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Review

The role of long chain fatty acids and their epoxide metabolites in nociceptive signaling

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ABSTRACT

Lipid derived mediators contribute to inflammation and the sensing of pain. The contributions of omega-6 derived prostanoids in enhancing inflammation and pain sensation are well known. Less well explored are the opposing anti-inflammatory and analgesic effects of the omega-6 derived epoxyeicosatrienoic acids. Far less has been described about the epoxidized metabolites derived from omega-3 long chain fatty acids. The epoxide metabolites are turned over rapidly with enzymatic hydrolysis by the soluble epoxide hydrolase being the major elimination pathway. Despite this, the overall understanding of the role of lipid mediators in the pathology of chronic pain is growing. Here, we review the role of long chain fatty acids and their metabolites in alleviating both acute and chronic pain conditions. We focus specifically on the epoxidized metabolites of omega-6 and omega-3 long chain fatty acids as well as a novel strategy to modulate their activity *in vivo*.

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1. Introduction

The role of arachidonic acid (ARA) metabolites in pain and inflammation has received great attention over the last 45 years. This has expanded the understanding of lipids as cell signaling molecules with biological actions not limited to their role as energy stores. The exploration of bioactive lipid metabolites has largely remained focused on the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid ARA. Recently, this has extended to investigating the biological fate of the omega-3 fatty acids through the same enzymatic pathways of the ARA cascade. The omega-6 and omega-3 PUFAs differ in the position of the double bonds from the methyl end of the molecules. Nevertheless, both are formed from the essential fatty acids linoleic acid and α-linolenic acid respectively which cannot be synthesized *de novo* by humans or other mammals. However, the conversion of α-linolenic acid to long chain omega-3 PUFAs is limited, and they are more efficiently taken in through dietary supplementation [1]. The PUFAs affect membrane fluidity, modulate inflammation, hemostasis and vascular tone [2]. Roles for omega-3 PUFAs in the central nervous system (CNS) and CNS development have been well described [3,4] and more recently the role in CNS pathology has attracted focus [5,6]. Here we examine the bioactivity of these omega-3 PUFAs compared to omega-6 ARA and their metabolites with special attention paid to their role in modulating nociceptive signaling and pain. While new evidence is emerging regarding the role of omega-3 PUFA metabolites of lipoxygenase enzymes in pain [7], this review will focus on the epoxidized PUFA metabolites (epoxy fatty acids, EpFAs) formed by cytochrome P450 enzymes.

1.1. PUFA metabolism

The ARA cascade is typically simplified and described as three enzymatic pathways including the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP450) enzyme families that convert the parent PUFAs to multiple bioactive lipid metabolites. It is now established that the omega-3 fatty acids, both eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), in addition to omega-6 ARA (20:4 n-6), are substrates for each of the major catalyzing enzymes of these three branches of the cascade [8,9]. While use of the parent PUFAs as dietary intervention has been more often investigated, there is a broadening exploration of the activity of individual metabolite species. The COX produced prostaglandins from ARA are well studied and prostaglandin E_2 specifically is known to have a prominent role in pain and inflammation [10]. The COX-2 enzyme acts on EPA and DHA to form the respective E and D series resolvins in the presence of aspirin, and the LOX enzymes form neuroprotectin-1 from DHA [11,12]. The LOX produced leukotrienes from ARA have roles in inflammation and lung related pathologies such as asthma [13]. The CYP450 produced metabolites from ARA are less well described and their biological activity is still being explored. The CYP450 oxidases also act on the omega-3 substrates forming multiple species of EpFAs [14] as well as numerous hydroxylated metabolites. Thus, a new nomenclature such as the PUFA cascade is more applicable.

1.2. EpFA synthesis

The enzymes responsible for the *de novo* production and degradation of EpFAs are broadly distributed through tissues and are present in neurons and glial cells in both rodents and humans [15,16]. The omega-6 ARA and omega-3 EPA and DHA PUFAs are cellular membrane lipids esterified primarily at the sn-2 position of glycerophospholipids [9,17,18]. All three classes are released from these stores by phospholipases [19] and other enzymes. They are subsequently transformed by CYP450 enzymes into regioisomers of the EpFAs, the epoxydocosapentanoic acids (EDPs also known as EpDPEs), eicosatetraenoic acids (EEQs also known as EpETEs) and epoxyeicosatrienoic acids (EETs) from DHA, EPA and ARA respectively. The CYP450 oxidases convert all of these substrates with higher catalytic turnover of EPA and DHA to ARA [20,21]. CYP2C8, CYP2C9 and CYP2J2 are reported to be largely responsible for the formation of the EpFAs. However several other P450 oxidases thought to be principally responsible for the oxidative metabolism of the lipophilic xenobiotics may also produce EpFAs. All of these CYP450s make several regioisomers of EpFAs and all of them are involved to varying degrees in allylic, omega, and omega-1 hydroxylation in addition to epoxidation. The PUFA substrates can be epoxidized at any of several double bonds resulting in several regioisomer metabolites and the corresponding enantiomers for each class of PUFAs. Thus, there is a large metabolite pool when all possible outcomes are considered. EETs and omega-3 EpFAs are also esterified in phospholipids present in cellular membranes and plasma lipoproteins [22–24]. Therefore, they are immediately available upon release from these membranes.

1.3. EpFA metabolism

EETs are endogenous substrates of the soluble epoxide hydrolase (sEH, *EPHX2*, EC 3.3.2.10) an enzyme downstream of the CYP450s in the ARA cascade. The sEH appears primarily responsible for the hydration of EpFAs to their corresponding diols when it is present. These endogenous substrates are efficiently degraded by the sEH making it an important regulatory enzyme (Fig. 1). In his classical review of the epoxide hydrolases Oesch pointed out that the microsomal epoxide hydrolase (mEH, *EPHX1*, EC 3.3.2.9) can hydrolyze epoxysteric acid [25]. The hydrolysis of fatty acid epoxides by the mEH has been observed by numerous subsequent workers [26,27], however compared to sEH the K_m is higher, the V_{max} or k_{cat} values are much lower, and in general the abundance of the mEH is much lower. Thus, except possibly in rare tissues where the sEH level is exceptionally low and mEH level is high, the sEH is the predominant enzyme responsible for the hydrolytic degradation of fatty acid epoxides. However, other mechanisms such as reincorporation into the cellular membrane and beta oxidation also limit the *in vivo* residence time of EETs. Several studies have attempted to determine the maximum possible hydrolysis of EpFAs in tissues that is not due to the sEH ranging from the early work of Moody to more recent investigations [28,29]. The conclusion is that no substantial hydrolysis of EpFAs is due to enzymes other than the sEH. There are several genes that could code for proteins with sEH activity, with EH3 and EH4 the most well studied [30]. While there are reports of EpFA hydrolysis by EH3 [31] many laboratories have difficulty in reproducing this work possibly due to expression problems.

The sEH converts EpFAs of all three classes into the vicinal diols. There is evidence of substrate preference, for example sEH has the strongest preference for the 14,15 isomer of the EETs, and increasing the distance between the terminal carbon and the epoxide reduces this preference [23]. However, omega-3,4 epoxides of