor of CYP eicosanoids. However, AA metabolizing CYP enzymes show rather broad substrate specificities and are able

to function as hydroxylases or epoxygenases with virtually all PUFAs of both the n-6 and n-3 families (Fig. 6.2) [115]. Which of the various PUFAs becomes accessible and is actually metabolized largely depends on (1) the relative abundance of the individual PUFAs; (2) the substrate specificity of the phospholipases that release free PUFAs from membrane phospholipids and thus initiate their metabolism by CYP enzymes and other eicosanoid generating oxygenases; and (3) the substrate and reaction specificities of the CYP enzymes expressed in a given tissue.

To (1) Mammals unlike plants, marine phytoplankton and nematodes are unable to produce and interconvert n-6 and n-3 PUFAs [116–118]. Accordingly, these two PUFA families are essential components of the mammalian diet and the relative abundance of individual PUFAs in the body is determined by their dietary intake and subsequent tissue-specific mechanisms of metabolism, distribution and uptake [119]. "Western diets" typically contain n-6 and n-3 PUFAs in a ratio of about 15:1, whereas the genetic constitution of our ancestors presumably evolved in a nutritional environment with an n-6/n-3 PUFA ratio of nearly 1:1 [120]. Importantly, the relative deficiency of n-3 PUFAs in the modern human diet has been linked to an increased risk of cardiovascular disease and inflammatory disorders [116, 121–123].

AA is directly available from meat and dairy products or can be synthesized from linoleic acid (LA; 18:2 n-6) that is abundant in vegetable oils (Fig. 6.2). In line with the prevalence of n-6 PUFAs in the "Western diet", AA is indeed the predominant long-chain PUFA in most organs and tissues, except the brain and retina that are able to largely maintain high levels of docosahexaenoic acid (DHA; 22:n-6) even when the diet provides only small amounts of n-3 PUFAs [124–126]. Long-chain n-3 PUFAs, such as DHA and eicosapentaenoic acid (EPA; 20:5 n-3), can be synthesized from alpha-linolenic acid (ALA; 18:3 n-6) that is contained among others in leafy green vegetables (Fig. 6.2). However, the enzymatic steps converting ALA to EPA and further to DHA have limited efficiencies in human [119]. Fish oil and other seafood are a rich direct source of EPA and DHA due to the marine food chain starting with EPA/DHA producing phytoplankton [117]. Based on the accumulating evidence showing that EPA and DHA have beneficial effects in various cardiac disorders, the use of EPA/DHA supplements is recommended for the management of patients after myocardial infarction and for the treatment of hyperlipidemia [123, 127].

To (2) Under basal conditions, AA is predominantly esterified into the sn-2 position of membrane phospholipids and thus not accessible to CYP enzymes and other eicosanoid generating oxygenases. However, free AA becomes readily available in response to extracellular stimuli that activate phospholipases A2 (PLA2) that in turn release AA from the membrane stores [128]. In most tissues, extracellular signal-induced activation of the cytosolic calcium-dependent cPLA2 initiates AA release and eicosanoid formation. EPA and DHA are also incorporated into the sn-2 position of membrane phospholipids and thereby partially replace AA. The classical cPLA2 releases AA and EPA with almost equal efficiencies but is largely inactive in liberating DHA [129]. In the brain, AA and DHA are released by different mechanisms using cPLA2 for AA and a calcium-independent phospholipase A2 (most likely iPLA2β) for DHA [130, 131]. The identity of the PLA2 enzymes releasing DHA in other tissues remains to be elucidated. Recently, the endogenous levels of oxidized PUFA metabolites were compared in the livers of iPLA2γ knockout and WT mice. Interestingly, deletion of iPLA2γ was associated with a marked decrease of DHA- but not of LA- or AA-derived CYP epoxygenase metabolites [132].

To (3) The capacity of CYP enzymes to oxidize EPA and DHA was first shown with rat renal and hepatic microsomes [133, 134]. Recent studies with recombinant CYP enzymes clearly demonstrate that, in fact, all major AA metabolizing CYP enzymes accept these n-3 PUFAs as

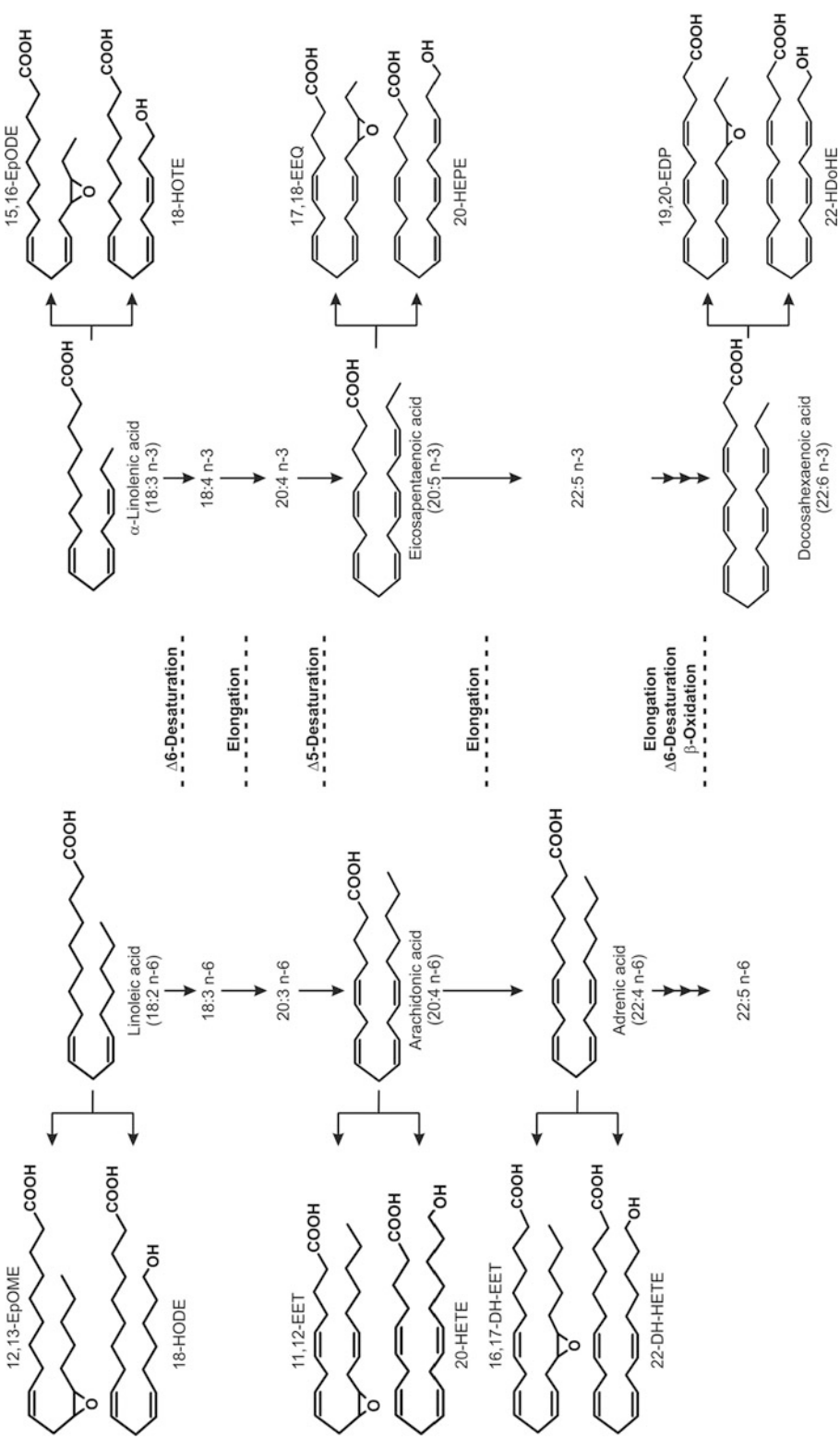

Fig. 6.2 Biosynthesis of long-chain n-6 and n-3 PUFAs. The biosynthetic pathway of long chain n-6 and n-3 polyunsaturated fatty acids provides a series of substrates that can be metabolized by CYP epoxygenases and CYP hydroxylases (For further details, compare Sect. 6.2.5)

Table 6.1 AA-metabolizing CYP enzymes known to accept the fish-oil omega-3 fatty acids EPA and DHA as efficient alternative substrates

	Main metabolites			
Enzyme	AA	EPA	DHA	Refs.
Human				
CYP1A1	19-HETE	17,18-EEQ[a], 19-HEPE	19,20-EDP[a]	[59, 101, 137]
CYP1A2	11,12-EET	17,18-EEQ[a]	19,20-EDP[a]	[59, 137]
CYP2C8	11,12-EET, 14,15-EET	17,18-EEQ[b], 14,15-EEQ	19,20-EDP[b]	[59, 60, 136, 137]
CYP2C9	14,15-EET, 11,12-EET 8,9-EET	14,15-EEQ, 17,18-EEQ[a], 11,12-EEQ	10,11-EDP	[59, 60, 136, 137]
CYP2C18	8,9-EET, 11,12-EET, 14,15-EET	11,12-EEQ, 17,18-EEQ	19,20-EDP	[59]
CYP2C19	14,15-EET, 19-HETE	17,18-EEQ[a], 19-HEPE	7,8-EDP, 10,11-EDP, 19,20-EDP[a]	[59, 60, 137]
CYP2E1	19-HETE	19-HEPE, 17,18-EEQ[a]	21-HDoHE, 19,20-EDP[a]	[31, 59, 60, 137]
CYP2J2	14,15-EET	17,18-EEQ[a]	19,20-EDP[a]	[59, 60, 137]
CYP4A11	20-HETE	19-HEPE	22-HDoHE, 21-HDoHE	[31, 60]
CYP4F2	20-HETE	20-HEPE	22-HDoHE	[31, 60]
CYP4F3A	20-HETE	20-HEPE	22-HDoHE, 21-HDoHE	[31]
CYP4F3B	20-HETE	20-HEPE	22-HDoHE	[31, 276]
CYP4F8	18-HETE		19,20-EDP,	[105]
CYP4F12	18-HETE		19,20-EDP	[105]
CYP2S1	11,12-EET, 14,15-EET	14,15-EEQ, 17,18-EEQ		[100]
Rat				
CYP2C11	11,12-EET, 14,15-EET	17,18-EEQ[a]	10,11-EDP, 19,20-EDP	[60, 136]
CYP2C23	11,12-EET	17,18-EEQ[a]	10,11-EDP	[60, 136]
CYP4A1	20-HETE	19-HEPE, 20-HEPE, 17,18-EEQ[a]		[135]
Mouse				
Cyp4a12a	20-HETE	20-HEPE, 17,18-EEQ[a]	22-HDoHE	[42, 60]
Cyp4a12b	20-HETE	17,18-EEQ	20-HDoHE	[42, 60]
C. elegans				
CYP-33E2	11,12-EET, 19-HETE, 20-HETE	17,18-EEQ, 19-HEPE, 20-HEPE		[277]

[a]R,S-enantiomer

[b]S,R-enantiomer

efficient alternative substrates (Table 6.1) [31, 59, 60, 135, 136].

CYP2C and CYP2J enzymes that epoxidize AA to EETs also metabolize EPA to epoxyeicosatetraenoic acids (EEQs) and DHA to epoxydocosapentaenoic acids (EDPs). The (ω−3) double bond distinguishing EPA and DHA from AA is the preferred site of attack by most of the classical CYP epoxygenases. CYP2C enzymes are in general almost equally efficient when utilizing AA, EPA or DHA as substrates. Surprisingly, however, EPA is the clearly preferred substrate of CYP2J2, which is the predominant AA epoxygenase in the human heart. Moreover, CYP2J2 shows only a moderate regiospecificity when metabolizing AA but predominantly produces 17,18-EEQ from EPA.

CYP4A and CYP4F enzymes, hydroxylating AA to 20-HETE, metabolize EPA to 20-hydroxyeicosapentaenoic acid (20-HEPE) and DHA to 22-hydroxydocosahexaenoic acid (22-HDoHE). Human CYP4A11 is most active with EPA, whereas CYP4F2 prefers DHA over

AA and EPA [60]. CYP4A enzymes display remarkably increased (ω−1)-hydroxylase activities when metabolizing EPA or DHA instead of AA. Moreover, some of them even attack the (ω−3) double bond. For example, CYP4A11 metabolizes AA to 20-HETE and 19-HETE in a ratio of 82:18, EPA to 20-HEPE, 19-HEPE and 17,18-EEQ in a ratio of 28:62:10 and DHA to 22-HDoHE, 21-HDoHE and 19,20-EDP in a ratio of 48:44:8 [60]. Similarly, murine recombinant Cyp4a12a hydroxylates AA to 20-HETE and 19-HETE (80:20) but metabolizes EPA to 20-HEPE, 19-HEPE, and 17,18-EEQ in a ratio of 12:32:56 [42]. CYP1A1, CYP2E1 and other enzymes converting AA predominantly to 19-HETE or 18-HETE (CYP4F8 and CYP4F12) show pronounced (ω−3)-epoxygenase activities with EPA and DHA (for references, see Table 6.1).

Taken together, it can be concluded that the capacity of utilizing EPA and DHA as alternative substrates is shared by virtually all of the AA-metabolizing CYP enzymes belonging to the subfamilies 1A, 2C, 2E, 2J, 2U, 4A and 4F. The CYP enzymes generally respond to the altered double-bond structure and chain-length of their fatty acid substrates with remarkable changes in the regioselectivity and, in part, also in the type of the catalyzed oxygenation reaction. Moreover, 17,18-EEQ and 19,20-EDP, the unique epoxy metabolites of EPA and DHA, are formed with pronounced stereoselectivities [137]. CYP1A1, CYP1A2, CYP2E1, CYP2C9, CYP2C11, CYP2C19, CYP2C23 and CYP2J2 as well as murine Cyp4a12a and rat CYP4A1 preferentially generate the corresponding *R*,*S* enantiomers, whereas CYP2C8 and CYP2D6 show stereoselectivities in favor of producing 17(*S*),18(*R*)-EEQ and 19(*S*),20(*R*)-EDP [42, 135–137]. Noteworthy, CYP1A1 metabolizes AA to 19-HETE as the main product and epoxidizes EPA to 17(*R*),18(*S*)-EEQ with an optical purity greater than 98 % [101]. These substrate-dependent features of the PUFA metabolizing CYP enzymes may have important physiological implications, considering that the biological activities of CYP eicosanoids are dependent on the regio- and stereoisomeric position of their functional epoxy or hydroxy groups.

6.2.6 Effect of Dietary Omega-3 Fatty Acids on the Endogenous CYP-Eicosanoid Profile

First studies investigating the effects of marine omega-3 fatty acids (EPA and DHA) on eicosanoid formation were focused on potential changes in the production and activity of COX- and LOX-dependent metabolites. These studies were stimulated by the seminal observation in the 1970s of significantly lower myocardial infarction rates in Greenland Inuit's, who traditionally live on EPA/DHA-rich sea food, compared to Danish controls [138]. Subsequent world-wide epidemiological studies revealed the general existence of striking cardiovascular mortality differences between populations living on n-6 PUFA- versus n-3 PUFA-rich diets [121]. Giving first insight into the mechanisms that might explain the low myocardial infarction rate among Inuit's, EPA was shown to compete with AA yielding less proaggregatory (thromboxane A3 versus thromboxane A2) and less proinflammatory eicosanoids (leukotriene B5 versus leukotriene B4) via the COX- and LOX-dependent pathways [138, 139]. In contrast, prostacyclin I3, formed from EPA, acts with the same potency as vasodilator and inhibitor of platelet aggregation as its AA-derived counterpart prostacyclin I2. Indeed, a favorable shift of the thromboxane/prostacyclin ratio to a more antiaggregatory and vasodilatory state was shown in Intuits as well as in persons after long-term intake of high amounts of EPA (10–15 g/day) [140, 141]. Many of these studies were performed before the discovery of CYP eicosanoids and of other novel classes of lipid mediators, such as the resolvins, that could bring new twists in the search for EPA- and DHA-derived metabolites mediating the

Table 6.2 Effect of the dietary n-6/n-3 PUFA-ratio on the fatty acid and CYP eicosanoid profiles in different organs of the Rat

		Precursor PUFA			Epoxy metabolites			Hydroxy metabolites		
Organ	Diet	AA:	EPA:	DHA	EET:	EEQ:	EDP	HETE:	HEPE:	HDoHE
Left ventricle	n-6	87	0	13	86	0	14	42	1	57
	n-3	28	5	67	26	13	61	6	6	88
Kidney	n-6	95	0	5	93	0	7	61	0	39
	n-3	57	25	18	49	34	17	9	40	51
Cerebral cortex	n-6	45	0	55	43	0	57	41	0	59
	n-3	37	0	63	40	1	59	26	1	73
Lung	n-6	95	1	4	39	0	8	66	0	34
	n-3	34	24	42	23	36	41	8	8	84
Liver	n-6	88	0	12	82	1	17	32	0	68
	n-3	35	19	46	27	38	35	2	18	80
Pancreas	n-6	96	1	3	94	1	5	59	0	41
	n-3	28	41	31	18	55	27	2	11	87
Plasma	n-6	85	1	14	94	0	6	28	0	72
	n-3	29	23	48	15	47	38	1	13	86

Rats were fed either an n-6-rich diet or received a diet supplemented with the fish-oil n-3 PUFAs EPA and DHA for 3 weeks. Different tissues were analyzed as described previously [60]. Shown are the relative ratios of AA, EPA and DHA serving as potential CYP-eicosanoid precursors (AA:EPA:DHA) and the ratios of the corresponding epoxy- (EETs:EEQs:EDPs) and hydroxy metabolites (20-HETE:20-HEPE:22-HDoHE) formed in the different tissues

cardiovascular benefits of marine omega-3 fatty acids [142].

First evidence for the in vivo formation of EPA- and DHA-derived CYP epoxygenase metabolites was provided by the detection of EEQs and EDPs in human urine and plasma samples [143, 144]. Marked increases in the plasma levels of EPA- and DHA-derived epoxides and their vicinal diols were observed in healthy volunteers treated for 4 weeks with 4 g of an EPA/DHA-supplement [144] and in asthmatic patients who received for 3 weeks 4 g EPA + 2 g DHA per day [145]. Considering that AA remained the predominant long-chain PUFA despite EPA/DHA supplementation, these studies indicate that EPA and DHA were metabolized in vivo with significantly higher relative efficiencies compared to AA. Even without dietary intervention, the individual differences in the serum concentrations of EPA-derived CYP epoxygenase metabolites correlated well with the EPA content in red blood cells as shown in a recent study comparing the metabolite profiles in hyper- and normolipidemic humans [146].

We analyzed EPA/DHA-supplementation induced tissue-specific changes of the endogenous CYP-eicosanoid profile in the rat [60]. The animals received standard chow supplemented with 5 % sunflower oil (n-6 PUFA-rich diet), or additionally with 2.5 % OMACOR®-oil (a formulation of EPA/DHA-ethylesters containing 480 mg EPA and 360 mg DHA/g). The n-6 PUFA-rich diet resulted in a 10–20-fold excess of AA over EPA and DHA in most organs and tissues except the brain that maintained an almost 1:1 ratio of AA and DHA. In the heart, the AA content was about sevenfold higher than that of EPA + DHA. After EPA/DHA supplementation, the AA levels were generally reduced by 40–50 % and partially replaced by EPA and DHA in a tissue-specific manner. These changes in the relative PUFA levels correlated with marked changes in the endogenous CYP-eicosanoid profile (Table 6.2). For example, the ratio of EETs:EEQs:EDPs was shifted from 93:0:7 to 49:34:17 in the kidney and from 86:0:14 to 26:13:61 in the left ventricle. EPA/DHA-supplementation also modulated the endogenous formation of ω-hydroxylase

products and resulted in a tissue-specific replacement of 20-HETE for 20-HEPE and 22-HDoHE (Table 6.2). The corresponding metabolite/precursor fatty acid ratios indicate that the CYP epoxygenases expressed in the different tissues metabolized EPA with a two to fourfold higher efficiency and DHA with almost the same efficiency, compared with AA [60]. Noteworthy, 17,18-EEQ and 19,20-EDP became the predominant CYP epoxygenase metabolites in most tissues. This finding is in line with the intrinsic feature of many individual CYP enzymes to catalyze preferentially the epoxidation of the (ω−3) double bond when having access to EPA and DHA as substrates (compare Sect. 6.2.5). Moreover, also a recent study in growing piglets identified the vicinal diols of 17,18-EEQ and 19,20-EDP as the epoxygenase-derived metabolites most markedly increased upon dietary n-3 PUFA supplementation [147].

Taken together, these studies demonstrate that the formation of endogenous CYP eicosanoids is highly susceptible to changes in the dietary n-6/n-3 PUFA ratio. Thus, the traditional view that AA is the main source of biologically active epoxy and hydroxy metabolites applies primarily to human populations and laboratory animals living on n-6 PUFA-rich ("Western") diets. However, EPA and DHA may readily become superior sources of CYP-dependent eicosanoids upon n-3 PUFA supplementation or a high dietary intake of fish oil and other marine foodstuffs.

The currently known biological activities of EPA- and DHA-derived CYP metabolites partially resemble those of their AA-derived counterparts, appear in part unique or can even produce opposite effects [148]. The epoxy metabolites of all three PUFAs share vasodilatory properties. However, the potencies of EEQs and EDPs may largely exceed those of EETs in some vascular beds [135, 149]. Interestingly, Cyp1a1 knockout mice display increased blood pressure presumably due to a reduced capacity of producing vasodilatory metabolites from n-3 PUFAs [150]. Potential candidates generated by Cyp1a1 are 17,18-EEQ and 19,20-EDP that efficiently relax murine aortic segments when added at picomolar concentrations [150]. Antiinflammatory effects were first revealed for 11,12- and 14,15-EET but are also exerted by EPA epoxides as exemplified by 17,18-EEQ [151, 152]. 17,18-EEQ and 19,20-EDP inhibit the Ca^{2+}- and isoproterenol-induced increased contractility of neonatal cardiomyocytes, indicating that these metabolites may act as endogenous antiarrhythmic agents [60]. Whereas certain EET regioisomers promote tumor angiogenesis and metastasis, 19,20-EDP and other regioisomeric DHA epoxides inhibit these crucial events in cancerogenesis [153, 154]. Moreover, CYP-dependent EPA- and DHA-derived epoxy metabolites were identified as potent antihyperalgesic agents in an animal model of pain [155]. These findings suggest that EPA- and DHA-derived CYP eicosanoids may serve as mediators in a variety of beneficial effects attributed to fish oil n-3 PUFAs, such as protection against cardiovascular disease, sudden cardiac death and tumor development [123, 156].

6.3 De Novo Biosynthesis and Metabolic Fate of CYP Eicosanoids

6.3.1 CYP Eicosanoids as Second Messengers

As already discussed in Sect. 6.2.5, AA, EPA or DHA become only accessible as substrates to the CYP enzymes after being released from membrane phospholipids. In this way, in vivo generation of CYP eicosanoids is normally strictly coupled to extracellular signals that trigger the activation of phospholipases, which in turn release the potential substrates from intracellular membrane stores. Accordingly, CYP eicosanoids are typically formed as second messengers of diverse hormones, cytokines and growth factors [17]. Examples include bradykinin-induced EET formation in endothelial cells as part of the vasodilatory response [53], VEGF-induced EET formation in angiogenesis [157], and angiotensin II-induced 20-HETE formation in vasoconstriction of renal arterioles [158]. After de novo

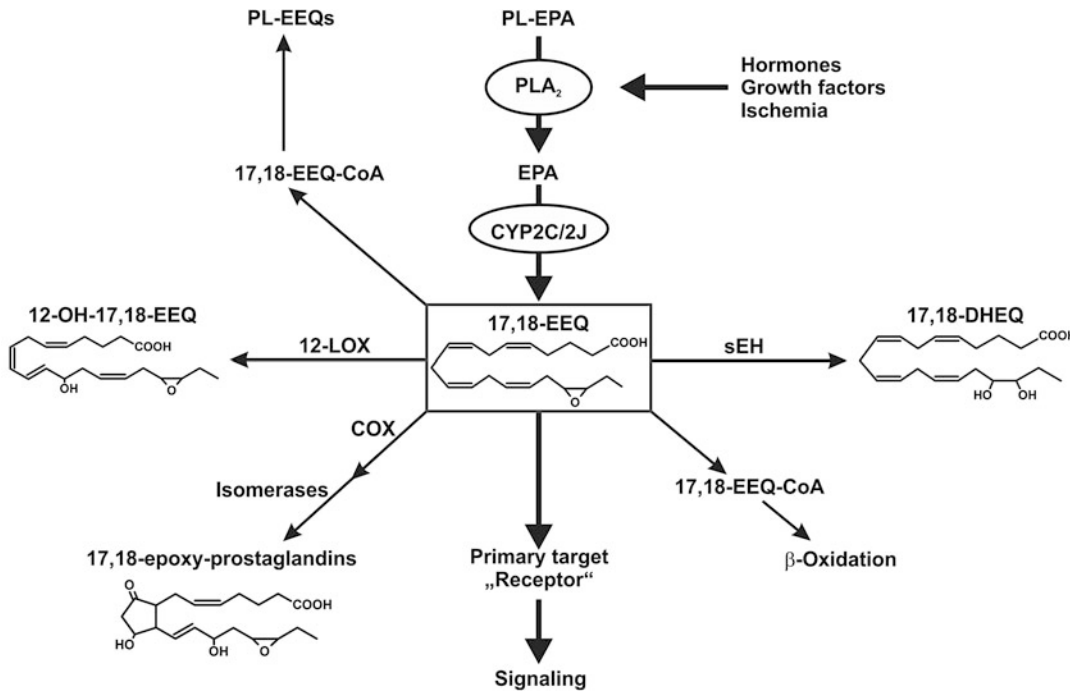

Fig. 6.3 Biosynthesis and metabolic fate of 17,18-EEQ. The biosynthesis of 17,18-EEQ is initiated by extracellular signals that activate phospholipases A2 (PLA2) in the given tissue. The PLA2 enzymes liberate eicosapentaenoic acid (*EPA*) from phospholipid (*PL*) stores and make free EPA accessible as substrate to the CYP enzymes. After its de novo synthesis, 17,18-EEQ triggers intracellular signaling pathways and can be further metabolized via various routes leading to its storage in membrane phospholipids, the formation of secondary metabolites with novel biological activities or to inactivation and degradation (For further details, see Sect. 6.3)

synthesis, CYP eicosanoids elicit cell type specific signaling pathways but are also subject to rapid further metabolism that may lead to (1) the generation of membrane pools of preformed CYP eicosanoids; (2) the formation of secondary metabolites with new biological activities; or (3) inactivation and degradation (Fig. 6.3).

6.3.2 Storage and Release

Unlike COX-dependent prostanoids, CYP-dependent hydroxy and epoxy metabolites are partially re-esterified into the sn-2 position of glycerophospholipids, generating a membrane pool of preformed CYP eicosanoids that is also accessible to PLA2 enzymes [159–161]. This unique feature of CYP eicosanoids is particularly important for their release and action in ischemia/reperfusion injury (compare Sect. 6.4).

6.3.3 Formation of Secondary Metabolites with New Biological Activities Through Actions of COX, LOX and CYP Enzymes

Several CYP eicosanoids such as 20-HETE, 5,6-EET or 17,18-EEQ still contain the double bond structure required for cyclooxygenation and can indeed serve as substrates of COX enzymes [16, 162, 163]. Depending on the COX enzymes and isomerases expressed in a given tissue, this route can result in the formation of 20-hydroxy, 5,6-epoxy or 17,18-epoxy analogs of diverse prostanoid subfamilies

including prostaglandins, prostacyclins and thromboxanes. COX-dependent 20-HETE transformation has been proposed as an important mechanism in the regulation of renal microvascular tone [164]. A recent study showed that the proadipogenic effect of 20-HETE depends on its COX-2 mediated transformation to 20-OH-PGE2 [165]. The vasoactivity of 5,6-EET is dependent on the vascular bed and may consist of a vasodilator component of the primary metabolite and a vasoconstrictor component due to COX-dependent secondary metabolite formation [166, 167].

17,18-EEQ provides a thus far unique example of a CYP epoxygenase metabolite that can be further metabolized by LOX enzymes. Recently, 8-OH, 12-OH and 15-OH-17,18-EEQ were identified as endogenous metabolites in the peritoneal fluid of mice after feeding the animals an EPA-rich diet [168]. In vitro, the individual regioisomers can be enzymatically synthesized incubating 17,18-EEQ with purified 8-LOX, 12-LOX and 15-LOX, respectively. Among them, 12(*S*)-OH-17,18-EEQ (compare Fig. 6.3) displays highly potent antiinflammatory action by limiting neutrophil infiltration in experimental murine peritonitis. In vitro, 12(*S*)-OH-17,18-EEQ inhibits neutrophil chemotaxis with an EC_{50} of 0.6 nM [168]. These remarkable findings suggest that the combined actions of CYP epoxygenases and 12-LOX are an important component of the metabolic cascade mediating the antiinflammatory effects of dietary EPA intake. Another EPA-initiated antiinflammatory pathway uses 18-HEPE as a precursor and leads to formation of the E-series of resolvins that are highly potent mediators in the resolution of inflammation [169]. The enzymatic origin of 18-HEPE may include COX (after binding aspirin) or CYP enzymes, whereby the identity of the latter remains to be clarified in humans and mammals [170].

As shown in Fig. 6.4, CYP epoxygenases and CYP hydroxylases also can cooperate in producing secondary metabolites with unique biological activities. EETs are metabolized by CYP4A and CYP4F enzymes to hydroxy EETs (HEETs) and the same class of metabolites is also efficiently produced by CYP2C-catalyzed epoxidation of 20-HETE [71, 171, 172]. Among the CYP enzymes expressed in the rat kidney, CYP4A1 preferentially hydroxylates 11,12-EET [171], whereas CYP2C23 predominantly epoxidizes the 8,9 double bond of 20-HETE [71]. Importantly, the HEETs formed via both pathways act as high-affinity ligands of the peroxisome proliferator-activated receptor alpha (PPARα) that is involved in the regulation of lipid metabolism as well as the control of inflammation [71, 171].

6.3.4 Inactivation and Degradation

EETs and related epoxy metabolites derived from other PUFAs are rapidly degraded to the corresponding vicinal diols by the soluble epoxide hydrolase (sEH) [173, 174]. This mechanism leads to a loss of most of the biological activities attributed to EETs, although the vicinal diols can show, in part, overlapping net effects. EETs incorporated into membrane phospholipids or bound in the cytosol to fatty acid binding proteins are largely protected from enzymatic hydrolysis [175, 176]. The sEH enzyme is highly expressed in all major organs and throughout the cardiovascular system [174]. Its expression is further induced by angiotensin II and thus contributes to decreased EET levels in hypertension and cardiac disease [177, 178]. Over the last decade, pharmacological inhibition of sEH-mediated EET hydrolysis became a highly active field of research with great promise for the prevention and treatment of cardiovascular disease [179] (compare Sect. 6.4).

Resembling the metabolic fate of fatty acids, CYP eicosanoids can also become subject to peroxisomal and mitochondrial β-oxidation but also to chain elongation. Thereby, partial β-oxidation as well as chain elongation may produce metabolites with novel biological activities [180].

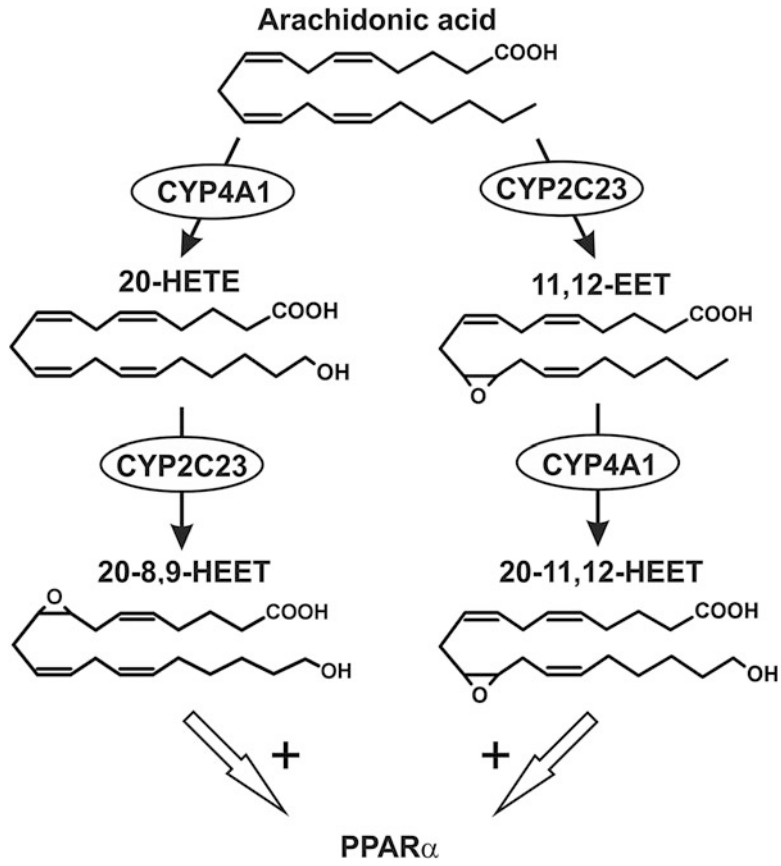

Fig. 6.4 Renal arachidonic acid (*AA*) metabolism by CYP4A1 and CYP2C23. CYP4A and CYP2C enzymes produce 20-HETE and EETs as primary products. However, they can also cooperate to generate secondary hydroxy-epoxy metabolites (HEETs) that function as high-affinity ligands of the transcription factor PPARα (For further details, compare Sect. 6.3)

6.4 CYP Eicosanoids in Cardiovascular Function and Disease

As described in the Introduction, studies in rat models of genetic hypertension led to the concept that imbalances in CYP eicosanoid formation contribute to the pathogenesis of hypertension and target organ damage. Subsequent studies proved this hypothesis in various other animal models of hypertension (see Table 6.3) and provided mechanistic insight into the partially opposing roles of EETs and 20-HETE in the regulation of vascular, renal and cardiac function [16, 17, 54, 180]. The basic concept was successfully extended and specified to a series of other disease conditions such as ischemia-induced injury of the heart, kidney and brain (Table 6.4), cardiac hypertrophy and arrhythmia (Table 6.5), inflammatory disorders, and atherosclerosis [179, 181–186].

6.4.1 Hypertension and Target Organ Damage

Trying to understand the mechanisms linking CYP-eicosanoid formation to blood pressure regulation, it is helpful to distinguish three major types of alterations in CYP-dependent AA metabolism that are associated with the development of hypertension (compare Table 6.3): (**1**) increased vascular CYP hydroxylase expression and 20-HETE formation resulting in vasoconstriction and vascular inflammation; (**2**) decreased renal tubular CYP4A expression and 20-HETE formation resulting in impaired renal function; and (**3**) decreased CYP epoxygenase and/or increased sEH expression

Table 6.3 Role of CYP eicosanoids in animal models of hypertension

Model	Genetic or pharmacological intervention	Effect	Refs.
Spontaneously hypertensive rats (SHR)	Treatment with $SnCl_2$	Inhibits 20-HETE formation and prevents the development of hypertension	[13]
	Adenovirus-mediated overexpression of CYP epoxygenases	Prevents development of hypertension	[278]
	sEH inhibition	Persistent reduction of blood pressure in female SHR when sEH inhibitor is administered in perinatal phase	[279]
Salt-sensitive hypertension	Salt-resistant Dahl rats -high dietary salt intake combined with CYP epoxygenase inhibitor or A2AR antagonist	Loss of the salt-resistant phenotype due to the inability of upregulating the adenosine-A2AR-EET axis	[14, 210, 211]
	Normal rats with high salt diet and CYP4A inhibitor	Normal rats are rendered salt-sensitive	[202]
Angiotensin II-induced hypertension	High salt diet combined with AngII-infusion in rat	Inability to upregulate renal CYP2C/EET expression is associated with hypertension and renal injury	[280]
	Rats overexpressing human renin and angiotensinogen	Reduction in renal microsomal AA epoxygenase and hydroxylase activities	[69]
	Rats overexpressing human renin and angiotensinogen treated with fenofibrate	Fenofibrate restores renal CYP2C23 expression and protects against hypertension and renal damage	[71]
	Renin transgenic rats treated with CYP4A- and/or sEH inhibitor	Attenuates the development of hypertension and target organ damage	[254]
	Pharmacologic sEH inhibition in mice	sEH-inhibition prevents and reverses angiotensin II-infusion hypertension	[212]
High fat diet and metabolic syndrome	High fat diet	Reduction of renal hydroxylase and epoxygenase activity	[281]
	Obese HO-2 KO mice treated with EET agonist or sEH inhibitor	Reduction in blood pressure and body weight gain, increased insulin sensitivity	[282]
Preeclampsia and Pregnancy	CYP-epoxygenase inhibition in rat models of preeclampsia	Amelioration of hypertension and endothelial dysfunction likely via reduced trophoblast-mediated 5,6-EET formation	[283]
	CYP epoxygenase inhibition in normal pregnancy of rat	Hypertension and impaired renal function	[284]
Androgen-induced hypertension	CYP4A inhibitor	Prevents androgen-induced 20-HETE overproduction and protects against hypertension and renal injury	[193, 194]
Cyclosporine-induced nephrotoxicity	CYP4A inhibitor	Amelioration of cyclosporine A-induced nephrotoxicity and hypertension	[285]
TG and KO mouse models of hypertension	CYP4F2 overexpression in mice	Enhanced 20-HETE production and increased blood pressure	[286, 287]
	Cyp4a14 KO and Cyp4a12 TG mice	Development of 20-HETE-dependent hypertension	[43, 288]
	Cyp2c44 KO mice	Impaired sodium reabsorption and hypertension in response to high dietary potassium intake	[81]

leading to decreased EET levels and resulting in impaired vasodilation and renal salt excretion.

Classical genetic models of hypertension or complex models of secondary hypertension such as angiotensin II-infusion hypertension show combinations of these three basic imbalances in CYP-eicosanoid formation. Moreover, these models frequently do not allow deriving the actual cause-and-effect relationships between the disease state and the associated changes in

Table 6.4 Role of CYP eicosanoids in animal models of ischemia/reperfusion injury

Model	Genetic or pharmacological intervention	Effect	Refs.
Heart			
Ex vivo global I/R in isolated rat hearts	11,12-EET, 14,15-EET, 19-HETE	Improved postischemic functional recovery after treatment with 11,12-EET, but not with 14,15-EET or 19-HETE	[75]
Left anterior descending (LAD) artery occlusion in dogs and rats	11,12-EET, 14,15-EET	Reduced infarct size	[225, 226]
LAD occlusion; ischemic preconditioning (IPC) in canine hearts	CYP ω-hydroxylase inhibitor; 20-HETE antagonist	Reduction in infarct size; synergistic beneficial effect with IPC	[222, 223]
In vivo I/R and ischemic pre- and postconditioning in rats	CYP epoxygenase inhibitor	Inhibition of CYP epoxygenase prevents the beneficial effect of postconditioning	[228]
In vivo I/R remote preconditioning of trauma (RPCT) in rats	CYP epoxygenase inhibitor; EET antagonist	Inhibition of EET formation or action abolishes the protective effects of RPCT	[229, 289]
Ex vivo global I/R in isolated mouse hearts	Cardiomyocyte- or endothelial cell-specific overexpression of CYP2J2	Improved functional recovery in mice with cardiomyocyte-, but not endothelial cell-specific overexpression of CYP2J2	[56, 182, 224]
Kidney			
Transient occlusion of renal artery and vein	CYP hydroxylase inhibitor; 20-HETE antagonist	Inhibition of 20-HETE formation or action ameliorates I/R-induced renal injury	[183]
Brain			
Middle cerebral artery occlusion (MCAO)	CYP ω-hydroxylase inhibitor	Reduction of ischemic infarct size	[234]
MCAO	sEH inhibitors in rats or sEH KO mice; Estradiol	Protective effect of estradiol is partially mediated by downregulation of cerebral sEH expression	[290]
Intracerebral hemorrhage in rats	Inhibitor of 20-HETE synthesis	Inhibition of 20-HETE synthesis reduced infarct size	[234]

Table 6.5 Role of CYP eicosanoids in animal models of cardiac hypertrophy

Model	Genetic or pharmacological intervention	Effect	Refs.
Transverse aortic constriction (TAC)	CYP2J2 TG mice	Prevention of ventricular connexin 43 delocalization and arrhythmia	[248]
	sEH inhibitor	Prevention and reversal of cardiac hypertrophy	[246]
	sEH knockout mice	Improved cardiac function	[247]
Chronic β-adrenergic stimulation by isoproterenol	CYP2J2 TG mice	Prevention of atrial fibrosis and atrial fibrillation	[248]
Angiotensin II-induced hypertrophy	sEH inhibitor	Reduction in left ventricular hypertrophy	[178]
	sEH knockout mice	Improved cardiac function	[247]
Doxorubicin-induced cardiotoxiticy	CYP2J2 TG mice	Reduced cardiotoxicity and improved cardiac function	[249]

CYP-eicosanoid formation. These problems have been partially overcome due to the recent progress in developing suitable pharmacological tools that specifically target the formation and action of 20-HETE and EETs. These tools include selective inhibitors of CYP hydroxylases [23],

CYP epoxygenases [187], and the sEH [179] as well as synthetic agonists and antagonists of 20-HETE [188, 189] and EETs [190–192]. Moreover, genetic engineering has been increasingly used to dissect the tissue-specific actions of CYP eicosanoids and to prove their significance in the development of cardiovascular disease (compare Table 6.3).

To (1) Androgen-induced hypertension provides a good example of how pharmacological and genetic interventions can be successfully combined for elucidating the prohypertensive and proinflammatory role of 20-HETE [193]. First, androgen treatment was shown to elevate blood pressure in rats. Indicating an important role of 20-HETE, androgen-induced hypertension was associated with increased vascular CYP4A expression and could be ameliorated by treating the animals with an inhibitor of CYP4A-mediated 20-HETE synthesis [194]. Moreover, adenovirus-mediated vascular overexpression of a 20-HETE generating CYP4A enzyme was alone sufficient to cause hypertension and renal injury in rats [195]. Partially explaining these in vivo observations, 20-HETE has been identified (1) as a potent vasoconstrictor by inhibiting calcium-activated potassium (BK) channels in vascular smooth muscle cells [196] and (2) to promote endothelial dysfunction by uncoupling endothelial nitric oxide synthase (eNOS) and activating the proinflammatory transcription factor NF-κB [197]. Beyond these mechanisms, 20-HETE is able to induce angiotensin-converting enzyme expression resulting in enhanced local and circulating angiotensin II-levels that contribute to the systemic prohypertensive effects of vascular 20-HETE overproduction [198, 199].

Other animal models, where hypertension may rely on similar 20-HETE-mediated mechanisms, include cyclosporine-induced hypertension in rats, androgen-induced hypertension in mice, and blood pressure elevation in Cyp4a14 knockout mice that is associated with an upregulation of androgen-inducible Cyp4a12 (for references, see Table 6.3). Increased urinary 20-HETE levels are associated with endothelial dysfunction in humans indicating that 20-HETE may also play an important role in human vascular pathophysiology [200]. Interestingly, the same study found significantly higher 20-HETE levels in men compared to women.

To (2) There are two major sites of 20-HETE generation in the kidney: (1) preglomerular microvessels where 20-HETE mediates vasoconstriction by inhibiting BK channels and (2) the renal tubule where 20-HETE promotes salt excretion by inhibiting Na^+-K^+-ATPase in proximal tubules and the Na^+-K^+-$2Cl^-$ cotransporter in the thick ascending loop of Henle [16, 17, 201]. Accordingly, renal tubular 20-HETE deficiency may contribute to the development of hypertension as first suggested based on studies with salt-sensitive Dahl rats [15]. Proving this hypothesis, treatment with a selective inhibitor of 20-HETE formation was sufficient to promote salt-sensitive hypertension in normal Sprague-Dawley rats [202]. Tubular 20-HETE deficiency is obviously also involved in the development of DOCA-salt induced hypertension in mice [203, 204] and some other animal models.

In humans, the T8590C polymorphism leads to the expression of a functional variant of CYP4A11 with reduced AA ω-hydroxylase activity [26]. Carriers of the C-allele show an increased risk of developing essential and salt-sensitive hypertension [26, 205]. Moreover, the CYP4A11 T8590C genotype was suggested to predict responses to medications that affect sodium homeostasis in hypertensive patients [206]. Functional polymorphisms exist also in the human CYP4F2 gene and were recently shown to associate with hypertension and other components of the metabolic syndrome [207].

To (3) Under physiological conditions, EETs are involved in the regulation of renal blood flow and salt excretion. EETs mediate vasodilator responses and represent the major EDHF in renal arterioles [53, 201, 208]. In distal tubules, EETs inhibit sodium reabsorption by reducing ENaC activity [79, 209]. Adenosine acting via the adenosine A2A receptor (A2AR) promotes renal EET formation in response to high dietary

salt increase. The inability to upregulate this pathway is associated with the development of salt-sensitive hypertension in Dahl salt-sensitive rats [210]. Proving the importance of the adenosine-A2AR-EET axis, salt-resistant rats are rendered hypertensive inhibiting the key components of this pathway [211].

EET deficiency caused by downregulation of CYP epoxygenases and/or upregulation of sEH is also an important feature and mediator of angiotensin II-induced hypertension (Table 6.3). For example, in double transgenic rats overexpressing the human angiotensinogen and renin genes, fenofibrate restored CYP2C23-mediated renal EET formation and prevented the development of hypertension and renal injury [71]. Pharmacological inhibition of the sEH enzyme prevented and reversed angiotensin II-infusion hypertension in mice [212]. Direct evidence for the protective role of CYP epoxygenases in angiotensin II-induced hypertension comes from recent studies using transgenic mice with endothelial specific overexpression of the human enzymes CYP2C8 and CYP2J2 [55, 64].

In humans, circulating levels of 20-HETE are increased and those of EETs are decreased in renovascular disease, whereas the urinary excretion of 20-HETE is reduced [213]. Moreover, as reviewed by other authors, genetic association studies indicate that certain functional polymorphisms in the human CYP2J2 and sEH (EPHX2) genes may be linked to an increased risk of developing hypertension, coronary artery disease and stroke [214–216].

6.4.2 Ischemia-Reperfusion Injury

Ischemia-reperfusion (I/R) induced organ damage is a common feature of myocardial infarction, acute kidney injury and stroke. Early events initiating the pathophysiological cascade include ATP depletion and Ca^{2+}-overload followed by a rapid activation of PLA2 enzymes. Ischemia-induced PLA2 activation plays a critical role in I/R injury of the heart and brain [217–219] and has also been demonstrated in the kidney [220]. PLA2 activation results in the generation of potentially toxic lyso-phospholipids as well as accumulation of free AA that in turn may trigger disturbances in eicosanoid formation in the reperfusion phase. Moreover, the activated PLA2 is able to release preformed CYP eicosanoids from their membrane stores as shown for 20-HETE in the kidney [183]. I/R-induced excessive 20-HETE formation was also shown in the heart [221]. Accumulating evidence from various animal models suggests that 20-HETE plays a major detrimental role in I/R-injury, whereas measures increasing EET formation and action exert strong protective effects (Table 6.4).

Heart First studies leading to the recognition of the detrimental role of 20-HETE in myocardial infarction were performed in dogs. In canine hearts subjected to coronary artery ligation, inhibition of endogenous 20-HETE formation reduced infarct size, whereas exogenous 20-HETE administration exacerbated the injury [222]. Moreover, inhibition of 20-HETE formation enhances the beneficial effects of ischemic preconditioning (IPC) on the severity of myocardial infarction [223].

Initiating research on the protective role of EETs in the heart, exogenous EET administration to isolated perfused hearts was found to improve postischemic functional recovery and also to prevent electrocardiogram abnormalities in the reperfusion phase [75, 224]. EET pretreatments also efficiently reduced myocardial infarction size after transient coronary artery occlusion [182, 225, 226]. Further studies revealed an essential role of EETs in mediating the beneficial effects of pre- and postconditioning [227–229]. Mimicking the effects of exogenous EET administration, cardiomyocyte-specific overexpression of human CYP2J2 in transgenic mice improved recovery of pump function, and ventricular repolarization after ischemia [63]. In line with the cardioprotective effects of EETs, pharmacological inhibition of the sEH enzyme as well as sEH

gene deletion ameliorated myocardial I/R-injury in mice [230]. Moreover, a synthetic EET analog was successfully used for protecting isolated murine hearts against global ischemia-induced loss of pump function and myocardial injury [231].

The potential mechanisms underlying the opposing roles of 20-HETE and EETs have been discussed in recent reviews [182, 232, 233]. Accordingly, the detrimental role of 20-HETE in myocardial I/R-injury is certainly multifactorial and involves vasoconstrictor and proinflammatory actions similar to those discussed above in the context of hypertension and vascular injury (compare Sect. 6.4.1). Moreover, 20-HETE induces inherent mechanisms of apoptosis in cardiomyocytes probably by inhibiting the mitochondrial ATP-sensitive potassium channel. EETs oppose the vasoconstrictor and proinflammatory action of 20-HETE and are able to induce prosurvival mechanisms in cardiomyocytes.

Brain Studies in rat models of brain I/R-injury demonstrated that blockade of 20-HETE synthesis ameliorates cerebral vasospasm following subarachnoid hemorrhage, and reduces infarct size in ischemic stroke [234, 235]. Pharmacological inhibition as well as genetic deletion of the sEH enzyme is protective in mouse models of ischemic stroke [236]. Interestingly, sex-specific expression of the sEH (male>female) has been linked to the pronounced sex difference in the extent of brain injury after cerebral artery occlusion in mice [237]. Based on these and further findings, sEH has been proposed as a novel therapeutic target in stroke [238]. A recent review gives further information on the potential mechanisms of cerebral I/R-injury that are beneficially modulated upon inhibiting 20-HETE or increasing EET levels by inhibiting the sEH [185].

Kidney I/R-induced acute kidney injury (AKI) leads to increased morbidity and mortality, particularly after cardiovascular surgery and kidney transplantation [239–241]. I/R-induced mechanisms in the kidney include persistent vasoconstriction, inflammation, endothelial dysfunction, and tubular injury [242, 243]. As analyzed in a rat model of AKI, 20-HETE released in the ischemic phase plays an important role in setting the stage for the subsequent events leading to renal failure. Inhibiting the formation or action of 20-HETE during ischemia improved the recovery of renal tissue perfusion and oxygenation in the early reperfusion phase and protected against subsequent inflammatory cell infiltration, tubular epithelial cell apoptosis and decline of renal function [183]. 20-HETE overproduction also exacerbates the cytotoxic and proapoptotic effects of chemical hypoxia on cultured primary renal tubular epithelial cells [244]. In contrast, protective effects of 20-HETE were observed in another rat model of AKI. In this model, systemic long-term inhibition of 20-HETE formation aggravated and antagonizing 20-HETE action in the reperfusion phase ameliorated renal I/R injury [184]. These apparently contradictory results probably reflect the unique dual role of 20-HETE in the kidney that unlike other organs requires 20-HETE for its normal function.

6.4.3 Cardiac Hypertrophy and Arrhythmia

Maladaptive cardiac hypertrophy is associated with structural and electrical remodeling eventually leading to heart failure and increased propensity to ventricular tachyarrhythmia and sudden cardiac death [245]. This disease may occur upon chronic pressure overload due to aortic stenosis but also develops frequently in more complex disease and stress conditions such as hypertension and myocardial infarction or adrenergic overdrive (Table 6.5).

Indicating an important protective role of CYP epoxy metabolites in pressure overload-induced cardiac hypertrophy, pharmacological sEH inhibition prevents and reverses left ventricular hypertrophy after transverse aortic constriction (TAC) in mice [246]. Pressure overload as well as angiotensin II-induced maladaptive cardiac hypertrophy is ameliorated in sEH knockout compared to wild-type mice [247]. Genetic

analysis in rats identified the sEH gene (EPHX2) as a susceptibility factor for heart failure [247]. Interestingly, some of the rat strains used for experimental studies carry an EPHX2 promoter variant that decreases basal expression of the sEH enzyme and abolishes its angiotensin II-inducibility [247]. In mice, upregulation of cardiac sEH expression was shown to be essential for angiotensin II-induced cardiac hypertrophy [178].

Direct experimental evidence for a cardioprotective role of enhanced endogenous EET biosynthesis was provided comparing the development of TAC-induced cardiac hypertrophy in CYP2J2-transgenic mice and corresponding wild-type littermates [248]. Cardiomyocyte-specific overexpression of the human CYP epoxygenase markedly improved the survival of the animals and prevented the development of ventricular tachyarrhythmia vulnerability. Reduced arrhythmia susceptibility was related to CYP2J2-mediated protection against hypertrophy-induced delocalization of left ventricular connexin-43. CYP2J2 transgenic mice also displayed improved electrical remodeling in β-adrenergic stimulation-induced cardiac hypertrophy. In this model, CYP2J2 overexpression specifically prevented the development of fibrosis and atrial fibrillation susceptibility [248]. Other studies with CYP2J2 transgenic mice demonstrate that enhanced cardiac EET biosynthesis also protects against doxorubicin-induced cardiotoxicity [249] and the development of heart failure upon long-term infusion of angiotensin II or isoproterenol [250]. In vitro, exogenous administration of 14,15-EET inhibited the hypertrophic response of cultured cardiomyocytes to isoproterenol, whereas 20-HETE was alone sufficient to induce cellular hypertrophy [251].

Taken together, these studies revealed an important role of CYP eicosanoids in the pathogenesis of cardiac hypertrophy, heart failure and arrhythmia. The mechanisms are only partially understood but obviously include opposing roles of 20-HETE and EETs in mediating or suppressing prohypertrophic, proinflammatory and proapoptotic signaling pathways in cardiomyocytes [252]. Moreover, 20-HETE and EETs modulate ion channel activities in cardiomyocytes and thus influence cardiac electrophysiology and Ca^{2+}-handling [253]. Recently, combined inhibition of 20-HETE formation and of EET degradation was shown to attenuate hypertension and cardiac hypertrophy in Ren-2 transgenic rats [254]. This study provides an example for the role of 20-HETE and EETs in conditions of severe hypertension and end-organ damage and also of the promising therapeutic potential of approaches targeting the CYP-eicosanoid pathway in such complex disease states.

6.5 Conclusions

During the last three decades, the work of many laboratories improved our understanding of the physiological and pathophysiological relevance of the CYP-eicosanoid pathway. Extending the initial discoveries showing important roles in hypertension and renal failure, novel and previously unexpected implications have been revealed in myocardial infarction, maladaptive cardiac hypertrophy, acute kidney injury and stroke. Recent progress in CYP-eicosanoid profiling as well as genetic association studies suggest that much of what has been learned from animal experiments is also relevant to human cardiovascular disease. The list is steadily growing and we have to apologize for not explicitly covering in this review many other exciting findings regarding for example the role of CYP-eicosanoids in the gastrointestinal tract [255], lung [256, 257] and liver [258]. There is also significant progress in understanding the contribution of CYP-eicosanoids to the regulation of neurohormone release and pain sensation [259, 260]. Another important avenue of research led from recognizing EETs as stimulators of insulin secretion in isolated rat pancreatic islets [261] via the identification of CYP2J2 as a major epoxygenase in this cell type [262] to the hypothesis that EETs may be the link between endothelial dysfunction and insulin resistance [263]. The most recent paper in this chain demonstrates beneficial effects of sEH inhibition on glucose

homeostasis and islet damage in a streptozotocin-induced diabetic mouse model [264].

Beyond their nowadays well-established roles in cardiovascular health and disease, CYP eicosanoids have been recently recognized as mediators of physiological and pathophysiological forms of angiogenesis [153, 265–269]. On the one hand, these novel findings improve our understanding of repair mechanisms and may open new opportunities for promoting wound healing. On the other hand, these findings indicate that alterations in the CYP-eicosanoid pathway may contribute to tumor proliferation and metastasis, age-related macular degeneration and other disease states associated with pathological angiogenesis. They also suggest that interventions into the CYP-eicosanoid pathway aimed at protecting vascular, cardiac and renal function may have detrimental side effects in promoting cancer progression. This concern was specifically raised against therapeutic strategies that increase the endogenous EET levels because, in particular, these AA-derived metabolites could function as "double-edged swords" [270].

The balance of n-6 and n-3 PUFAs in the diet has been recognized as one of the most important modifiable risk factors for the development of cardiovascular disease but also to influence cancerogenesis and pathologic neovascularization in ocular disease. Studies on the substrate and reaction specificity as well as on diet-induced changes in the endogenous CYP-eicosanoid profile clearly demonstrate that CYP enzymes do not metabolize only AA but also a wide range of other n-6 and n-3 PUFAs. In particular, the CYP-dependent metabolism of EPA and DHA generates sets of epoxy metabolites with superior vasodilatory, antiinflammatory and cardioprotective properties compared to the AA-derived counterparts. First studies also suggest that these n-3 PUFA-derived metabolites have unique biological activities in exerting antiarrhythmic effects [60] and suppressing tumor angiogenesis [154]. It is tempting to speculate but remains to be directly shown that the CYP-eicosanoid pathway mediates a variety of the beneficial effects attributed to diets rich in EPA and DHA.

However, there are also important gaps of knowledge. In particular, our understanding of the CYP-eicosanoid induced signaling pathways is incomplete and hampered by the fact that the primary cellular targets of the diverse epoxy and hydroxyl metabolites have not yet been identified. Eicosanoids generated via the COX- and LOX-dependent pathways exert their biological functions by activating G-protein coupled receptors [18]. Accumulating evidence suggests that there are also receptor-like membrane proteins specifically interacting with individual CYP eicosanoids [271–273] and it will be exciting to learn their molecular identities in the near future. Unexpected help in unraveling the components of CYP-eicosanoid mediated signaling pathways in mammals may also come from studies on PUFA-derived signaling in small animal models such as *Caenorhabditis elegans* and *Drosophila melanogaster* [118]. Indicating the existence of evolutionary conserved mechanisms, CYP-33E2, a CYP enzyme resembling the human cardiac epoxygenase CYP2J2, is expressed in *Caenorhabditis elegans* and contributes there to the regulation of pharynx activity, an organ continuously pumping in nematodes [274]. Moreover, CYP eicosanoids are obviously essential for mediating the behavioral response of *Caenorhabditis elegans* to hypoxia-reoxygenation perhaps via signaling pathways partially resembling those expressed in mammals and mediating the effects of CYP eicosanoids in ischemia-reperfusion injury of the heart, brain and kidney [275].

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