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Review

Cytochrome P450-dependent metabolism of ω-6 and ω-3 long-chain polyunsaturated fatty acids

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Abstract:
Dietary fish oil ω-3 fatty acids (n-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect against arrhythmia and sudden cardiac death using largely unknown mechanisms. EPA and DHA may serve as efficient alternative substrates of arachidonic acid (AA) metabolizing cytochrome P450 (CYP) enzymes. For many of the CYP isoforms, the n-3 PUFAs are the preferred substrates. Moreover, the CYP enzymes oxygenate EPA and DHA with largely different regioselectivities compared to AA. In particular, the ω-3 double bond that distinguishes EPA and DHA from AA is a preferred site of CYP-catalyzed epoxidation reactions. Given the pivotal role of CYP-dependent AA metabolites in the regulation of vascular, renal and cardiac functions, their replacement by unique sets of epoxy- and hydroxy-metabolites derived from EPA and DHA may have far-reaching physiological implications. The currently available data suggest that some of the vasculo- and cardioprotective effects attributed to dietary n-3 PUFAs may be mediated by CYP-dependent metabolites of EPA and DHA.

Key words:
cytochrome P450, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, eicosanoids, ω-3 fatty acids, cardiovascular disease

Cardiovascular protective effects of ω-3 fatty acids

Polyunsaturated fatty acids (PUFAs) of both the ω-6 (n-6) and ω-3 (n-3) class are essential dietary components because they are necessary for human health but cannot be synthesized *de novo* in the body. The genetic constitution of human beings likely evolved on a diet with a ratio of n-6 to n-3 PUFAs of about 1:1, whereas in recent Western diets, the typical ratio is 15:1 [80]. The general hypothesis that this imbalance increases the susceptibility to cardiovascular and other chronic diseases has been supported by numerous experimental, epidemiological and clinical studies performed over the last three decades. In particular, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), the major n-3 PUFAs contained in fish oil, have anti-inflammatory, antithrombotic, vasodilatory, hypolipidemic and antiarrhythmic properties and thus exert pleiotropic beneficial effects on the cardiovascular system [44, 73].

Protection against sudden cardiac death

The beneficial health effects of fish oil n-3 PUFAs were first recognized while searching for conditions leading to lower cardiovascular mortality in populations with traditionally high sea food intake, such as the Greenland Inuit's, compared with those living on

a typical n-6 PUFA-rich Western diet [5, 45, 46]. Later, clinical trials revealed that n-3 PUFAs reduce cardiovascular mortality predominantly by decreasing the risk of sudden cardiac death from ventricular arrhythmias [49]. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto-Prevenzione Study showed a 45% reduction in sudden cardiac death in patients with recent myocardial infarction by treatment with a daily dose of 850 mg of EPA plus DHA [1, 56]. Based on this and other positive clinical studies, certain formulations of n-3 PUFAs have received approval for clinical use and were generally recommended for cardiovascular disease patients by the American Heart Association [44]. Importantly, n-3 PUFAs also protect against atrial fibrillation in cardiac surgical settings [9] and promote plaque stabilization [90]. Additional clinical and epidemiological outcomes on the use of n-3 PUFAs are available in a series of recent reviews [27, 29, 48, 62, 94]. An extensive meta-analysis summarizing 27 studies performed prior to 2005 on the impact of n-3 PUFAs on ischemia/reperfusion (I/R)-induced cardiac arrhythmias in animal models is also available [57]. In this context, it is interesting to note that ischemic preconditioning and n-3 PUFAs are both cardioprotective and may share the same basic mechanisms proposed by a recent animal study [2]. Moreover, we found that EPA and DHA protect angiotensin (Ang) II-hypertensive rats from arrhythmia and sudden cardiac death, suggesting that the cardioprotective effects of n-3 PUFAs are not restricted to disease settings related to I/R [17].

Potential molecular mechanisms

The various beneficial effects exerted by dietary n-3 PUFAs are expressed with different dose-dependencies. The anti-thrombotic, anti-inflammatory and triglyceride-lowering effects of n-3 PUFA require relatively high doses of DHA and EPA (3 to 4 g/day), whereas the anti-arrhythmic effects and reduction of sudden cardiac death are achieved at lower doses (0.5 to 1 g/day) [48]. Recently, the so-called ω-3 index was introduced to provide a quantitative biomarker for the actual bioavailability of EPA and DHA. The index represents the ratio of EPA + DHA in percent of total fatty acids in red blood cell membranes. An evaluation of epidemiological and clinical studies estimates that an ω-3 index of less than 4% is associated with a 10-fold higher risk of sudden cardiac death in comparison with an ω-3 index greater than 8% [27, 93].

The molecular mechanisms of n-3 PUFA action are only partially understood and include changes in membrane structures, direct interactions with ion channels and transcription factors, and alterations in eicosanoid biosynthesis [8, 40, 49]. EPA and DHA compete with arachidonic acid (20:4 n-6; AA), the physiologically most important n-6 PUFA, for binding and conversion by cyclooxygenases (COX) and lipoxygenases (LOX) and thus modulate the production and bioactivity of prostanoids and leukotrienes. The importance of alterations in eicosanoid signaling was first recognized while studying the antithrombotic effects of EPA. Pioneering work performed during the late 1970s by John R. Vane, Jorn Dyerberg, Richard J. Gryglewski and others, showed that EPA inhibits the conversion of AA to thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) [14, 24, 25]. Importantly, the analogs produced from EPA show reduced pro-aggregatory properties (TXA_3 *vs*. TXA_2) but well preserved anti-aggregatory properties (PGI_3 *vs*. PGI_2). Numerous subsequent studies confirm that AA and EPA compete at all steps of prostanoid biosynthesis resulting in specific alterations in metabolite production and action [95]. Similarly, the competition between AA and EPA for the production of leukotrienes yields the first explanation for the anti-inflammatory effect of dietary n-3 PUFAs. AA is metabolized by the 5-lipoxygenase to leukotriene B_4 (LTB_4) that induces inflammation and acts as a powerful chemoattractant of neutrophils. In contrast, the same pathway yields LTB_5 from EPA, a metabolite at least 30 times less potent than LTB_4 [89]. However, recent studies reveal that the anti-inflammatory effects of EPA and DHA are not only due to an exchange of the classical AA-derived pro-inflammatory eicosanoids for their less potent n-3 counterparts. EPA and DHA are the precursors of novel lipid mediators, termed resolvins and protectins, that have highly potent anti-inflammatory and pro-resolution properties and may play an essential role in the protection against various inflammatory diseases [76].

n-3 PUFAs also interfere with the third branch of eicosanoid production that is catalyzed by cytochrome P450 (CYP) enzymes. As described in detail below, CYP-dependent AA metabolites play a pivotal role in the regulation of cardiovascular function. The same CYP isoforms that metabolize AA accept EPA and DHA as efficient alternative substrates. These interactions may have important physiological implications and provide novel insight into the mechanisms

of the vasculo- and cardioprotective effects of n-3 PUFAs.

Role of cytochrome P450-dependent arachidonic acid metabolites in the cardiovascular system

AA is oxygenated by COX, LOX and CYP enzymes to different classes of biologically active metabolites collectively termed eicosanoids. COX enzymes initiate the formation of prostaglandins and thromboxanes and LOX enzymes form leukotrienes and lipoxins [21]. These metabolites activate rhodopsin-like seven membrane-spanning G-protein coupled receptors (GPCRs) [28, 69]. The COX- and LOX-dependent pathways are clinically targeted in the treatment of inflammation, cardiovascular disease, asthma, fever and pain [21].

The CYP-catalyzed hydroxylation and epoxidation of AA was established recently as the so-called third branch of the AA cascade (for reviews see [11, 58, 71]). Members of the CYP2C and CYP2J subfamilies function as AA epoxygenases and produce isoform-specific sets of regio- and stereoisomeric epoxyeicosatrienoic acids (EETs). In contrast, CYP4A and CYP4F isoforms are AA ω/(ω-1)-hydroxylases and produce 20-hydroxyeicosatetraenoic acid (20-HETE) as their main product. EETs and 20-HETE are second messengers of various hormones and growth factors and play partially opposing roles in the regulation of vascular, renal and cardiac function. Whether EETs and 20-HETE initiate signaling through specific membrane receptors is largely unclear. Analogous to the mode of action of prostaglandins and leukotrienes, the existence of a novel "GPCR-family" may be postulated whose members show distinct preferences for the binding of individual CYP-eicosanoids. In favor of this hypothesis, U937 cells express a surface high-affinity binding site for 14,15-EET [104].

Physiological and pathophysiological roles of EETs

In general, EETs are involved in antihypertensive and organ-protective mechanisms [85]. EETs are the major endothelium-derived hyperpolarizing factor (EDHF) in several vascular beds [10, 18] but may mediate vasodilation also by activating endothelial nitric oxide synthase (eNOS) [31, 96] or by modulating other agonist-induced mechanisms in endothelial cells [20, 53]. EETs promote renal salt excretion [88]. EETs also mediate anti-inflammatory and anti-apoptotic mechanisms that protect against cell and organ injury [13, 68, 103]. Moreover, EETs promote the growth of endothelial cells but inhibit the migration of vascular smooth muscle cells indicating an important role in vascular healing and in the prevention of uncontrolled vascular remodeling [19, 86]. Substantiating the physiological relevance of EET formation, hypertension and end-organ damage are associated with EET-deficiency and can be ameliorated by increasing the EET levels in various animal models [35, 51, 64, 71].

The soluble epoxide hydrolase as a novel target to treat cardiovascular disease

EETs are rapidly metabolized to corresponding dihydroxyeicosatrienoic acids (DHET) by the soluble epoxide hydrolase (sEH), an enzyme widely expressed in the cardiovascular system [37]. Expression of the sEH gene (EPHX2) is induced by angiotensin (Ang) II in the vasculature and in the heart [3, 4], providing a rationale for sEH-activity inhibition in Ang II-induced hypertension and cardiac hypertrophy. Recently developed sEH-inhibitors lower blood pressure in Ang-II hypertensive rats and mice as well as in spontaneously hypertensive rats (SHR) [36, 41, 106]. Even desoxycorticosterone acetate (DOCA)-salt hypertension is ameliorated with sEH inhibition [52]. Cardiac hypertrophy is prevented in both Ang II-induced and pressure-overload models treated with sEH inhibitors [4, 102]. Moreover, these compounds are also effective in protecting the heart and brain against experimental ischemic injury [61, 81] and the lung against smoke-induced inflammation and monocrotaline-induced pulmonary vascular remodeling and hypertension [70, 84].

Despite the amazing beneficial effects of sEH inhibitors, whether the effects are due to enhanced tissue EET levels or whether they are, in part, related to off-target actions or to unknown sEH-dependent mechanisms remains unclear. Moreover, concerns about potential adverse effects of sEH inhibitors merit increased attention: (i) EETs are known to mediate hypoxic pulmonary vasoconstriction [43], (ii) EETs promote tumor growth and metastasis [39], and (iii)

Ephx2 knockout mice show reduced survival after cardiac arrest and resuscitation [34]. Confirming an important role of sEH in the development of maladaptive cardiac hypertrophy, we found in collaboration with other groups, that Ephx2-knockout mice were protected from Ang II- and pressure overload-induced heart failure and cardiac arrhythmias [59]. Thus, in the case of cardiac hypertrophy, pharmacological sEH-inhibition and Ephx2 gene disruption yielded the same beneficial effect.

Physiological and pathophysiological roles of 20-HETE

20-HETE is involved in both pro- and antihypertensive mechanisms, depending on the site of its formation [58]. When produced in the vasculature, 20-HETE is a potent endogenous vasoconstrictor. Moreover, vascular overproduction of 20-HETE induces endothelial dysfunction and hypertension by enhancing vasoconstriction, uncoupling of eNOS, and activation of the pro-inflammatory transcription factor NF-κB [12, 38, 97]. These features of 20-HETE action contribute to the development of hypertension and end-organ damage in several animal models including spontaneously hypertensive rats, and androgen- and cyclosporin A-hypertensive rats [7, 82, 83]. A second major site of 20-HETE production is the renal tubular system. Here, 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 [58, 71]. A deficiency in tubular 20-HETE formation may be associated with hypertension as exemplified by Dahl salt-sensitive rats [72], DOCA-salt hypertensive mice [33] and the development of salt-sensitive hypertension in normal rats treated with a CYP4A inhibitor [32].

Role of CYP-eicosanoids in the regulation of cardiac function

In the heart, EETs regulate L-type Ca^{2+}-, ATP-sensitive potassium (K_{ATP}) and Na^+-channels [50, 54, 100]. In isolated cardiomyocytes, 11,12-EET induces Ca^{2+} influx and contraction *via* a cAMP/PKA-dependent mechanism [99]. In isolated hearts, 11,12-EET perfusion prior to global ischemia improves functional cardiac recovery in the subsequent reperfusion phase. Moreover, transgenic mice with heart-specific overexpression of the human cardiac EET-synthase (CYP2J2) show improved functional recovery after ischemia/reperfusion (I/R) [77]. Cardiomyocytes isolated from these animals display enhanced L-type Ca^{2+} currents [101]. Activation of K_{ATP} channels is essential for improved functional recovery after I/R as shown by pharmacological inhibition experiments in CYP2J2 transgenic mice. Moreover, activation of p42/p44 mitogen activated protein kinases contributes to the improved recovery in this model [77]. Recent results demonstrate that EETs have cardioprotective properties beyond functional recovery [79]. In canine hearts, perfusion with 11,12- and 14,15-EET significantly reduces the infarct size after I/R. This effect is blocked by K_{ATP} inhibition [23]. The beneficial effects of EETs are not shared by their sEH-mediated hydrolysis products, DHETs, as shown *in vitro* for infarct size reduction in isolated hearts. Moreover, recent studies also reveal improved cardiac functional recovery after I/R in sEH-knockout compared with wildtype mice. This effect was inhibited by the EET antagonist 14,15-EEZE and by K_{ATP} channel inhibitors [78].

20-HETE shows detrimental effects under the same conditions. I/R-induced myocardial infarction is significantly ameliorated in canine and rat hearts by pretreatments with CYP4A inhibitors or 20-HETE antagonists [22, 67]. In contrast, infarct size is increased by 20-HETE pretreatment. The protective effect of 20-HETE inhibition is abolished by pharmacological blockade of the sarcolemmal K_{ATP} channel. The beneficial effect of preconditioning is also enhanced by CYP4A inhibition [66]. Taken together, these results demonstrate a strong negative role of 20-HETE and an important beneficial role of EETs in myocardial infarction. The essential components of EET-mediated cardioprotection are probably sarcolemmal and mitochondrial K_{ATP} channels.

Cytochrome P450-dependent metabolism of EPA and DHA

EPA and DHA can partially replace AA at the sn-2 position of glycerophospholipids and may become accessible to CYP enzymes in the same way as AA after extracellular signal-induced activation of phospholipases (Fig. 1). However, the possibility that EPA and DHA may indeed serve as alternative substrates of

AA metabolizing CYP isoforms has been recognized only recently. Early studies performed in the 1980s revealed that liver and renal microsomal CYP enzymes convert EPA and DHA, like AA, by epoxidation and hydroxylation [91, 92]. The principal CYP-dependent metabolites derived from EPA include 5 regioisomeric epoxyeicosatetraenoic acids (EETeTrs) and ω/(ω-1)-hydroxyeicosapentaenoic acids (19- and 20-HEPE), whereas DHA can be metabolized to 6 regioisomeric epoxydocosapentaenoic acids (EDPs) and ω/(ω-1)-hydroxydocosahexaenoic acids (21- and 22-HDoHE) (Fig. 1). Each of the major EET- and 20-HETE generating CYP isoforms in human, rat and mouse accept EPA and DHA as efficient alternative substrates. In the majority of CYP isoforms, EPA is the preferred substrate and DHA is metabolized with similar rates compared to AA. Moreover, as discussed in detail below, the CYP enzymes respond to the altered double bond structure and chain-length of their fatty acid substrates with changes in the regio- and stereoselectivity of product formation (Fig. 2).

CYP1A1 and CYP2E1

Human CYP1A1 functions predominantly as a subterminal hydroxylase when metabolizing AA and produces 19-HETE, followed by 18-, 17-, and 16-HETE, as main products. In addition, small amounts of EETs, mainly 14,15-EET, account for about 10% of the total product. In contrast, CYP1A1 acts both as an epoxygenase and ω-1 hydroxylase when converting EPA and produces 17,18-EETeTr and 19-OH-EPA, representing 68 and 31% of total metabolites, respectively [75]. Remarkably, EPA is epoxidized by CYP1A1 with almost absolute regio- and stereoselectivity to 17(*R*),18(*S*)-EETeTr. Similarly, with DHA as substrate, CYP1A1 exclusively epoxidizes the ω-3 double bond and produces 19,20-EDP as the main prod-

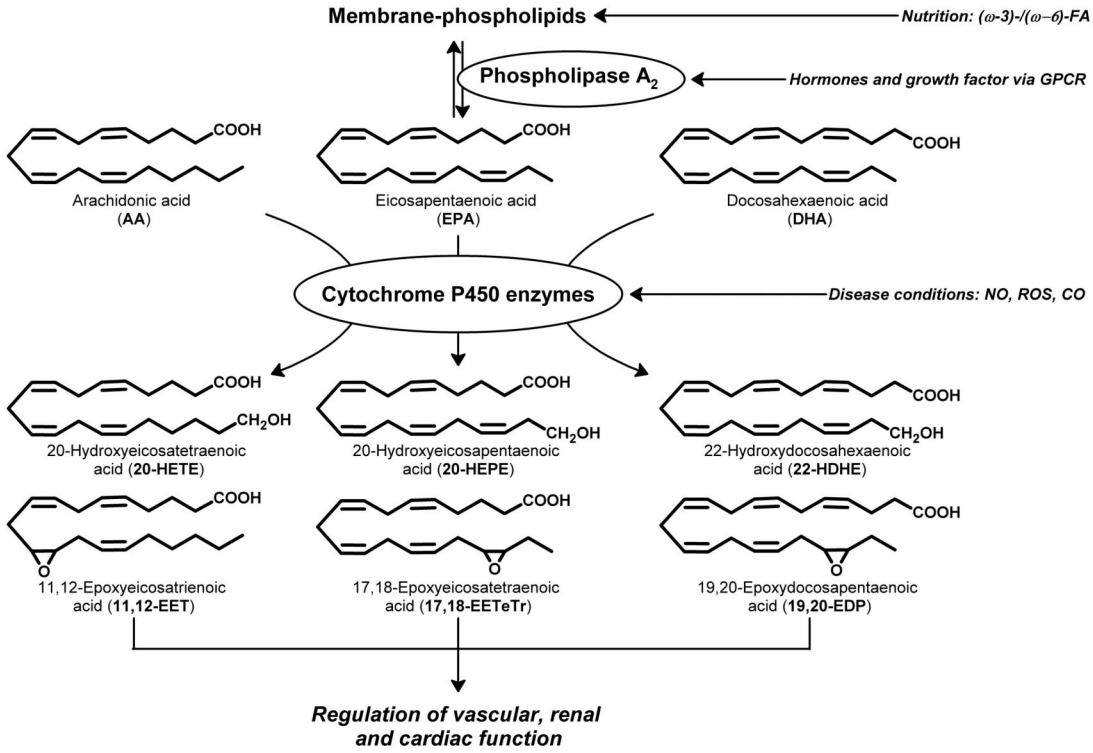

Fig. 1. Generation and function of cytochrome P450 (CYP)-dependent eicosanoids in the cardiovascular system. Nutritional uptake of essential fatty acids (FA) and their cell-type specific incorporation determines the acyl-chain composition of membrane phospholipids. AA and, after appropriate dietary supplementation, ω-3 PUFAs, such as EPA and DHA, are released by phospholipase A_2 which is activated by various hormones and growth factors *via* G-protein coupled receptors (GPCR). Free AA, EPA and DHA are now accessible and compete for conversion by CYP enzymes that function as hydroxylases or epoxygenases. CYP enzymes are inhibited by nitric oxide (NO), carbon monoxide (CO) and reactive oxygen species (ROS) which are produced in variable amounts depending on inflammation and other disease states.The CYP-dependent eicosanoids (and docosanoids) serve as second messengers in various signaling pathways regulating vascular, renal and cardiac function

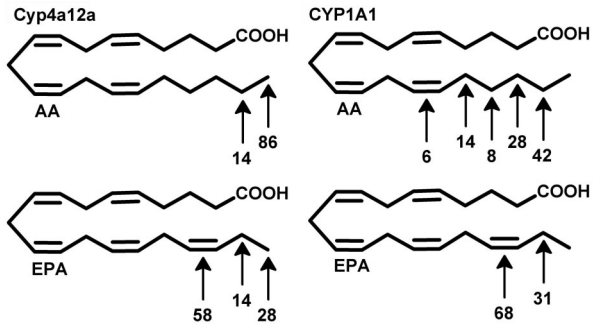

Fig. 2. Cytochrome P450 enzymes oxygenate arachidonic acid and eicosapentaenoic acid with different regioselectivities. For example, mouse renal Cyp4a12a (left panel) functions predominantly as ω-hydroxylase with AA as a substrate and produces 20- and 19-HETE in a ratio of 86:14. In contrast, the same enzyme shows a high 17,18-epoxygenase activity when metabolizing EPA and produces 17,18-EETeTr, 20-HEPE and 19-HEPE in a ratio of 56:28:14. Human CYP1A1 (right panel) metabolizes AA to a mixture of subterminal hydroxy-derivatives (16- through 19-HETE) and minor amounts of 14,15-EET. Using EPA as substrate, CYP1A1 attacks the ω-3 double bond with high regio- and stereoselectivity and forms 17(R),18(S)-EETeTr and 19-HEPE in ratio of 68:31

uct [16]. Moreover, the Ile462Val polymorphism in human CYP1A1 affects both the catalytic activity and regioselectivity of this enzyme towards EPA [74].

Human CYP2E1 is another isoform that functions predominantly as an ω-1 hydroxylase when converting AA, but CYP2E1 preferentially produces 17,18-EETeTr and 19,20-EDP when using EPA and DHA as substrates [16].

Members of the CYP2C subfamily

Members of the CYP2C subfamily are considered the most potent AA epoxygenases in human and rat. Actually, most of the CYP2C isoforms show a 1.5 to 2-fold increase in catalytic activities when converting EPA instead of AA. This fact applies to human CYP2C isoforms 2C8, 2C9 and 2C18, except 2C19 [6, 16], and to the rat CYP2C isoforms 2C11 and 2C23 [6]. Human CYP2C8 metabolizes AA mainly to 11,12- and 14,15-EET. The analogous regioisomers (11,12- and 14,15-EETeTr) are also produced from EPA. However, the 17,18-double bond that distinguishes EPA from AA is a preferred site for CYP2C8-catalyzed EPA epoxidation. Similarly, CYP2C8 produces a set of regioisomeric EDPs when converting DHA and produces 19,20-EDP as the main product [16]. The other three human CYP2C isoforms show a less strict regioselectivity when converting AA and in their metabolism of EPA and DHA. Remarkably however, all CYP2C isoforms epoxidize the ω-3 double bonds of EPA and DHA.

CYP2C11, the major EET synthase in male rat liver, produces a relatively broad pattern of metabolites when converting EPA. EETeTrs represent about 60% of the total primary products and consist of the 8,9-, 11,12-, 14,15-, and 17,18-regioisomers in a ratio of 20:20:27:33. CYP2C23, the predominant EET-generating enzyme in rat kidney, displays a significantly different regioselectivity when converting EPA instead of AA. EETs represent more than 90% of the total primary AA metabolites, and 8,9-, 11,12-, and 14,15-EET are produced in a ratio of about 1:2:0.6. With EPA as substrate, the 17,18-double bond is clearly the preferred site of epoxidation (about 60% of total EETeTrs), and 8,9-, 11,12-, and 14,15-EETeTr are produced in almost equal quantities (13%, 14%, and 15% of total EETeTrs) [6]. Data on the metabolism of DHA by the rat CYP2C isoforms are not available.

CYP2J2

The substrate specificity of CYP2J2 is of particular interest because it represents the predominant AA epoxygenase in the human heart [98]. CYP2J2 shows a rather weak regioselectivity with AA as substrate and produces 5,6-, 8,9-, 11,12- and 14,15-EET in a ratio of about 37:18:24:21 [98]. Remarkably, CYP2J2 converts EPA and DHA at rates 9- and 2-times higher compared to that of AA epoxidation. Moreover, CYP2J2 displays a clearly pronounced regioselectivity when metabolizing the n-3 PUFAs. EPA is converted preferentially to 17,18-EETeTr (about 40% of the total epoxygenase product) and DHA almost exclusively to 19,20-EDP [16].

Members of the CYP4A and CYP4F subfamilies

CYP isoforms belonging to the CYP4A/CYP4F subfamilies are the major 20-HETE producing enzymes in mammals including human. CYP4A/CYP4F enzymes show a clear regioselectivity in favor of hydroxylating the terminal methyl group when converting AA. For example, CYP4A1, the most active 20-HETE-generating CYP isoform in rat, hydroxylates AA to 20- and 19-HETE in a ratio of approximately 12:1 [65]. This enzyme also accepts EPA as a substrate and converts it with the same rate as AA when both substrates are tested at a concentration of 10 μM

[47]. Surprisingly however, CYP4A1 hydroxylates EPA to 20- and 19-HEPE in a ratio of approximately 4:3 and additionally epoxidizes the ω-3 double bond to yield 17,18-EETeTr (about 20% of the total product). An even more pronounced substrate-dependent change in the regioselectivity of the oxygenation reaction is observed with the mouse Cyp4a12a [63]. The catalytic efficiency (V_{max}/K_m) of this enzyme is about 1.5-fold higher with EPA compared with AA as substrate. AA is hydroxylated to 20- and 19-HETE in a ratio of 8:2 and EPA to 20- and 19-HEPE in a ratio of 3:1. Moreover, Cyp4a12a functions exclusively as an ω/(ω-1)-hydroxylase when converting AA but predominantly as a 17,18-epoxygenase with EPA (17,18-EETeTr accounted for 56% of total EPA metabolites) [63]. Human CYP4A11 provides a further example for a dramatic substrate-dependent shift in the ω/(ω-1)-hydroxylase activity ratio: 1) AA is hydroxylated to 20- and 19-HETE in a ratio of 3:1, 2) EPA to 20- and 19-HEPE in a ratio of 1:3, and 3) DHA to 22- and 21-HDoHE in a ratio of 1:2 [15]. At a concentration of 10 μM, the relative rates of total product formation from AA, EPA and DHA are approximately 2.2:1.4:1.

Other highly active 20-HETE producing isoforms involve the human CYP4F subfamily members CYP4F2, 4F3A and 4F3B. The ability of these enzymes to produce 20-HEPE and 22-HDoHE from EPA and DHA, respectively, was shown first for CYP4F3B using liquid chromatography/mass spectrometry [26]. Another study analyzing CYP4F2, 4F3A and 4F3B provided the remarkable finding that CYP4F2 hydroxylates DHA with more than 2 and 3 times higher rates than AA and EPA, respectively, suggesting that DHA may be the preferred substrate of this enzyme [15]. CYP4F3A and CYP4F3B show high and almost equal hydroxylase activities with AA and DHA but were significantly less active with EPA. In contrast to the CYP4A isoforms described above, all three CYP4F isoforms function predominantly as ω-hydroxylases independent of the substrate. However, a clear shift towards increased (ω-1)-hydroxylation is obvious comparing the metabolite patterns produced from AA, EPA and DHA [15].

Other CYP isoforms

The capacity to metabolize not only AA, but also EPA and DHA, is certainly shared by almost all CYP isoforms involved in the metabolism of long-chain fatty acids. Actually, numerous other n-3, n-6 and n-9 PUFAs, such as α-linolenic acid, linoleic acid and oleic acid, are potential alternative substrates and it may largely depend on the physiological conditions whether or not these PUFAs interfere with the CYP-dependent metabolism of AA. Recently, a novel human CYP isoform was identified and classified as CYP2U1 [42]. This enzyme is specifically expressed in the thymus and brain and functions as an ω/(ω-1)-hydroxylase of α-linolenic acid, AA, EPA and DHA. Moreover, CYP isoforms primarily involved in prostaglandin hydroxylation, such as CYP4F8 and CYP4F12, may contribute to EPA and DHA metabolism. Interestingly, these two enzymes metabolize AA by (ω-2)/(ω-3)-hydroxylation, but EPA and DHA are metabolized by epoxidation of the ω-3 double bond [87].

Conclusions and hypothesis on the physiological implications

The studies described above demonstrate that the CYP isoforms involved in the production of physiologically active AA metabolites accept EPA and DHA as efficient alternative substrates. Several of the enzymes show higher catalytic rates when converting EPA or DHA instead of AA. Important examples for a preference towards EPA involve CYP2J2, the major EET producing isoform in the human heart, CYP2C23, the predominant EET producing isoform in the rat kidney and Cyp4a12a, the mouse renal 20-HETE-producing isoform. Among the human isoforms producing 20-HETE, CYP4A11 apparently prefers AA over EPA and DHA, whereas CYP4F2 is most active with DHA.

Comparing the metabolites produced from AA, EPA and DHA, it is obvious that the regioselectivity of the CYP-catalyzed oxygenation reactions is dependent on the substrate. Providing an impressive example, CYP2J2 produces an almost equal mixture of 5,6-, 8,9-, 11,12- and 14,15-EET when metabolizing AA but predominantly converts EPA to 17,18-EETeTr and DHA to 19,20-EDP. Also, for many other CYP isoforms, including several members of the CYP4A subfamily, the ω-3 double bond that distinguishes EPA and DHA from their n-6 PUFA counterparts represents a major site of epoxidation. Moreover, it is of particular note that the CYP4A and CYP4F enzymes

show largely increased (ω-1)-hydroxylase activities when utilizing EPA and DHA instead of AA as substrates.

In vivo studies of the effect of dietary EPA and DHA supplementation on the endogenous CYP-eicosanoid profile are not available. However, it seems reasonable to assume that the nutrition-induced partial replacement of AA by EPA and DHA in membrane phospholipids will be followed by a corresponding and tissue-specific substitution of the AA-derived CYP metabolites by those derived from EPA and DHA. More specifically, it may be predicted that the EETs can be largely replaced by EETeTrs and EDPs and 20-HETE by 20-HEPE and 22-HDoHE. Due to the substrate-dependent regioselectivity of the CYP epoxygenases, we can expect that 17,18-EETeTr and 19,20-EDP, the products of ω-3 epoxidation, will become the predominant epoxy-metabolites in a given tissue. Moreover, 20-HETE may not be simply replaced by its EPA- and DHA-derived homologues, 20-HEPE and 22-HDoHE. Simultaneously an increased level of corresponding (ω-1)-hydroxy-metabolites may occur due to the substrate-dependent regioselectivity of CYP hydroxylases.

Studies on the physiological effects of CYP-dependent EPA and DHA metabolites are only beginning. The few publications available provide the first evidence that a shift of the CYP-eicosanoid profile from AA- to EPA/DHA-derived metabolites contributes to the beneficial effects of n-3 PUFAs on cardiovascular function.

In the vasculature, EETs and 20-HETE play opposite roles in the regulation of vascular tone by serving as second messengers of vasodilator and vasoconstrictor hormones, respectively (see above). Mimicking the effects of EETs, EETeTrs and EDPs are powerful activators of BK channels in VSMCs and mediate the dilation of coronary and mesenteric microvessels [30, 47, 105, 107]. In rat cerebral artery VSMCs, BK channel activation is highly regio- and stereoselective and the effect of 17(R),18(S)-EETeTr largely exceeds that of 11(R),12(S)-EET, the most powerful AA metabolite [47]. DHA-derived epoxides are up to 1000-fold more potent than EETs in activating BK channels in myocytes isolated from rat coronary arterioles [105]. Thus, an exchange of EETs for EETeTrs and EDPs could explain the observation that n-3 PUFAs improve vascular function by increasing the response to vasodilator hormones. In addition, it is tempting to speculate that a shift from 20-HETE to the ω/(ω-1)-hydroxylase metabolites of EPA and DHA may reduce vasoconstrictor responses. Recently, 19(R)-HETE was shown to act as a strong antagonist of 20-HETE [12], raising the possibility that the enhanced formation of (ω-1)-hydroxylase products from EPA and DHA may interfere with vasoconstrictor signaling.

In the human lung, 17,18-EETeTr relaxes and hyperpolarizes both pulmonary artery and bronchial smooth muscle cells [60]. These effects are related to the activation of BK and K_{ATP} channels in both tissues and to a decrease in Ca^{2+} sensitivity of myofilaments. In the lung, CYP1A1 is constitutively expressed and highly inducible by xenobiotics. As described above, human CYP1A1 epoxidizes EPA with an exceptionally high regio- and stereoselectivity to 17(R),18(S)-EETeTr. It will be interesting to learn whether or not the relaxing effect of this metabolite is also stereoselective in the lung.

In the heart, EETs reduce infarction injury and improve functional recovery after I/R, whereas 20-HETE plays an opposite detrimental role under the same pathological conditions (compare above). Providing the first clue to an underlying mechanism, mitochondrial and sarcolemmal K_{ATP} channels, that are known to play a key role in cardiac protection, are activated by EETs, whereas the same channels are obviously blocked by 20-HETE. Importantly, one study has shown that the capacity of EETs to activate sarcolemmal K_{ATP} channels in cardiomyocytes is largely exceeded by their EPA- and DHA-derived counterparts [55]. Thus, the beneficial effects of n-3 PUFAs on cardiac function may be partially mediated by the removal of 20-HETE from the tissue and the replacement of the AA-derived EETs by the more powerful EETeTrs and EDPs. If true, the substrate and reaction specificity of CYP2J2 described above indicates that this enzyme has the potential to provide the human heart with highly potent cardioprotective metabolites after dietary EPA/DHA supplementation.

Additional research is needed to establish the role and potential therapeutic implications of CYP-dependent metabolites of EPA and DHA in the cardiovascular system. Considering the substrate and reaction specificity of the CYP enzymes involved in AA metabolism, EPA and DHA are able to efficiently compete with AA and are themselves oxygenated to unique sets of alternative metabolites. These alternative metabolites may supplant the role of EETs and 20-HETE as second messengers and thereby enhance or dampen various signaling pathways regulating vas-

cular, renal and cardiac function. However, future studies will probably modify this simple model. In particular, efforts in different laboratories may successfully identify the putative CYP-eicosanoid receptors and their specific interactions with the individual epoxy- and hydroxy-metabolites derived from n-6 and n-3 PUFAs.

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