Soluble epoxide hydrolase as a therapeutic target for pain, inflammatory and neurodegenerative diseases☆

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Abstract

Eicosanoids are biologically active lipid signaling molecules derived from polyunsaturated fatty acids. Many of the actions of eicosanoid metabolites formed by cyclooxygenase and lipoxygenase enzymes have been characterized, however, the epoxy-fatty acids (EpFAs) formed by cytochrome P450 enzymes are newly described by comparison. The EpFA metabolites modulate a diverse set of physiologic functions that include inflammation and nociception among others. Regulation of EpFAs occurs primarily via release, biosynthesis and enzymatic transformation by the soluble epoxide hydrolase (sEH). Targeting sEH with small molecule inhibitors has enabled observation of the biological activity of the EpFAs in vivo in animal models, greatly contributing to the overall understanding of their role in the inflammatory response. Their role in modulating inflammatory pain has been demonstrated in disease models including cardiovascular pathology and inflammatory pain, but extends to neuroinflammation and neuroinflammatory disease. Moreover, while EpFAs demonstrate activity against inflammatory pain, interestingly, this action extends to blocking chronic neuropathic pain as well. This review outlines the role of modulating sEH and the biological action of EpFAs in models of pain and inflammatory diseases.

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Keywords:
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Epoxy-fatty acids (EpFAs)
Inflammatory pain
Neuropathic pain
Depression
Alzheimer’s disease

1. Introduction

Eicosanoids are a group of lipid mediators generated from arachidonic acid (ARA) by activity of cyclooxygenases (COX), lipoxygenases (LOX) and cytochrome P450 (CYP450) enzymes. These fatty acid metabolites are implicated in critical biological processes throughout the body in most cells, tissues and organs (Funk, 2001; Xu et al., 2016). Eicosanoids have been intensely investigated for their role in the inflammatory response and more recently the complexity of the pro and anti-inflammatory as well as other non-inflammatory roles for these metabolites have been recognized (Dennis & Norris, 2015). Knowledge of the complex signaling networks that the eicosanoids comprise now

Abbreviations: AD, Alzheimer’s disease; ARA, arachidonic acid; COX, cyclooxygenase; CYP450, cytochrome P450; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EETs, epoxyeicosatrienoic acids; EpFAs, epoxy-fatty acids; ER Stress, endoplasmic reticulum stress; IBD, inflammatory bowel disease; LOX, lipoxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD, Parkinson’s disease; PGE2, prostaglandin E2; sEH, soluble epoxide hydrolase; sEHI, soluble epoxide hydrolase inhibitors; ROS, reactive oxygen stress.

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extends to include the metabolites of other long chain polyunsaturated acids (LC-PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that are recognized to flow through the same enzymatic pathways. Much is known about the bioactivity of prostaglandin metabolites of ARA, and similarly, the leukotriene metabolites have well described, potent biological action. There is less known about the metabolites of the CYP450s, the epoxy-fatty acids (EpFAs), though the body of knowledge regarding their bioactivity is growing. Moreover, when addressing the EpFAs specifically as a class of lipid mediators, the epoxide metabolites of all LC-PUFAs can be included. It is in examining the biology of this EpFA class that the importance of the soluble epoxide hydrolase (sEH) enzyme was revealed because it is a major regulator of EpFA biology. Uncovering the physiologic role of the EpFAs has been greatly aided by the ability to inhibit the sEH enzyme. Because the effects of maintaining EpFA titers in vivo has been largely beneficial, small molecule inhibitors of sEH (sEHI) have become a novel approach to altering disease pathologies including cardiovascular diseases, inflammation, neurodegenerative disorders and chronic pain among others.

1.1. EpFA biosynthesis and regulation

LC-PUFA are 14–26 long carbon chains with several double bonds imparting their polyunsaturated nature. The term “ecosa” refers to 20 carbon length fatty acids formed mostly from 20:4(n-6) ARA which, along with the omega-3 metabolites of EPA (20:5, n-3) and longer chain DHA (22:6, n-3) fatty acids, are the major focus of this review. The CYP450 enzymes act on LC-PUFA to form EpFAs by epoxidation of the double bonds (Konkel & Schunck, 2011). Multiple regioisomers of EpFAs are produced from the parent LC-PUFA depending on the location of the epoxidized double bond. There is also a high degree of enantiofacial selectivity (R/S regioisomer) conferred in this process (Spector, Fang, Snyder, & Weintraub, 2004). The epoxidized metabolites, epoxyeicosatrienoic acids (EETs) from omega-6 ARA, epoxyeicosatetraenoic acids (EEQs) from omega-3 EPA, and epoxydocosapentaenoic acids (EDPs) from omega-3 DHA are all classified as EpFAs and are principally anti-inflammatory eicosanoids (Morisseau et al., 2010). The relative contribution of different CYP450s to the total production of the EpFAs will vary with substrate availability and concentration. Also, the expression of the CYP450 monooxygenases that produce them vary depending on sex, species, organ and proportion of the regioisomer of epoxide they produce. However, both the CYP450s that produce the EpFAs and the sEH that is their principal regulatory enzyme are expressed at some level in most tissues. This demonstrates the biological relevance of these metabolites because all types of EpFAs are transformed by the sEH into diols (Fig. 1) and in the case of EETs the diols are less active (Spector, 2009).

sEH (EC:3.3.2.10) is part of the α/β hydroxylase fold super family and is a 120 kD homodimer enzyme with a C-terminal hydroxylase and N-terminal phosphatase (Beetham, Tian, & Hammock, 1993; Cronin et al., 2003). The phosphatase domain hydrolyzes phosphorylated lipids such as isoprenoid phosphates and lysophosphatidic acid that stimulate cell growth but far less is known about the biological role of this activity (Oguro & Imaoka, 2012; Oguro, Sakamoto, Suzuki, & Imaoka, 2009). The C-terminal domain hydrolyzes the epoxides by addition of water to the three membered oxirane ring (Spector, 2009). sEH expression is well conserved among species from simple chordates to preclinical rodents and all mammals tested to date indicating its fundamental role in biology (Harris & Hammock, 2013). sEH is widely distributed throughout the body with the most concentrated expression in the liver, kidney, intestine and vasculature in mammals (Enayetallah, French, Thibodeau, & Grant, 2004). However, sEH is also found in the brain and in C57Bl/6 mouse is observed more strongly in the cortex, hippocampus, amygdala and striatum (Marowsky, Burgener, Falker, Fritschy, & Arand, 2009). sEH expression has been found in neurons along with the CYP450 enzymes that produce EpFAs (Iliff, Wang, Zedlin, & Alkayed, 2009) and in astrocytes including astrocytic end feet (Marowsky et al., 2009). In human naïve brain, sEH is expressed in neurons, oligodendrocytes, astrocytes and ependymal cells (Sura, Sura, Enayetallah, & Grant, 2008).

Potent selective inhibitors of sEH were first described in the early 1980’s as a mechanism to identify the biological importance of the enzyme (Mullin & Hammock, 1982). The diols formed from sEH action generally lack the activity of the epoxidized precursors however they are dramatically more polar, move rapidly out of cells, and are easily
EETs and other EpFAs are known to reduce inflammation through multiple mechanisms which have been demonstrated in animal models (Fig. 2). One such mechanism of EET regioisomers in cardiovascular biology is to inhibit VCAM-1, E-selectin and ICAM-1 expression (Node et al., 1999; Zhao et al., 2012). EETs also decrease TNF-α secretion from mononuclear cells (Bystrom et al., 2011) and may also inhibit their adherence (Node et al., 1999). Other mechanisms by which EETs reduce inflammation include blocking the nuclear translocation of NFκB (Bystrom et al., 2011; Fife et al., 2008; Node et al., 1999) which in turn downregulates several enzymes including calcium-insensitive nitric oxide synthase (iNOS), lipoxigenase-5 (LOX-5), and cyclooxygenase-2 (COX-2) that are upregulated in inflammation (Schmelzer et al., 2005; Schmelzer et al., 2006). sEH administration also blocked increases in phospho-IkBα levels which activate NFκB and thus inhibited NFκB signaling in a murine model (Xu et al., 2006). Activation of signal transducer and activator of transcription 3 (STAT3) (Williams, Bradley, Smith, & Foxwell, 2004) and other nuclear receptor activation such as peroxisome proliferator activated receptor (PPAR) alpha and gamma are additional mechanisms that have been described for EETs (Fang, 2006; Ng et al., 2007). In vivo sEH gene deletion and the resulting increase in EETs lowered inflammatory gene expression and neutrophil recruitment, though these effects displayed some organ specificity being more robust in lung (Deng et al., 2011). EpFAs derived from omega-3 LC-PUFAs are less well described but recent studies demonstrate they also have generally anti-inflammatory properties (Isobe & Arita, 2014; Morin, Sirois, Echave, Albadine, & Rousseau, 2010). However, it is critical that EpFAs and their regio and optical isomers be treated as distinct compounds.

Determining the mechanisms of EpFA action has been complicated by the lack of a defined receptor. There has been a considerable effort in the last decade to identify a receptor, or more likely receptors, for the EETs with little progress. The COX and LOX systems are perhaps better exploited because prostanoids and leukotrienes have identified G protein coupled receptors (GPCR) and selective compounds for...
pharmacological agonism and antagonism. Although lacking a defined receptor, G proteins have been implicated in the action of EETs in coro-
nary smooth muscle (Li & Campbell, 1997) and EETs have been antago-
nized with a synthetic antagonist (Gauthier et al., 2002; Gross et al.,
2008). In cerebral artery smooth muscle cells, EETs bind to transient re-
ceptor potential cation channel subfamily V member 4 (TRPV4) chan-
nels (Earley, Heppner, Nelson, & Brayden, 2005; Vriens et al., 2005;
Watanabe et al., 2003). However, there is evidence that EETs can act
on more than one TRP channel (Loft & Fleming, 2011), and that they
have effects that are independent of calcium signaling (reviewed in
Sudhahar, Shaw, and Imig [2010]). Thus, there are multiple possible ac-
tions of EpFAs and their mode of action may differ depending on com-
pound, tissue type and receptor expression.

The sEH reduce the severity of a variety health problem in animal
models. In many cases inflammation could be seen as a common mecha-
anism as introduced above, but in other cases it is hard to understand
how a single mechanism could address such diverse illnesses as atiral fi-
brillation and pancreatitis. It now appears that modulation of endoplas-
mic reticulum stress and specifically the pathological axis from
mitochondrial dysfunction through ROS generation and activation of
the ER Stress pathway leading to cell damage is an event common to
many of the beneficial effects of EpFAs and sEH.

The bioactivity of EpFAs is transient in vivo principally due to the ac-
tion of sEH. The primary route of EpFA transformation to inactive diols is
blocked by inhibiting the sEH enzyme to increase their residence time
and observe their bioactivity. Several commonly used sEH are outlined
in Table 1 including their chemical structures (Table 1). Even with sEH
inhibited or removed, EpFAs are metabolized at a somewhat slower rate
by beta oxidation or chain elongation, CYP450 oxidation, reincorpo-
rating into glycerides and other pathways (Spector et al., 2004). Inhibiting
sEH has demonstrated anti-inflammatory action in several studies using
animal models (Liu, Tsai, et al., 2009; Liu, Yang, et al., 2010; Schmelzer et
al., 2005). Anti-hyperalgesic activity in nociceptive assays has also been correlated with increased concentration of EpFAs
in vivo (Incoglu et al., 2012). In addition the demonstrated bioactivity
of EpFAs has been supported by advances made with the use of EET an-
alogues in vivo as an alternative experimental strategy (Sudhahar et
al., 2010).

2. sEH as a target for inflammatory diseases

Eicosanoids play a fundamental role in inflammation, and classical
pharmaceutical approaches have focused on blocking the formation of
metabolites or antagonising their receptor mediated action. This is the
case with most non-steroidal anti-inflammatory drugs (NSAIDs) and
leukotrien receptor antagonists. However, while formally described as
inflammatory, the pleiotropic effects of the eicosanoids are now
more deeply appreciated, and it is understood that many of the side ef-
facts of these therapies are due to blocking their production. More re-
cently, attention has been focused on alternative eicosanoid or
docosanoid metabolites that are anti-inflammatory or pro-resolving
including the EpFAs (for a review of specialized pro-resolving mediators
(SPMs) see Chiang & Serhan, 2017). Even compounds considered as
predominantly proinflammatory seem to regulate a series of events
leading to resolution of inflammation. Targeting the sEH is a novel strat-
ey in this scheme because inhibiting sEH sustains the endogenous
EpFAs to attain their biological effects. As mentioned above, other
degradation routes for the EpFA exist such as β-oxidation (Spector et
al., 2004). Thus, inhibiting sEH is unlikely to build a large or chronic
increase in EpFAs, enabling the beneficial effects of their modulation with-
out a large side effect profile.

2.1. Inflammatory bowel disease (IBD) and chronic peptic ulcer

EpFAs are also active against inflammatory disorders of the gas-
trointestinal tract. Both pharmacological inhibition and gene
ablation of sEH were investigated for their ability to reduce chronic
active inflammatory bowel disease (Zhang et al., 2012). In this
study using a genetically engineered IL-10 null mouse model of
IBD, both approaches of limiting sEH activity lowered the
number of ulcers and transmural inflammation. Quantitative real
time PCR demonstrated an increase in inflammatory cytokines and
chemokines including IFN-γ, TNF-α, MCP-1 and VCAM-1 in IL-10
null mice compared to double IL-10/sEH null and sEH reduced IFN-
γ, MCP-1 and VCAM-1 mRNA. Western blot analysis also showed
that phosphorylated NFκB was downregulated and oxygenin analysis
revealed increased EET to diol ratios, and a decrease in both leukotri-
ene B4 (LTB4) and 5-hydroxyeicosatetraenoic acid (5-HETE) metabo-
lites (Zhang et al., 2012).

The anti-inflammatory efficacy and physiological improvement
found in the IBD model are unique to sEH inhibition because the clin-
ical use of NSAIDs including COX-2 selective inhibitors exacerbates
IBD (Berg et al., 2002; Kaufmann & Taubin, 1987; Reuter, Asfaha,
Buret, Sharkey, & Wallace, 1996). In a later study it was determined that
sEH gene deletion decreased adenocarcinoma tumors in the IL
10 null mouse model of IBD (Zhang, Liao, et al., 2013). In this context,
the absence of sEH is anti-inflammatory and therefore limits the
transition of IBD to tumor formation in the bowel. However, the
role of EpFAs in tumor formation is still poorly understood and likely
complex. EETs have demonstrated angiogenic activity which is an
important contribution to their regulatory role in hyperemia and
modulation of neurovascular coupling in the cerebral vasculature.
Additionally, their angiogenic activity may be useful in improving
wound repair mechanisms (Sander, et al., 2011; Sander et al.,
2013). Angiogenesis is a critical biological process but pathological
angiogenesis can lead among other things to enhanced tumor
growth. However, in some preclinical cancer models, particularly
with solid tumors, sEH can increase tumor growth. For example,
with a solid tumor a 10× therapeutic dose of a sEH for 10 weeks re-
sulted in slightly increased tumor growth, angiogenesis and metas-
tasis (Panigrahy et al., 2012). In addition to the effects of inhibiting
sEH, when EETs were increased using other techniques including ge-
netic manipulation, multi-organ metastasis and tumor dormancy es-
cape were observed in mice. An interesting enigma is that, in Lewis
lung xenographs, high doses of sEH led to angiogenesis and tumor
growth but, if sEH were given with celecoxib (a NSAID) or an
omega-3 LC-PUFA, sEH demonstrated dramatically reduced angi-
genesis and tumor growth in both lung and breast tumor xenographs
(Zhang, Panigrahy, et al., 2013; Zhang et al., 2014). A partial hypothe-
sis to explain this is that an angiogenic metabolite of 8,9-EET can be
formed by COX enzymes but the metabolite does not form in the
presence of COX inhibitors or competing omega-3 lipids (Rand et
al., 2017). The EDPs which cannot form an analogous angiogenic me-
tabolite are inherently antiangiogenic and the EETs, in the presence of
celecoxib, are as well. At this point the proangiogenic effects of
sEH appear minor and the antiangiogenic effects in the presence of
celecoxib or an omega-3 dietary supplement appear major. Thus,
the homeostatic balance of angiogenesis in relation the EpFAs
needs to be further investigated for effects in cancer biology. In con-
trast, the role of sEH inhibition and EpFA in blocking inflammation in
the bowel and the subsequent reduction of adenocarcinoma appears
more robust. In addition to genetically induced models of IBD, sEH
inhibition has also demonstrated positive results in models of
NSAID-induced intestinal ulcer. Diclofenac induced ulcers were re-
duced by pretreatment of sEH with efficacy comparable to proton
pump inhibitors but at a far lower dose (Goswami et al., 2016). The
effect on ulcer correlated with increased EpFAs of both omega-6
and omega-3 classes. Furthermore, the efficacy of sEH in this study
suggests that reported beneficial effects of PUFA for ulcer relief
(Pineda-Pena, Jimenez-Andrade, Castaneda-Hernandez, & Chavez-
Pina, 2012) are potentially mediated by the EpFA metabolites. It
also appears that part of the efficacy of proton pump inhibitors

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Table 1
Commonly used sEH inhibitors.

Most commonly used sEH in recent publications. High activity on the primate sEH, good activity with rodent sEH and often poor activity on sEH of other species. High oral availability and good PK-ADME with PK data available in many species. Centrally active in mice. Lipophilic and high melting requiring a long dissolution time in water and careful formulation (Inceoglu et al., 2013; Rose et al., 2010).

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Activity</th>
<th>Toxicity</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPPO, UC1770</td>
<td><img src="TPPO.png" alt="" /></td>
<td>1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea</td>
<td>Most commonly used sEH in recent publications. High activity on the primate sEH, good activity with rodent sEH and often poor activity on sEH of other species. High oral availability and good PK-ADME with PK data available in many species. Centrally active in mice. Lipophilic and high melting requiring a long dissolution time in water and careful formulation (Inceoglu et al., 2013; Rose et al., 2010).</td>
<td></td>
</tr>
<tr>
<td>APAU, UC1153, AR9281</td>
<td><img src="APAU.png" alt="" /></td>
<td>1-(1-Acetypiperidin-4-yl)-3-adamantanylurea</td>
<td>No toxicity at high doses in human Phase I and II trials. No clinically useful efficacy in a human hypertension trial. Short half-life in man and rodents. PK data available in multiple species. Surprisingly high water solubility and rapid dissolution. Potent inhibitor of rodent sEH, less active on primate sEH and poor activity in many other species. Poor target occupancy with human sEH (Chen, Whitcomb, et al., 2011).</td>
<td></td>
</tr>
<tr>
<td>t-AUCB, UC1471</td>
<td><img src="t-AUCB.png" alt="" /></td>
<td>trans-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid</td>
<td>Good potency on sEH from a variety of species. PK-ADME known in multiple species. Half-life is longer than AEPU but shorter than TPPO. Good water solubility off-sets lower potency and shorter half-life compared to TPPO (Hwang et al., 2007; Shaik et al., 2013).</td>
<td></td>
</tr>
<tr>
<td>t-TUCB, UC1728</td>
<td><img src="t-TUCB.png" alt="" /></td>
<td>trans-4-{4-[3-(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid</td>
<td>Good potency on sEH from a variety of species. PK-ADME known in multiple species. Longer half-life than t-AUCB above (Hwang et al., 2007; Liu, Tsai, et al., 2009).</td>
<td></td>
</tr>
<tr>
<td>AEPU, UC950</td>
<td><img src="AEPU.png" alt="" /></td>
<td>1-Adamantanyl-3-[5-{2-(2-ethoxyethoxy)ethoxy]pentyl</td>
<td>urea</td>
<td>Moderate potency on sEH from a wide variety of species. Short half-life but bioactive metabolites in mice increase efficacy. Very water soluble and dissolved directly in water. Most water soluble of the potent sEHI (Liu et al., 2015).</td>
</tr>
<tr>
<td>AUDA, UC700</td>
<td><img src="AUDA.png" alt="" /></td>
<td>12-(3-adamantan-1-yl-ureido) dodecanoic acid</td>
<td>Moderate potency on sEH from a wide variety of species. Short half-life, commercially available (A. N. Simpkins et al., 2009).</td>
<td></td>
</tr>
<tr>
<td>GSK 2256294A</td>
<td><img src="GSK.png" alt="" /></td>
<td>(1R,3S)-N-(4-cyano-2-(trifluoromethyl)benzyl)-3-[(4-methyl-6-(methylamino)-1,3,5-triazin-2-yl)amino]cyclohexanecarboxamide</td>
<td>Tested in human trials. Good PK-ADME in human. High melting with poor water solubility. Careful formulation is needed. Activity on multiple species not reported (Lazaar et al., 2016; Podolin et al., 2013).</td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td><img src="Sorafenib.png" alt="" /></td>
<td>4-[4-{3-[4-chloro-3-4-[4-[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl]amino]phenoxy]-N-methylpyridine-2-carboxamide</td>
<td>Potent inhibitor of the human and rodent sEH. A registered drug to treat cancer possibly as a Raf-1 or pan kinase inhibitor. Poor solubility needing complex formulation. Numerous side effects at high doses (Liu, Park, et al., 2009).</td>
<td></td>
</tr>
<tr>
<td>Regorafenib</td>
<td><img src="Regorafenib.png" alt="" /></td>
<td>4-[4-[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl]amino]-3-fluorophenoxy]-N-methylpyridine-2-carboxamide</td>
<td>Roughly an order of magnitude more potent on the human sEH than Sorafenib. Similar side effect spectrum and physical properties (Hwang et al., 2013).</td>
<td></td>
</tr>
</tbody>
</table>
such as omeprazol may be due to a reduction in inflammation caused by a dramatic increase in EpFA (Goswami et al., 2016).

2.2. Destructive bone diseases

2.2.1. Arthritis

Recently, an sEHI was evaluated in a randomized, blind preclinical study in aged dogs with natural osteoarthritic pain compared to vehicle control and is first reported on here. The pain was evaluated with a questionnaire adapted from the canine brief pain inventory (CBPI). The CBPI was originally developed for owners to assess the pain level of their pets with existing painful conditions. The questionnaire used for this experiment had 12 total questions to assess pain based on activities such as initiation of movement, walking, climbing stairs or jumping for a reward scored with a scale of 0 (not due to pain), 1 (not able to determine), and 2 (obviously due to pain) for the first 11 questions. The 12th question regarding overall pain was scored on a scale from 0 (no pain) to 4 (unwilling to participate). The dogs were assessed for five consecutive days prior to treatment to establish a baseline measurement of their pain. Treatments were with 5 mg/kg of the sEHI t-TUCB (Hwang, Tsai, Liu, Morisseau, & Hammock, 2007) in an oral capsule or placebo capsule for 5 days. Scores are reported as the percent of maximum possible pain score (score = observed / maximum = 100, maximum = 26 for the entire questionnaire). The 5 days of baseline measurements were pooled to compare 5 days of treatment with t-TUCB or placebo. This study revealed that in naturally occurring arthritis sEHI inhibition was effective in blocking pain (Fig. 3). The sEHI significantly lowered pain scores compared to both the pooled vehicle and placebo control (Kruskal-Wallis One Way Analysis of Variance on Ranks, H = 21.438 with 2 degrees of freedom, p ≤ 0.001, n = 7 dogs/group). Importantly the results in dogs reported here represent efficacy against naturally occurring arthritis and not an induced model. While the study is limited due to the number of dogs naturally presenting arthritis and therefore the absence of a positive control, the results of sEHI treatment was statistically significant and biologically meaningful by increasing activity in the dogs. This efficacy in a chronic condition is meaningful, particularly in dogs, because they are highly sensitive to the side effects of NSAIDs which can be lethal (Mathews, 2000). Inhibition of sEHI has been suggested as an anti-inflammatory strategy for chronic use in arthritis over NSAIDs and steroidal that have dose limiting side effects (Pillarsetti & Khanna, 2012). Schmelzer et al. demonstrated that sEHI and the resulting increased EETs transcriptionally down regulate a number of enzymes involved in mediation of inflammation including induced COX-2 (Schmelzer et al., 2005). sEHI have also been found by a number of studies to synergize with NSAIDs. Since sEHI reduce hypertension which is often associated with long term use of NSAIDs, as well as gastrointestinal erosion (Goswami et al., 2016) and NSAIDs mediated cardiovascular side effects, (Liu, Li, et al., 2010; Schmelzer et al., 2006) sEHI may act as safeners of NSAIDs when the two are combined.

2.2.2. Osteoporosis

Osteoporosis differs from arthritis in the amount of asymptomatic bone resorption often resulting in fragility fractures (Rachner, Kholsha, & Hofbauer, 2011). In a recent study, EETs blocked bone loss by effecting osteoclast differentiation in vitro via downregulation of ROS. In addition, EETs administration also reduced bone loss in ovariectomized mice as a model of estrogen deficiency-induced osteoporosis (Guan, Zhao, Cao, Chen, & Xiao, 2015). In these experiments EETs decreased ROS release, TNFα levels and NFκB activation. These results support the observation that sEHI inhibition has broad efficacy including lytic bone disease.

2.2.3. Chronic periodontitis

Some of the available data in periodontitis is focused on the role of eicosanoids in general. A recent study examined the oxylipins from human periodontitis samples and found inflammatory markers including increased prostaglandins and leukotrienes (Huang et al., 2014). EETs and diols were also measured in this study but did not exhibit as much change. In a preclinical model of periodontitis in mouse, both sEHI and sEHI null mice showed reduced bone loss when exposed to A. actinomycetemcomitans. In these animals, the chief osteoclastogenic molecules RANK/RANKL/OPG and the chemokine MCP-1 were downregulated and downstream inflammatory JNK and p38 kinase signaling was abated. In addition, this study demonstrated that ER Stress was upregulated with periodontal disease but was blocked by both sEHI administration and in the sEHI knockout mice (Trindade da Silva et al., 2017). Thus, given the role of sEHI and EpFAs action against inflammation combined with the effects on other bone loss conditions, sEHI inhibition may be a useful strategy to combat periodontitis.

Fig. 3. sEHI inhibition and EpFAs block natural arthritic pain. Male and female beagle dogs (ages 8–14 years) with naturally occurring osteoarthritides were administered the sEHI t-TUCB at 5 mg/kg orally in a capsule or placebo control for five days (n = 7 dogs/group). The results are presented as an average of 5 days of treatment (pooled baseline) compared to an average of 5 days of testing under treatment (placebo or t-TUCB). The sEHI significantly lowered pain scores compared to both pooled baseline and placebo control (p ≤ 0.001).

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2.3. Sepsis

Sepsis or endotoxemia in humans is a diverse syndrome of physiological (i.e., systemic arterial hypotension) and immunological responses to microbial infection that include increased circulating levels of cytokines TNF-α and IL-6 and high mobility group protein B1 (HMGB1). Historically, therapeutic approaches using antibodies to combat sepsis have had poor results (reviewed in Fink et al. (Fink, 2014)) and blunting of the innate immune response shows more promise. The approach of using sEH to block the inflammatory response in modeled sepsis prevented lipopolysaccharide (LPS) induced death, reduced pro-inflammatory cytokines, chemokines and prostaglandins (Schmelzer et al., 2005) and restored systolic blood pressure to near normal levels while increasing EETs to diole ratios (Liu, Tsai, et al., 2009). One can envision sEH as muting the cytokine storm and speeding resolution. The level of linoleic acid diols in the plasma may be a valuable marker for the onset of sepsis. Since they cause the may be causative as well (Moghaddam et al., 1997; Slim et al., 2001). Thus, although animal models of sepsis are inadequate to completely mirror the human immune response in this condition, they remain a critical path to the development of new therapeutic strategies and sEH inhibition shows great promise. The difficulty of designing a clinical trial for sepsis is due to its often unpredictable and rapid progression as well as defining a profitable clinical path makes sEH a difficult target. Possibly treating other conditions leading to a cytokine storm such as viral infections or the severe inflammation initiated by modern immunotherapy such as CAR-T for cancer represent more reasonable paths for agents as sEHI that can moderate the cytokine release syndrome (DeFrancesco, 2014).

2.4. Cardiovascular disease

The largest literature base on the anti-inflammatory properties of EpFAs is in the field of cardiovascular physiology and pathology. By the early 1980s there was a shift in cardiovascular research from a focus solely on the description of anatomic structures (e.g., the endothelium of blood vessels) to their actual function (Furchgott & Zawadzki, 1980). The action of aspirin had been elucidated by John Vane who shared the 1982 Nobel Prize in Physiology or Medicine with laureates Sune Bergstrom and Bengt Samuelson who described the prostaglandin lipid metabolites. As the biological activity of select prostaglandins in vascularization was further elucidated (Moncada & Vane, 1979) a series of papers on the sEH enzyme was published (Gill & Hammock, 1980). It was later found that sEH is a master regulatory enzyme of the endogenous EETs which were revealed to have vasodilatory effects (Singer & Peach, 1983). The effect of EETs on the modulation of vascular tone is more thoroughly reviewed in Sudhahar et al. (2010). In the 1990s the relationship between atherosclerosis and inflammation was recognized (Boring, Gosling, Cleary, & Charo, 1998). This underscored the importance of EpFAs action in the cardiovascular and implied that the anti-inflammatory properties may be a vital part of their antihypertensive action (Libby, Ridker, & Maseri, 2002). This was investigated in preclinical models where CYP2J2 epoxygenase overexpression and direct sEH inhibition prevented lipopolysaccharide (LPS) induced death, reduced pro-inflammatory cytokines, chemokines and prostaglandins (Schmelzer et al., 2005) and restored systolic blood pressure to near normal levels while increasing EETs to diole ratios (Liu, Tsai, et al., 2009). One can envision sEH as muting the cytokine storm and speeding resolution. The level of linoleic acid diols in the plasma may be a valuable marker for the onset of sepsis. Since they cause the may be causative as well (Moghaddam et al., 1997; Slim et al., 2001). Thus, although animal models of sepsis are inadequate to completely mirror the human immune response in this condition, they remain a critical path to the development of new therapeutic strategies and sEH inhibition shows great promise. The difficulty of designing a clinical trial for sepsis is due to its often unpredictable and rapid progression as well as defining a profitable clinical path makes sEH a difficult target. Possibly treating other conditions leading to a cytokine storm such as viral infections or the severe inflammation initiated by modern immunotherapy such as CAR-T for cancer represent more reasonable paths for agents as sEHI that can moderate the cytokine release syndrome (DeFrancesco, 2014).

3. sEH as a target for neurodegenerative diseases

With the general population ageing, the incidences of neurodegenerative diseases and chronic inflammatory conditions are both on the rise. Thus, there is an urgent need for new approaches to mitigate neuro-inflammation. The role of the EpFAs in regulating inflammatory conditions particularly in the brain is a potential target and inhibiting sEH as a strategy to sustain their biological activity is a novel approach with great promise. Here we outline the role of EpFA and sEH in several modeled neurological diseases with an inflammatory component.

3.1. Stroke

EpFAs, the EETs in particular, are known to be protective against cell death in ischemic stroke (Alkayed et al., 1996; Iliff et al., 2010; Simpkins et al., 2009). Importantly, evidence about sexually dimorphic gene expression and stroke outcomes demonstrate the protective effects of lowering sEH enzyme and increasing EpFA producing CYP450s (Alkayed et al., 1996; Gupta, Davis, Nelson, Young, & Alkayed, 2012; Koerner et al., 2007; Zhang, Otsuka, et al., 2008). A sEH genetic polymorphism in humans has been linked to stroke risk as well as cardiovascular disease (Fornage et al., 2005) and notably a G606A sEH polymorphism that reduces sEH activity and not CYP2J2 gene expression had a protective effect in nonsmoker stroke in China (Zhang, Ding, et al., 2008). Overexpression of sEH with a K55R polymorphism in Swedish men correlated with hypertension and increased ischemic stroke risk (Fava et al., 2010). In addition to expression of sEH in endothelial cells, sEH is expressed in astrocytes, oligodendrocytes, and neurons in human brain (Sura et al., 2008). In mice, sEH expression is found in astrocytes and neurons and has been characterized in brain regions providing background for preclinical models of stroke and other neurological disorders (Marowsky et al., 2009; Zhang et al., 2007). In rat, neurons and astrocytes produce EETs (Alkayed et al., 1996; Amruthesh, Falck, & Ellis, 1992) suggesting there is a fundamental regulation of EpFA bioactivity in the cells of the brain. As an example, it has been demonstrated that EETs are involved in neurovascular coupling (Farr & David, 2011; Higashimori, Blanco, Tuniki, Falck, & Filosa, 2010). Inhibiting sEH has also demonstrated protective effects against ischemic brain damage independent of effects on cerebral blood flow (Iliff et al., 2009; Zhang et al., 2007). It is important to note that using sEH as a strategy has often been favored over exogenous EETs administration in these studies because EETs are quickly metabolized (Imig, Simpkins, Renic, & Harder, 2010).
2011). Thus, these results reflect on the elevation of potentially several classes of EpFAs.

3.2. Seizure

Early attention for treating epilepsy was focused on anticonvulsants which regulate ion channels and affect mediator proteins to control seizures. While this approach greatly improved the quality of life for epileptics, they did little prevent seizures. Current research is focused on neuroinflammation and neural plasticity to potential targets to prevent recurrent seizures. The observation that blocking leukocyte infiltration blocked epileptogenesis clarified the role of inflammation in initiating epilepsy (Fahmida et al., 2008). However, inflammation is also a consequence of seizure and plays a role in the development of epilepsy after the first seizure event (Vezzani, French, Bartfai, & Baram, 2011). Thus, preventing neuroinflammation that promotes seizures or treating neuroinflammation post-seizure event are both current therapeutic targets.

The literature on the benefits of EpFAs for seizure reveals there is a substantial release of PUFA in the seizure events and neuroinflammation occurs secondary to them (Bazan, Birkle, Tang, & Reddy, 1986). An approach to mitigate the seizures and subsequent neuroinflammation by using sEH, gene ablation and direct administration of EETs to the brain demonstrated delayed onset of GABA mediated seizure (Inceoglu et al., 2013). Furthermore, sEH are efficacious in both chemical and electrical models of temporal lobe epilepsy. In pilocarpine induced status epilepticus (SE), sEH increased EETs and EET/DHET ratios, reduced neuroinflammation, as well as seizure frequency and duration (Hung et al., 2015). In the same study, sEH increased seizure-induction thresholds in epileptogenesis induced by electrical basolateral amygdala (BLA) kindling. In addition to controlling neuroinflammation, sEH inhibition has been shown to influence synaptic transmission and neuroplasticity by upregulating NMDA receptors (Wu et al., 2015). These data suggest that increasing EpFA levels by inhibiting sEH has potential to halt seizures by reducing neuroinflammation and increasing neural plasticity.

3.3. Alzheimer’s disease

There is consensus in the current literature that a large component of the pathogenesis of Alzheimer’s disease (AD) is neuroinflammation (Wysz-Coray & Rogers, 2012). The pathogenesis of AD is also related to oxidative stress and mitochondrial dysfunction (Sultana & Butterfield, 2010). These mechanisms are viewed as either contributing to the formation of or subsequently adding to the damage caused by β-amyloid plaques and tau-protein tangle deposition (Wyss-Coray & Rogers, 2012). The pathogenesis of AD is also related to the formation or subsequently adding to the damage caused by neuroinflammation (Bronzuoli, Iacomino, Steardo, & Scuderi, 2016). It has also been demonstrated that PUFAs concentrations decline with age in vivo and much study has been made of supplementation in humans (Dacks, Shineman, & Fillit, 2013; Freund-Levi et al., 2006). It has been hypothesized that the anti-inflammatory benefits of fish oil, which contains high levels of omega-3 fatty acids, are beneficial in limiting the damage of neurologically inflammatory leading to amyloid plaque formation (Barberger-Gateau et al., 2002; Fiala et al., 2015). Unfortunately, the results have been mixed with little improvement seen with omega-3 fatty acids for established dementia but some improvement for mild cognitive impairment (MCI) as a precursor to AD (Boudrault, Bazinet, & Ma, 2009). These inconsistent results might be due, in part, to poor characterization of dietary components or high levels of lipid peroxide in the omega-3 LC-PUFA used. It has been recently observed that this may relate to the role of APOE4 and delivery of DHA to the brain (Yassine et al., 2017). Despite this, omega-3 supplementation is still regarded as a meritorious strategy given that it is well tolerated and has minimal side effects.

There are opposing biological effects of omega-6 versus omega-3 LC-PUFAs (Schmitz & Eckert, 2008), but there are opposing effects within only the omega-6 derived metabolites as well. Several of the omega-6 generated prostaglandins have been implicated in contributing to inflammation including in the brain. Lowering amyloid precursor protein levels and limiting production of pro-inflammatory mediators by using NSAIDs to block COX metabolite production has been newly suggested as a strategy to protect against AD (Herbst-Robinson et al., 2015). However, this type of pharmacological intervention has been limited by the lack of central nervous system (CNS) penetration in addition to the well-known gastrointestinal and hematological side effects of NSAIDs.

The EETs, on the other hand, demonstrated interaction with mitochondria in the presence of β-amyloid. Select regioisomers of EETs were able to reduce mitochondrial membrane depolarization and fragmentation improving cellular respiration (Sarkar et al., 2014). The 14,15 EET regioisomer and sEH both increased cell viability in modeled reactive oxygen stress induced by hydrogen peroxide challenge. A CYP450 inhibitor, miconazole, decreased cell viability in co-cultured astrocytes and dopaminergic neurons (Terashvili, Sarkar, Van Nostrand, Falck, & Harder, 2012), β-amyloid also reduced EET synthesis in hippocampal astrocytes and neurons (Sarkar, Narayanan, & Harder, 2011), thus, supporting the levels of EETs by inhibiting sEH holds great promise by improving mitochondrial function in this pathological condition. More recently molecular mechanisms of this action have been revealed in human embryonic kidney 293 (HEK293)/Tau cells activated by hydrogen peroxide (Yao, Tang, Liu, & Wang, 2016). Cell viability was increased with sEH corresponding to a lower phosphorylation of tau protein, up-regulation of p-AKT and greater GSK-3β phosphorylation. Related to AD the investigation into the relationship of EpFAs and sEH, EETs specifically, in vascular cognitive impairment in humans revealed increased DHETs in VCI patients (Nelson et al., 2014). EETs and sEH have also increased axonal outgrowth in primary cultures of cortical, sympathetic and sensory neurons from rat (Abdu et al., 2010). Thus, both EpFA administration and sEH inhibition are viable strategies in blocking deleterious events contributing to AD.

3.4. Parkinson’s disease

Parkinson’s disease (PD) is a neurodegenerative disorder that has both genetic and environmental etiologies. There is substantial evidence for reactive oxygen species and oxidative stress in the damage that occurs to dopaminergic signaling neurons that give rise to the motor dysfunction characteristic of the disease (Dauer & Przedborski, 2003). PD is the second leading age related neurodegenerative disease after AD and the hallmarks of the disease are oxidative stress, mitochondrial dysfunction, and abnormal protein aggregation such as alpha-synuclein in Lewy bodies (Dauer & Przedborski, 2003; Halbach, Schuerer, & Krieglstein, 2004). More recently the role of secondary neuroinflammation related to oxidative stress has been investigated as the route to neuronal death in PD (Hirsch & Hnout, 2009; Hirsch, Vyas, & Hnout, 2012). In addition to the anti-inflammatory effect of EpFAs, EETs have demonstrated effects in preventing mitochondrial dysfunction (Liu et al., 2011). An early investigation of alyllic human sEH polymorphism and risk of developing PD was inconclusive (Farin et al., 2001), however, preclinical testing has demonstrated efficacy of both sEH and sEHI gene knockout against MPTP-induced Parkinsonism (Qin et al., 2015). In this model, sEH deficiency and inhibition prevented dopamine (tyrosine hydroxy-lase-positive) neuronal loss and improved motor performance. Furthermore, 14,15 EET administration protected dopaminergic neurons in mice treated with MPTP. Thus, because of the role neuroinflammation and mitochondrial dysfunction play in PD and the efficacy in preclinical models, it is hypothesized that sEH inhibition may have potential benefit in this condition in humans (Lakkappa, Krishnamurthy, Hammad, Velumurghan, & Bharath, 2016).

3.5. Depression

There is ample evidence that LC-PUFAs have a role in depression (Lin et al., 2012; Martins, Bentsen, & Puri, 2012). It is also understood that...
inflammation can be an integral part of depression. The rate limiting enzyme indoleamine 2,3-dioxigenase (IDO) that metabolizes tryptophan and thereby limits serotonin production is under regulation of inflammatory cytokines (O’Connor et al., 2009; Oxenkrug, 2010). In addition, increased inflammatory cytokines have been observed in human clinical patients as well as in post mortem brains from individuals with major depressive disorder (Dean, Tawadros, Scarr, & Gibbons, 2010; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimaki, 2015; Strawbridge et al., 2015). This is the background rationale for the use of inflammatory agents to induce depressive states in preclinical models (Frenois et al., 2007; Fu et al., 2010; Miller, Maletic, & Raison, 2009).

Recently, to exploit the anti-inflammatory properties of EpFAs, the effects of sEH inhibition and genetic ablation have been demonstrated in depression models. Use of the sEH lowered TNFα in LPS treated but not control mice (Ren et al., 2016). These experiments also investigated the effects of 14,15 EET and sEH on neuronal outgrowth in PC12 cells. Other antidepressants have an effect on neuronal plasticity and the results of elevating EpFAs in either manner showed increased NGF-induced neurite outgrowth in these cells (Ren et al., 2016). In behavioral tests, sEH administered both prophylactically and therapeutically resulted in less inactivity in LPS induced depression in mice. sEH inhibition was also effective against social defeat stress in these studies. sEH inhibition did not alter body weight in control mice but increased sucrose preference in social defeat mice. sEH gene deletion conferred resilience to social defeat stress with sEH knockout mice exhibiting similar social interaction time to non-stressed wild type controls. Importantly, when brain-derived neurotrophic factor - tropomyosin receptor kinase B (BDNF-TrkB) signaling was investigated for its role in the modeled depression, it was revealed that sEH knockout mice have higher BDNF levels in several brain regions compared to wild type controls. Additionally, although all groups had similar tissue levels of TrkB, there was a higher p-TrkB to TrkB protein ratio in sEH null mice as well as synaptogenesis markers (Glutamate receptor subunit GluA1 and post synaptic density protein PSD-95) by Western blot. Thus, these mechanisms may be involved in the resilience to social stress in sEH null mice.

This study also quantified sEH protein levels which were higher in both murine induced stress models compared to controls. This result paralleled with increases in post mortem human brain (pallidal cortex) samples from depressed, bi-polar or schizophrenic humans examined for sEH protein levels. An important finding in this work that merits highlighting is the rapid onset of anti-depressive action of sEH in both models of depression. This is noteworthy because pre-clinically as well as clinically, current anti-depressant therapies take several weeks to be at full effect (Gaynes et al., 2009; Krishnan & Nestler, 2010). This rapid anti-depressive action is without any observable side effects unlike ketamine which is now known to have rapid effects against depression but has major psychotomimetic side effects and abuse potential (Kirby, 2015). Thus, it is now suggested that sEH may be a promising new strategy to combat depression (Hashimoto, 2016).

Depression is the mental disorder with the highest numbers of affected individuals worldwide but there are other mental conditions that reveal similar changes in sEH and EpFA biology. Increased sEH expression has been observed with anorexia nervosa in human clinical patients which may be associated with depression and anxiety (Scott-Van Zeeland et al., 2014; Shih et al., 2016). Additionally, there may be a role in reducing inflammation plays in the pathogenesis of anxiety, post-traumatic stress disorder and obsessive compulsive disorder (Furtado & Katzman, 2015) and thus an opportunity for sEH inhibition to improve mental health as well as physical ailment is very broad.

4. sEH as a target for pain

The sEH and EpFAs have demonstrated greater potency than NSAIDs and synergism with inhibitors of both COX and LOX enzymes in reducing inflammation. It is therefore not surprising that this efficacy extends to pain, one of the hallmarks of inflammation. Pain is a complex signaling network that stems from noxious insult or tissue injury and release of inflammatory mediators such as cytokines, ions, bradykinins, prostaglandins and leukotrienes among others. These act on nociceptors directly and drive action potentials signaling the sensation of pain (Woold & Ma, 2007). Typically, pain is a signal to avoid further damage until the crisis resolves. However, there are situations where the alteration to nociceptors allows pain to persist beyond the inflammation or insult, and this signaling in the absence of stimulation is a pathological pain termed neuropathy.

The mechanisms driving neuropathic pain are still poorly understood and perhaps therefore also poorly treated. New approaches are urgently needed. While there are more available approaches to treat inflammatory pain, most of these therapies have dose or use limiting side effects and new approaches are desirable. Here we outline targeting the sEH as novel strategy with the unique property of being efficacious against both inflammatory and neuropathic pain.

4.1. Inflammatory pain

Targeting sEH for pain relief arose from initial investigations of the anti-inflammatory roles of sEH. In studies that investigated LPS induced sepsis a sophisticated mass spectrometry analysis revealed that inhibiting sEH altered not only the EETs and diol metabolite levels, but also several other metabolites in the COX and LOX metabolism pathways of the ARA cascade (Schmelzer et al., 2005). In particular, targeting the sEH with small molecule inhibitors lowered levels of PGE₂, a potent inflammation mediator and algogen, in addition to NO and other cytokines. This finding was groundbreaking because it demonstrated that stabilizing endogenous bioactive lipids was a novel strategy to limit inflammation compared to other enzyme inhibitors that blocked production of inflammatory metabolites. The observed shift in other prostaglandin metabolites and decrease in COX-2 protein levels with inhibiting sEH was the basis for hypothesizing that sEH inhibition would limit inflammatory pain which was investigated in several subsequent studies (Inceoglu et al., 2006; Schmelzer et al., 2006).

sEH were first tested in a model of inflammatory pain for their ability to synergize with COX-2 selective NSAIDs due to the previously observed effect of sEH suppressing the COX-2 enzyme (Schmelzer et al., 2006). This resulted in synergistic decreases in PGE₂ expression and increased thermal withdrawal latencies in mice. Importantly, these improvements occurred without an observable change in the prostacyclin to thromboxane ratio. Changes in the homeostatic balance of these COX metabolites are suspected to initiate thrombotic events that are adverse side effects of COX-2 selective NSAIDs (Fitzgerald, 2004).

The sEH were then tested as single administrations to determine if they were anti-hyperalgesic by themselves. Topical administration of two different sEH was effective in increasing both thermal withdrawal latencies and mechanical thresholds in an LPS inflammatory pain model in rat (Inceoglu et al., 2006). This study also investigated the direct topical administration of EETs which revealed the EpFA metabolites were anti-hyperalgesic by increasing thermal withdrawal latencies against LPS pain. Interestingly EETs demonstrated a slight pro-nociceptive effect in naïve rats compared to the sEH which had no effect in the thermal assay. The anti-hyperalgesic action was demonstrated to have a multifactorial mechanism. Interestingly, in this study COX-2 expression levels in spinal cord did not correlate with decreased pain behavior but sEH elicited anti-hyperalgesia which distinguishes them from glucocorticoids which repress inducible COX-2 to produce anti-hyperalgesia (Brostjan, Anrather, Csizmadia, Natarajan, & Winkler, 1997; Inceoglu et al., 2008). To further explore this, EETs were screened against a panel of cell receptors and were found to bind the translocator protein (TSPO) which transports cholesterol through mitochondrial membranes. The TSPO cooperates with steroids and other regulatory proteins (S3ARD1) which is upregulated by EETs (Inceoglu et al., 2008; Miller, 2007; Wang et al., 2006). It was evident from these experiments...
that the activity of sEH inhibition depends on a cellular factor present in inflammation that has a role in activating the TSPO/STARD1 pathway; this was identified as elevated cyclic adenosine monophosphate (cAMP).

Individual regioisomers of the EpFAs have also been examined for anti-hyperalgesic activity (Morisseau et al., 2010). This study demonstrated that all classes of EpFAs were efficacious against inflammatory pain, and that the EpFA had higher activity than the parent LC-PUFA. Importantly, the DHA derived EPDs appear to be more potent anti-hyperalgesics than the other classes. This merits using sEH as a strategy because it elevates the levels of several EpFA as opposed to single administering of one type of epoxidized metabolite or an epoxide mimic.

Importantly, sEH can also block the pain produced by PGE2 administration (Inceoglu et al., 2011). This was observed in examining the pain dependence of sEH inhibition which revealed several pieces of information. First, sEH is distinct from NSAIDs and steroids which act upstream of PGE2 and prevent its production, and second, that sEH activity depends on the amount of pain present and does not alter pain thresholds in the absence of a painful condition. Because the action of sEH was previously hypothesized to require the presence of cAMP, a phosphodiesterase inhibitor (PDEI) used to block the inactivation of cAMP was co-administered with sEH in this pain model. This strategy resulted in an enhanced response compared to single administration of the sEH or PDEI. Oxytalinin analysis revealed that PDEI increased EpFAs which may contribute to their action in the CNS (Inceoglu et al., 2011). Thus, the EpFAs are analgesic in several models of induced inflammatory pain and the anti-hyperalgesia relates to direct administration of the EpFA but also to sEH inhibition; both producing anti-hyperalgesic activity only in active pain states.

4.2. Neuropathic pain

The initial investigation of sEH efficacy in neuropathy was intended to compare a pain model with relatively low COX-2 induction to inflammatory pain. It was a great surprise when sEH inhibition blocked diabetic neuropathy, a chronic pain model that was proposed as the negative control model (Inceoglu et al., 2008). This was surprising because most NSAIDs that block inflammation have little efficacy against neuropathic pain (Gore, Dukes, Rowbotham, Tai, & Leslie, 2007; Wagner et al., 2013).

Exploring this efficacy against diabetic neuropathy in a preclinical model revealed that there were dose dependent improvements in mechanical pain thresholds and that the sEH outperformed the standard of care gabapentin (Inceoglu et al., 2012). This anti-hyperalgesia was independent of changes in glucose tolerance, insulin tolerance and glucose stimulated insulin secretion. Interestingly, the sEH enzyme activity was increased although the synthetic capacity to form EETs to compare a pain model with relatively low COX-2 induction to inflammatory pain and the anti-hyperalgesia relates to direct administration of the EpFA but also to sEH inhibition; both producing anti-hyperalgesic activity only in active pain states.

Recently the chronic constriction injury (CCI) model (Bennett & Xie, 1988) was used as another paradigm to test the efficacy of sEH against chronic pain. Surgical models of neuropathic pain allow the testing of the chronic nature of the developed pain state in contrast to acute nociceptive pain and offer an alternative to chemical agents that may alter metabolism. Both male and female adult Sprague Dawley rats underwent the surgical ligation with 4 loose ligatures of the sciatic nerve. Several weeks after surgery when the incision had healed and the neuropathy due to the ligatures had developed, the rats were dosed by oral gavage with vehicle (PEG300) or 3 mg/kg of the sEH TPU (Rose et al., 2010) and tested with the von Frey assay for their sensitivity to mechanical touch. An electronic aesthesiometer attached to a rigid tip was used to measure the response of both the ipsilateral (Fig. 4) and contralateral (not shown) hind paws. The points depicted in the graph represent the average score and standard error of mean for a group of rats that were assessed each 3–5 times per time point with a 1-minute interval between repeated stimulation. The TPPU treatment was highly significant in males (p < 0.001) and females (p < 0.010) compared to their respective vehicle controls (Two Way ANOVA, Holm-Sidak method post hoc, n = 3/group). The time course demonstrated that there is both long lasting efficacy and rapid action relieving pain by 30 min after oral gavage. The males also had a significantly more robust response (p < 0.001) than the females possibly due to the higher di-morphic sEH expression observed in male versus female rodents (Gill & Hammock, 1980; Wagner et al., 2017). Overall, the sEH was an effective strategy in both male and female CCI neuropathic rats which supports earlier data in streptozocin induced diabetes in rat and both induced and natural diabetes in mouse (Inceoglu et al., 2012; Wagner et al., 2014, 2017).

Adding to the evidence in preclinical species, sEH inhibition is successful in blocking neuropathic pain in veterinary patients presenting with pathological painful disease. The sEH t-TUCB was used to treat severe equine laminitis, a condition that often requires humane euthanasia of the horses. The first use of sEH in this condition was concurrent with failing standard of care therapy and allowed the horse to stand, eat and later recover (Guedes et al., 2013). Subsequent studies have demonstrated the continued success of sEH as a strategy to combat this condition (Wagner et al., 2017). Thus, sEH inhibition is active against severe neuropathic pain under conditions where standard of care therapies have failed and has broken the species barrier.

The efficacy of EpFA mediated analgesia in neuropathic pain revealed another independent mechanism of action. Diabetic rats were assessed for activation of ER Stress markers and the effect of sEH inhibition (Inceoglu et al., 2015). ER Stress occurs when the homeostatic
protein folding and trafficking in the cell is overwhelmed or unbalanced and leads to the unfolded protein response (UPR) and often to apoptosis (Hotamisligil, 2010). Investigation into the beginning pathogenesis of diabetes has long implicated misfolded proteins and ER Stress as precursors to the UPR and pancreatic beta islet apoptosis (Kozutsumi, Segal, Normington, Getling, & Sambrook, 1988; Oyadomari, Araki, & Mori, 2002). ER Stress is regulated by three main membrane associated sensors, protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1α (IRE1α) and activating transcription factor 6 (ATF6) which induce the UPR when activated (Fig. 5). Administration of sEH to diabetic rats was able to suppress these markers in skin and spinal cord and elicit anti-hyperalgesic effects in the animals (Inceoglu et al., 2015).

The role of ER Stress in the pathogenesis of diabetes is well established but there are also many links between ER Stress and inflammation including the activation of NfκB pathway, ROS production and c-Jun N-terminal kinase (JNK) signaling (Hotamisligil, 2010). In addition, there is evidence that the cellular dysfunction of ER Stress has consequences for neurodegeneration and progression of diseases such as AD (Lin, Walter, & Yen, 2008; Riederer, Leuba, Vernay, & Riederer, 2011; Sin & Nollen, 2015). Thus, protecting against the trio of ER Stress, ROS and mitochondrial dysfunction is the unifying action that allows the sEH to be beneficial in a wider variety of pathological conditions.

4.3. Potential side effects of targeting sEH

Importantly, the analgesic efficacy of sEH inhibition occurs without the common side effects of NSAIDs or narcotic analgescics. The well described gastrointestinal (GI) ulceration produced by NSAID use is not trivial given the significant rate of hospitalization and death associated with GI bleeding, particularly in elderly patients (Lazzaroni & Bianchi Porro, 2004; Shah & Mehta, 2012). There are also cardiovascular effects of NSAIDs including the selective COX-2 inhibitors that were thought to spare the GI side effects (Lazzaroni & Bianchi Porro, 2004). Interestingly, it has been demonstrated that the anti-inflammatory action of sEH inhibition does not have GI side effects, in fact as mentioned previously, sEH inhibition can mitigate GI ulceration caused by NSAIDs (Goswami et al., 2016). Inhibiting the sEH does not alter the thromboxane to prostacyclin ratio in vivo (Schmelzer et al., 2005) or clotting time (Liu, Yang, et al., 2010; Schmelzer et al., 2006) and thus are not anticipated to have the cardiovascular side effects associated with NSAIDs. The other intense side effects of NSAIDs included inducing hypersensitivity, NSAID-exacerbated cutaneous disease, respiratory disease and/or induced urticaria/angioedema have not been observed with sEH inhibition in preclinical models, though specific tests for these reactions have not been reported on.

It has also been demonstrated that the analgesia mediated by sEH inhibition does not exhibit the hallmarks of narcotic pain therapies. Typical opioid narcotics cause tolerance, withdrawal and addiction as on-target side effects and also have severe off-target side effects of respiratory depression and constipation. Recent studies of sEH inhibition in chronic pain states have used an operant conditioned preference assay to not only assess pain relief but also the addictive potential of sEH mediated analgesia (Wagner et al., 2014). This work demonstrated inhibiting sEH did not produce reward seeking behavior (a conditioned place preference) in naive and sEH null mice. Additionally, direct administration the EpFAs demonstrated both analgesic efficacy against neuropathic pain as well as a lack of rewarding side effects (Wagner et al., 2016).

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**Fig. 5.** sEH inhibition and EpFAs block Endoplasmic Reticulum Stress (ER Stress). A variety of biological signals can influence the ER Stress pathway such as unfolded and miss folded proteins, high glucose as in diabetes, or reactive oxygen species (ROS) which can be contributed by mitochondrial dysfunction and other sources. EETs reduce the effects of ROS on the ER Stress pathway and stabilize mitochondria (not shown). The three key protein sensors of the ER Stress are inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6) and PKR-like endoplasmic reticulum-resident kinase (PERK). Downstream of IRE1α X-box binding protein-1 when spliced (XBPs1) is activated, similarly ATF6 is cleaved to release the active NH2-terminal domain ATF6(N) and both enter the nucleus as transcription factors. Phosphorylated PERK results in the phosphorylation of eukaryotic initiation factor 2 (eIF2α), ATF4 activation and transcription of C/EBP homologous protein (CHOP), a major participant in genes involved in apoptosis. Apoptotic responses occur when ER Stress is excessive, prolonged, or insufficiently neutralized and is initiated through downstream pathways such as ER-associated protein degradation (ERAD) and CHOP. The enzymatic flow of a single EET regioisomer is depicted on the right. ARA released by phospholipase A2 from the phospholipid bilayer is acted on by CYP450 epoxygenase to form 14,15 EET and would be metabolized by the sEH into a less active 14,15 DHET. Inhibiting sEH maintains the EpFAs which block phosphorylation of PERK, eIF2α, and IRE1α and significantly decrease XBPs1 and ATF6(N) expression. In addition to this action, sEH also normalize phospho-p38 and phospho-JNK, kinase mediators of neuropathic pain. However, in healthy rats, sEH inhibition does not lead to changes in ER Stress pathways.

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5. Conclusion

Inhibiting sEH stabilizes endogenous EpaFs that have demonstrated beneficial effects in regulating inflammation including in neurological diseases in addition to combating chronic and inflammatory pain in preclinical models. sEH inhibitors have been optimized as experimental tools in the past several decades. These molecules designed as transition state mimics of sEH have improved in potency to the single nanomolar range. There are commercially available sEH inhibitors (AUDA, t-AUCB and TPPU) as well as published methods for their synthesis and a large body of literature describing the biological effects of their use in experimental models. In particular, the analgesia mediated by sEHI in experimental models is novel because it is non-narcotic, out performs NSAIDs without their side effects and has advantages for conditions with inflammatory and hypertensive comorbidities. Additionally, the ability of EpaFs to reduce neuroinflammation has great potential to intervene in the progression of neurodegenerative diseases. Overall, the robust analgesia in both inflammatory pain and chronic painful conditions typically refractory to most therapies offers promise for a new approach to alleviate pain. Building on the efficacy of sEH against pain and inflammation in preclinical models and clinical veterinary patients, sEH are being developed for use in humans. Previous clinical trials with sEH inhibitors such as GS2256294 have demonstrated that sEH inhibitors have the potential for broad application and targeting sEH is well tolerated. Optimized inhibitors of sEH are currently being advanced to the clinic for the treatment of diabetic neuropathy.

Conflict of interest statement

The University of California holds patents on the sEH inhibitors used in this study as well as their use to treat inflammation, inflammatory pain, and neuropathic pain. BD Hammock and CB McReynolds are co-founders and KM Wagner and WK Schmidt are employees of EcOsis LLC, a startup company advancing sEH inhibitors into the clinic.

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