



# HHS Public Access

Author manuscript

*Prostaglandins Other Lipid Mediat.* Author manuscript; available in PMC 2021 April 01.

Published in final edited form as:

*Prostaglandins Other Lipid Mediat.* 2020 April ; 147: 106385. doi:10.1016/j.prostaglandins.2019.106385.

## Prostaglandins and Other Lipid Mediators Cytochrome P450 derived epoxidized fatty acids as a therapeutic tool against neuroinflammatory diseases

Jogen Atone<sup>a</sup>, Karen Wagner<sup>a</sup>, Kenji Hashimoto<sup>b</sup>, Bruce D. Hammock<sup>a</sup>

<sup>a</sup>Department of Entomology and Nematology and UC Davis Comprehensive Cancer Center, University of California Davis, Davis, CA, United States

<sup>b</sup>Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan

### Abstract

Cytochrome P450 (CYP) metabolism of arachidonic acid (ARA) produces epoxy fatty acids (EpFAs) such as epoxyeicosatrienoic acids (EETs) that are known to exert protective effects in inflammatory disorders. Endogenous EpFAs are further metabolized into corresponding diols by the soluble epoxide hydrolase (sEH). Through inhibition of sEH, many studies have demonstrated the cardioprotective and renoprotective effects of EpFAs; however, the role of sEH inhibition in modulating the pathogenesis of neuroinflammatory disorders is less well described. In this review, we discuss the current knowledge surrounding the effects of sEH inhibition and EpFA action in neuroinflammatory disorders such as Parkinson's Disease (PD), stroke, depression, epilepsy, and Alzheimer's Disease (AD), as well as the potential mechanisms that underlie the therapeutic effects of sEH inhibition.

### Keywords

Stroke; Alzheimer's Disease (AD); Neuroinflammation; Depression; soluble epoxide hydrolase (sEH); Epoxyeicosatrienoic acid (EET); Parkinson's Disease (PD)

## INTRODUCTION

Cytochrome P450s (CYP450) are recognized to be a large family of heme containing enzymes active in the metabolism of both xenobiotics and endogenous substrates [1]. The enzymes were named due to their chromophore nature that was observed with an emittance peak at 450 nm when bound to carbon monoxide. Historically their importance was

\*Corresponding author: bdhammock@ucdavis.edu (B.D. Hammock).

**Conflict of interest statement:** BD Hammock is a founder and KM Wagner is an employee of EicOsis L.L.C., a startup company advancing sEH inhibitors into the clinic.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

demonstrated in mediating Phase I metabolism of xenobiotics including pharmaceutical therapies and pesticides, however their oxidation of endogenous substrates is now understood as an important cell signaling mechanism [2]. Here we review specifically the action of the epoxidized fatty acid metabolites resulting from CYP450 action on long chain parent polyunsaturated fatty acids (PUFAs). While a large body of literature describes the metabolites of arachidonic acid (ARA), we will expand this review to include what is known about the biological action of the omega-3 long chain PUFAs when available.

ARA metabolites have a wide range of actions, modulating inflammation, cell proliferation, angiogenesis, and immune response [3]. ARA and its 20-carbon metabolites are together termed eicosanoids, with much of research historically focusing on the role of prostaglandins (PGs) and leukotrienes (LTs) and their role in inflammation, vasodilation/vasoconstriction, apoptosis, and leukocyte trafficking among others, via a mechanism of activating G-protein coupled receptors (GPCRs) [4–6]. Prostaglandins and leukotrienes are synthesized from cyclooxygenase (COX) and lipoxygenase (LOX) metabolism of ARA, respectively, and COX or LOX inhibition have long been strategies of pharmacological intervention against inflammatory disorders, with varying degrees of success [7–11]. The biological functions of CYP450 derived epoxidized eicosanoids are less well described compared to prostaglandins; however, more evidence is accumulating in recent years regarding their anti-inflammatory and vasorelaxant properties [12]. These may include epoxyeicosanoids such as the epoxyeicosatrienoic acids (EETs) and the eicosapentaenoic acid (EPA) derived epoxyeicosatetraenoic acids (EEQs). Although distinct from eicosanoids given their 22-carbon chain length, the docosahexaenoic (DHA) derived epoxydocosapentaenoic acids (EDPs) are also considered CYP450 derived epoxy fatty acids (EpFAs). In addition, it is likely that similar metabolites of less abundant fatty acids will also demonstrate biological action. While these CYP450 synthesized EpFAs share similar biological activity as well as the hydrolytic pathway by the soluble epoxide hydrolase (sEH) enzyme and to a lesser degree the microsomal epoxide hydrolase (mEH) enzyme, there are differences in the substrate affinity and activity among the regioisomers of each PUFA metabolites [13–15]. Regardless, most of these EpFAs are relatively stable to chemical hydrolysis and nucleophilic substitution and are for the most part transformed by sEH activity [14]. However, in the absence of sEH,  $\beta$ -oxidation, chain elongation, and omega, omega-1 and other hydroxylation reactions become dominant. Increasingly, reincorporation into glycerides, membrane incorporation and other pathways are being found to be important.

CYP450 derived eicosanoids also include the  $\omega$ -hydroxylated 20-hydroxyeicosatrienoic acids (HETEs). The CYP4A and CYP4F subfamily derived 20-HETEs are noteworthy due to their vasoconstrictive role through inhibiting  $\text{Ca}^{2+}$  activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}}$ ) and inhibiting renal  $\text{Na}^+$ - $\text{K}^+$ -ATPases [16]. Abating 20-HETE synthesis has a therapeutic effect against hypertensive and ischemic injury, and therefore a clinical application of this strategy in disorders such as stroke and hypertension is under investigation [17, 18]. The omega and omega-1 hydroxylation occurs with omega-3 fatty acids as well but no biological role is yet established. In a similar vein, EETs have been demonstrated to regulate vascular tone through binding to  $\text{K}_{\text{Ca}}$  channels, TRPV4 channels,  $\text{Na}^+$ - $\text{K}^+$ -ATPases, thromboxane receptor activation, and activating the  $\text{G}_\alpha$  mediated cAMP/PKA pathway [19–23].

Production of EETs is well characterized, whereby ARA is first cleaved off of membrane phospholipids by calcium-independent phospholipase A2 (iPLA2) or inducible phospholipase A2 (cPLA2) [24, 25]. Less well studied is their release by other enzymes such as diacylglycerol lipase. Free ARA is then epoxidized by various CYP450s including the CYP2C and 2J subfamilies [26]. ARA is converted by CYP450s into four regioisomers of EETs depending on the location of the double bond where the oxygen is inserted namely, 14,15-EET, 11,12-EET, 8,9-EET, and 5,6-EET. In the case of the CYP2J, it has been demonstrated that the oxidation of ARA to 14,15-EET is preferred over other regioisomers [27]. The EETs are subsequently hydrolyzed by the sEH (Figure 1) [28]. The sEH enzyme is a major regulatory enzyme for all the EpFAs converting them into corresponding vicinal diols, specifically for EETs into the dihydroxytrieneoic acids (DHETs) which lack the biological function of EETs. The sEH catalysis of EETs and all EpFAs is rapid and the major route of their conversion, thereby altering the biological effects they exert. Alternatively, EETs have other elimination pathways as mentioned above where they undergo  $\beta$ -oxidation to form a 16-carbon hexadecadienoic acid or elongate to form a 22-carbon docosatrienoic acid [29].

Genetic ablation of sEH as well as pharmacological inhibition of sEH through highly potent and selective inhibitors increases the bioavailability of EpFA by blocking the conversion of EpFA to diols. Significant effort in increasing the bioavailability and stability of sEH inhibitors has resulted in molecules such as 1-(4-trifluoro-methoxy-phenyl)-3-(1-propionylpiperidin-4-yl)urea (TPPU) give sub-nanomolar potency even following oral administration [30]. TPPU, as an example of the optimized compounds, has demonstrated low to moderate blood brain barrier (BBB) penetration with distribution into the brain, though oral administration results in a higher percentage of TPPU in the liver and the heart [31]. While sEH inhibitors are thought to increase the local concentration of bioactive EETs, it is important to note that they also increase levels of epoxygenated EPA and DHA metabolites systemically and diminish diol concentrations. Although DHETs are usually regarded as the inactive metabolite of EETs, DHETs may have some biological functions. For example, 11,12- and 14,15-DHETs have been shown to contribute to monocyte chemotaxis in presence of MCP-1 [32], indicating the possibility that the effect of sEH inhibition may be partially mediated by the lack of DHET activity. Inhibition of sEH can also prevent formation linoleate derived leukotoxin diols, which have been shown to exert greater toxicity than their corresponding epoxides through opening of mitochondrial pore transition and facilitating the release of cytochrome c [33, 34]. They also lead to increased permeability of vascular endothelium and pulmonary epithelium. Thus, investigation of the biological function of PUFA diols will result in a more comprehensive understanding of the underlying mechanism of sEH inhibition. Furthermore, sEH efficiently hydrolyzes DHA and EPA derived epoxides as well as ARA epoxides [14]. While the *in vivo* concentration of these fatty acids are dependent on diet, ARA and DHA in a brain are roughly similar in proportion compared to the concentration of EPA, which is lower than the ARA or DHA [35, 36]. The brain and plasma concentrations of EPA, DHA and their metabolites, however, are greatly responsive to dietary omega-3 supplementation [35, 37]. Therefore, biological effects of EDPs and EEQs should be taken into consideration when assessing the effect of sEH inhibition. Furthermore, the proportion of linoleic acid has increased dramatically in the

Western diet since the early 20<sup>th</sup> century, and sEH inhibition may have a notable effect on the levels of linoleate diols which are known to exert toxic effect on cells [33, 34, 38].

### Presence of sEH and bioactive epoxide in the central nervous system

Relative abundance of sEH can vary depending on species, sex, cell type, exposure to hormonal signals and inflammatory conditions among other factors [39–43]. For example, testosterone and the peroxisome proliferator-activated receptor (PPAR) agonist clofibrate have been shown to increase sEH levels in mouse kidney and liver [39]. Other PPAR agonists such as rosiglitazone and troglitazone have also been shown to increase sEH levels in murine adipose tissue but not in kidney or liver [40]. In the central nervous system (CNS), postmortem analyses of patients with disorders such as depression, bipolar disorder, schizophrenia, and Lewy body dementia show increased sEH protein expression, suggesting a possible link between sEH expression and neuroinflammation [41, 42]. A similar increase in sEH in inflamed tissue is so widely observed that the sEH protein may emerge as a marker of inflammation. Investigation of the baseline sEH expression in human CNS using immunohistochemical (IHC) staining indicates a wide distribution of sEH in brain regions including the thalamus, hypothalamus, cerebellum, hippocampus, basal ganglia, as well as in the brain stem and spinal cord which implicates a wide range of spinal and supraspinal targets for sEH inhibition [44]. In addition, sEH is present in various cell types including oligodendrocytes, endothelial cells, astrocytes, and neural cell bodies [44]. In contrast, Marowsky et al. 2009 has demonstrated through IHC staining of mouse brain that sEH appears in cerebral cortex, hippocampus, and striatum, though co-localized mostly in astrocytes rather than neurons, with the exception of the central amygdala [43]. While microglial sEH is not yet well characterized, sEH has been shown to be expressed in the BV2 microglial murine cell line [45]. Interestingly, sEH inhibition or ablation can suppresses microglial activation both *in vitro* and *in vivo*, indicating that sEH expression in microglia has a functional relevance in neuroinflammatory disorders [45]. The lack of neural sEH stain by Marowsky et al. 2009 may indicate species-specific differences in sEH localization, though it is possible that the results may also vary depending on the antibody used [46]. While sEH expression is confirmed in rat cortical astrocytes [47], recent experiments in our laboratory using methods per Morisseau et al. 2000 [48] reveal sEH activity in rat primary cortical neurons comparable to that of astrocytes (Figure 2). Inhibition of sEH has a demonstrable anti-inflammatory effect in the CNS and has been shown to act on microglia, astrocytes, and neurons to prevent neuroinflammation [42, 45, 49].

Neuroinflammation is a complex process and can be necessary for clearance of debris, glial scar, and  $\beta$ -amyloid plaque among other cellular debris that could further the inflammatory process if left unmitigated [50, 51]. While understanding the scope of sEH inhibition in the CNS is not yet complete, a growing number of investigations are now focusing on the therapeutic potential of sEH inhibition in neuroinflammatory diseases. The current knowledge surrounding the effect of sEH inhibition in various neuroinflammatory disease models is summarized in Table 1. The most recent of these has demonstrated improvements in a preclinical model of autism spectral disorder specifically murine maternal immune activation [52]. In this review, we will present the current knowledge surrounding the effect

of EpFAs and sEH inhibition in neuroinflammatory diseases with an emphasis on the possible cellular mechanisms that could underlie such effects.

## NEUROVASCULAR DISORDER

While the site, duration, and the subtype (ischemic or hemorrhagic) of stroke may differ, all cases cause a temporary disruption of cerebral blood flow. This results in a reduction of ATP synthesis and Na<sup>+</sup>/K<sup>+</sup> imbalance, glutamate excitotoxicity, and release of damage associated molecular patterns (DAMPs) by neurons that aid in recruitment of pathogenic lymphocytes such as NK cells [53–55]. In addition, stroke is associated with compromised BBB integrity, leukocyte adhesion and infiltration, iNOS upregulation, COX-2 upregulation, and inflammatory cytokine secretion [56].

### Stroke

There is evidence indicating that inhibition of sEH elicits a protective effect against ischemic stroke in both rat and mice middle cerebral artery occlusion (MCAO) models [57, 58]. For example, oral administration of sEH inhibitors AUDA or *t*-AUCB results in a significantly diminished neural apoptosis and infarct size upon MCAO of stroke-prone spontaneously hypertensive and Wistar-Kyoto rats [59, 60]. In addition, Shaik et al. 2013 observed that rats pretreated with *t*-AUCB exhibit higher behavioral and neurological scores after MCAO even though they did not observe changes in cerebral blood flow [61]. Interestingly, although Dorrance et al. 2005 did not detect higher plasma and urinary EETs concentration after AUDA treatment [59], Shaik et al. 2013 did measure higher cortical EET to DHET ratios in brain cortices of MCAO rats treated with *t*-AUCB [61]. This discrepancy between plasma and cortical EETs level could be explained by the upsurge of PLA2 activity in cortex observed during cerebral ischemia [62], which could release membrane bound EETs or ARA for CYP450 metabolism into EETs. Furthermore, Tu et al. 2018 demonstrated that TPPU treatment upon focal ischemia in rats reduces the infarct volume, improves behavioral/neurological score, and reduces the upregulation of cytokine mRNA levels TNF- $\alpha$  and IL-1 $\beta$  caused by the ischemic stress [57]. These results suggest that the reduction in inflammation may mediate the effect of sEH inhibition on the infarct size reduction, although sEH inhibition also could be preventing the neural apoptosis directly. While the post-treatment effect on the infarct size is less robust than that observed through pre-treatment using *t*-AUCB [61], the results still suggest that sEH inhibitors are viable pharmacological tools for potential use in a clinical setting to mitigate damages associated with ischemic stroke.

**EETs Increase VEGF and BDNF**—The increased expression of vascular endothelial cell growth factor (VEGF) and brain derived neurotrophic factor (BDNF) can mediate the effect of sEH inhibition. VEGF overexpression is neuroprotective against intracerebral hemorrhage in mice [63], likely through the activation of the neuronal VEGFR2 and PI3K/AKT pathway [64]. Primary rat astrocytes treated with TPPU or *t*-AUCB releases significantly more VEGF under oxygen glucose deprivation (OGD) compared to the control conditions, and the effect is blocked by the addition of the EET antagonist 14,15-EEZE, suggesting that VEGF expression is EET dependent [49]. Furthermore, treatment of endothelial progenitor cells

with *t*-AUCB increases VEGF and hypoxia-inducible factor (HIF-1 $\alpha$ ) in a dose dependent manner, an effect partially blocked by the PPAR- $\gamma$  inhibitor GW9662 [65]. Since EPA dependent increase in VEGF expression involves a GPCR and PPAR- $\gamma$  activation [66], it is plausible that EETs may also be acting in a similar fashion to induce VEGF expression. In addition, BDNF can also mediate the neuroprotective effect of sEH inhibition. For example, administration of AUDA for seven days following a MCAO treatment in mice increases the protein expression of mature BDNF, and sEH KO mice exhibit higher basal levels of BDNF receptor neuronal tropomyosin receptor kinase B (TrkB) and phosphorylated TrkB levels compared to wild type controls [67]. The effect of AUDA treatment on the MCAO infarct is abrogated by an intracerebroventricular injection of K252a, a Trk receptor antagonist [67]. While this evidence supports the view that induction of mature BDNF mediates the effect of sEH inhibition, more research is needed to exclude the possibility that other neurotrophins such as neurotrophin-3 (NT-3) and nerve growth factor (NGF) may also be involved.

Regulation of BDNF is thought to act through a positive feedback loop involving phosphorylation of TrkB, cAMP response element-binding protein (CREB), calcium response element (CaRF1) dependent factors, along with various kinases [68, 69]. As such, an increase in BDNF observed upon AUDA treatment may be mediated by or TrkB phosphorylation combined with GPCR activation and calcium influx into the cells. Interestingly, MCAO of sEH KO mice does not result in increased BDNF expression despite increased TrkB phosphorylation [67], suggesting that EETs may affect the TrkB phosphorylation state independent of BDNF.

**EETs and Leukocyte Trafficking**—Ischemic stroke can cause an accumulation of neutrophils, T-cells, and macrophage that facilitate the inflammatory response and exacerbate the outcome of a stroke [70]. The infiltration by activated T-cells and monocytes across the endothelial layer is facilitated by endothelial expression of adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin [71] as well as by chemokines such as CCL2, CCL3, CINC-1, CXCL-12, and CX<sub>3</sub>CL1 [72]. While exposure to cytokines such as TNF- $\alpha$  increase endothelial cell surface expression of VCAM-1, ICAM-1, and E-selectin, co-treatment with 11,12-EET can significantly reduce the expression of these adhesion molecules [73]. Furthermore, pretreatment of pulmonary artery endothelial cells with 11,12-EET or 14,15-EET prior to stimulation with inflammatory oxidized low-density lipoprotein results in reduction of ICAM-1, VCAM-1, and E-Selectin, as well as upregulation of CCL-2/MCP-1, both in terms of mRNA and protein expression [74]. This in turn may diminish accumulation of harmful leukocytes such as NK cells into the ischemic brain. In contrast, 5,6-EET can induce VCAM-1 and ICAM-1 expression in B-lymphocytes through TRPV4 dependent Ca<sup>2+</sup>-permeant nonselective cation channel (NSCC) activation [75], though this effect is not observed in endothelial cells [73]. Endothelial cells are also known to express TRPV4, suggesting that 5,6-EET have a cell-type specific effect on for adhesion molecule expression [76–78].

Interestingly, EETs may influence peripheral immune cells themselves, facilitating the resolution of neuroinflammation from the periphery. Incubation of THP-1 monocytic leukemia cells with 11,12-EET can attenuate IL-1 $\beta$  and phorbol-12-myristate-13-acetate

(PMA) induced COX-2 protein induction, an effect likely mediated through the activation of PPAR- $\alpha$  [79]. This result is consistent with the fact that CYP450 inhibitor SKF525A or PPAR- $\alpha$  inhibitor GW6471 are independently capable of inducing COX-2 in these cells [79]. Furthermore, a study by Gilroy et al. 2016 has shown that CYP450 inhibition disrupts the resolution of zymosan induced inflammation by facilitating monocyte and polymorphonuclear cell accumulation, explained in part by the increased mRNA expression of chemotactic proteins: CCR2, CXCR1, and CCL2 [80]. The upregulation of CCL2 is also seen *ex vivo* in cultured peritoneal lavage cells treated with zymosan, and application of 14,15-EETs significantly reduces CCL2, iNOS, and IL-12 expression but not TNF- $\alpha$  [80]. Future studies may elucidate the degree to which EpFAs modulate inflammation and leukocyte infiltration through a detailed study characterizing the leukocyte inactivation.

## NEURODEGENERATIVE DISORDERS

Neuroinflammation is a necessary process for both developing and mature brains, because it contributes to neurodevelopment during developmental stages, debris clearing upon injury, and immune conditioning against invasive pathogens in both developing and mature brains [81]. Nonetheless, neuroinflammation is thought to exacerbate the pathogenesis of neurodegenerative diseases such as the Parkinson's Disease, Alzheimer's Disease, epilepsy, and depression.

### Parkinson's Disease

Parkinson's Disease (PD) is characterized by a significant loss of dopaminergic neurons along with increased inflammatory cytokines including interleukin 1 $\beta$  (IL-1 $\beta$ ), interferon  $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor alpha (TNF- $\alpha$ ) [82, 83]. This is congruent with the fact that PD etiology includes the mutation of genes such as leucine-rich repeat kinase 2 (LRRK2) and environmental toxins such as the mitochondrial complex I inhibitor rotenone, factors that could intensify the inflammatory response in PD [84, 85]. Given this, several investigators have attempted to elucidate the therapeutic effect of sEH inhibition on PD pathogenesis.

Qin et al. 2015 has demonstrated that upon treating mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a precursor compound for the mitochondrial complex I inhibitor and redox cyler 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), the number of dopaminergic cells stained positive for tyrosine hydroxylase (TH) diminish and sEH immunoreactivity increases 14 days post treatment [86]. This neurotoxic effect of MPTP is attenuated by sEH gene knock out, pretreatment with the sEH inhibitor AUDA, and to a lesser degree 14,15-EET pretreatment. Interestingly, pretreatment with 14,15-EET *in vivo* does not elicit any additional benefit in mitigating dopaminergic TH<sup>+</sup> cell loss when administered to a sEH KO mice [86]. Ren et al. 2018 has replicated these finding using TPPU [42]. TPPU decreases the endoplasmic reticulum (ER) stress protein phosphorylation response, decreases nitric oxide synthase (iNOS) protein expression, and increases superoxide dismutase (SOD) protein expression to levels comparable to the baseline [42]. In addition, genetic ablation of sEH mitigates the OX42 expression in striatum which represents microglia, macrophage, and leukocyte accumulation in response to MPTP treatment [42]. Furthermore, *in vitro* studies

suggest that TPPU has a therapeutic effect directly on induced pluripotent stem cells (iPSC) with a parkin RBR E3 ubiquitin protein ligase (PARK2) mutation, preventing the increase in caspase-3 cleavage [42]. Finally, the effect of TPPU is prominent when administered after MPTP treatment whereas Qin et al. 2015 were unable to show therapeutic effect of the more rapidly metabolized AUDA 24-hour post MPTP [42, 86]. This may be due to the differences in the physical and chemical properties between the two sEH inhibitors affecting their pharmacokinetic profiles and bioavailability. This is reasonable considering that unlike the current optimized sEH inhibitor TPPU, AUDA was synthesized to mimic 14,15-EET with a longer alkyl side chain, giving it a unique structure distinct from modern sEH inhibitors. [87] Interpretations of biological data using AUDA as a probe is complex because it is an EET mimic as well as an sEH inhibitor.

The sEH inhibitors could act either through mitigating microglial activation or preventing the initial neural damage caused by the MPTP. In a rotenone model of PD, *in vivo* rotenone treatment causes microglia activation as well as microglial dependent neuronal damage of dopaminergic neurons [88, 89]. MPP<sup>+</sup> can also induce microglial COX-2 expression and PGE<sub>2</sub> production in a primary neuron-glia culture [90]. Genetic ablation of sEH reduces traumatic brain injury (TBI) induced microglial activation in mice, and lipopolysaccharide (LPS) and IFN- $\gamma$  induced inflammation in BV2 microglial cells is mitigated through AUDA treatment [45]. These lines of evidence suggest that sEH inhibition can act through modulating microglial activation. On the other hand, sEH inhibition can also act directly on neurons as well. For example, Lakkappa et al. 2019 has reported that N27 dopaminergic cells treated with an sEH inhibitor following a rotenone treatment exhibit a marked reduction in COX-1, COX-2, and IL-6 mRNA [91]. In addition, the authors have reported improved cell viability and increased expression of redox genes such as superoxide dismutase (SOD) and catalase (CAT) [91].

In addition to increased expression of redox genes, improved mitochondrial membrane potential may also underlie the effect of sEH inhibition. It is known that the entry of Ca<sup>2+</sup> into cells through Ca<sub>v</sub>1.3 L-type Ca<sup>2+</sup> channels intensifies mitochondrial oxidation in dopaminergic neurons, and inhibition of these Ca<sup>2+</sup> currents by a Ca<sup>2+</sup> channel blocker such as isradipine reduces mitochondrial oxidation in neurons [92]. Since 11,12-EETs block Ca<sub>v</sub>1.3 L-type Ca<sup>2+</sup> channels in a porcine cardiac muscle membrane enriched in L-type Ca<sup>2+</sup> channels [93], improvement in mitochondrial membrane potential may be mediated by a diminished Ca<sup>2+</sup> entry into dopaminergic cells [94]. Future studies should determine the relative contribution of EET mediated Ca<sub>v</sub>1.3 blockade is the modulator of neuronal mitochondrial function.

## Epilepsy

Inhibition of sEH may also play a role in mitigating seizures, since epilepsy is associated with increased inflammatory cytokine expression along with dysregulation of glutamatergic/GABAergic neurotransmission [95]. In a pilocarpine model of status epilepticus (SE) in rats, IL-1 $\beta$  protein expression increases in the frontal cortex, hippocampus, entorhinal cortex, and amygdala after SE onset, while immune cell presence such granulocytes and MHC-class II presenting microglia increase in the prefrontal cortex and hippocampus [96]. There is



evidence of cytokines such as TNF- $\alpha$  inducing seizure in human patients during a clinical trial [97] and intensifying seizures in rats that have undergone amygdala kindling [98]. If inflammation does in fact contribute to the etiology of epilepsy, then sEH inhibition may mitigate seizure propensity or intensity. To test this hypothesis, Hung et al. 2015 treated mice that had undergone pilocarpine treatment or amygdala kindling with sEH inhibitors AUDA or TPPU, as well as with sEH genetic ablation [99]. As expected, AUDA and TPPU both diminished the increase in protein expression of IL-1 $\beta$  and IL-6, and reduced I $\kappa$ B $\alpha$  phosphorylation 7 days after SE onset [99]. However, the authors observed the threshold for seizure induction in kindled mice decreased only with AUDA treatment (a joint EET mimic/sEH inhibitor) but not with treatment of the pure sEH inhibitor TPPU [99]. In addition, sEH KO mice did not exhibit decreased inflammatory cytokine levels nor mitigate the ietogenic effect in either the pilocarpine model or the kindling model [99]. Conversely, Inceoglu et al. 2013 demonstrated that sEH KO and the sEH inhibitors TPPU and TUPS are all capable of delaying GABA<sub>A</sub> receptor antagonists pentylentetrazole (PTZ) and picrotoxin (PIC) induced seizures increasing the threshold for tonic seizures, which the authors hypothesized to be due to modulation of GABAergic signaling [100]. There is evidence suggesting that 11,12-EET can induce G-protein activated inwardly rectifying K<sup>+</sup> (GIRK) channel activation in CA1 pyramidal neurons thus mitigating their excitatory post synaptic currents (EPSCs) [101]. However, such an effect is contrary to the excitatory effect of 14,15-EET in the CA1 region [102]. It may be that excitatory amino acid transporters (EAATs) are playing a role in mitigating seizures, because EAATs clear synaptic glutamate and minimize inappropriate firings and excitotoxic injuries [103, 104]. EAAT levels can be modulated by PPAR- $\gamma$  agonists rosiglitazone or 15d-PGJ<sub>2</sub> [103]. Given this, the delayed seizure onset observed by Inceoglu et al. 2013 could be explained by increased EAAT expression on astrocytes. However, further research is needed to understand how EETs and PPAR- $\gamma$  agonism may influence EAAT2 expression, and the dynamic relationship between PPAR- $\gamma$  agonists and sEH levels in the brain, because PPAR agonists are known to induce sEH in some cells [39, 40]. It should be noted that the action of the sEH inhibitors is complicated by the lack of BBB penetration of different molecules. It also could be the sEH inhibitors are acting peripherally to increase EpFA which enter the brain as free fatty acids, glycerides, or even in macrophage. The role of inflammation in seizures is not well understood, and while sEH inhibitors appear anti-inflammatory and pro-resolving molecules, they could exacerbate seizure damage at one in time and mitigate it at another.

## Depression

Inflammation is not a universal biomarker for depression; nonetheless, elevation of inflammatory cytokines in serum such as IL-6, IL-1 $\beta$ , and TNF- $\alpha$  is associated with an increased propensity for depression [105]. Neuroinflammation in depressed patients may play a role in impaired neurogenesis, activation of the hypothalamic-pituitary-adrenal axis, and increased rate of tryptophan degradation by inducing indoleamine 2,3-dioxygenase (IDO), which decreases the availability of serotonin and melatonin [106]. Genetic risk for depression may include cytokine gene polymorphs. Bull et al. 2009 show that genetic polymorphism in IL-6 genotype make patients with chronic hepatitis C viral infection less susceptible to depressive symptoms induced by TNF- $\alpha$  treatment [107]. In addition, RNA microarray of post-mortem frontal lobes of patients diagnosed with major depression has

indicated that cytokines including IFN- $\gamma$ , IL-1 $\alpha$ , IL-2, -3, -4, -5, -8 are upregulated, though interestingly the authors did not find any change in IL-6 expression [108].

The therapeutic mechanisms of antidepressants aim to increase the synaptic availability of monoamines, improve neuroplasticity and neurogenesis, increase BDNF, improve GABAergic neurotransmission, and reduce neuroinflammation [109]. Given that EETs are involved in reducing inflammation, facilitating hippocampal synapse formation, modulating neurogenesis, and increasing the production of growth factors such as BDNF and VEGF, it is reasonable to assume that sEH inhibition and/or exogenous administration of EETs may help alleviate depressive symptoms. To test this hypothesis, Ren et al. 2016 induced depression like behavior in mice through repeated social defeat stress with and without TPPU to assess the antidepressant effect of the sEH inhibitor. They observed that the mice treated with TPPU spend more time swimming in the forced swim test, have diminished social avoidance behavior compared to vehicle controls, and show increase in BDNF production in their hippocampus [41, 110]. Accordingly, the authors observed an increase in BDNF and phosphorylated TrkB level in sEH KO mice, suggesting that the antidepressant effect of sEH inhibition/ablation is mediated through the BDNF-TrkB pathway. This research was followed by Wu et al. 2017, who showed that the mice treated with TPPU exhibit increased markers of neurogenesis in the dentate gyrus as well as diminished forced swim scores upon treatment with the TrkB inhibitor K252a, confirming the results obtained by Ren et al. 2016. It is important to note, however, that Ren et al. did not find any changes in EETs concentration in hippocampus or frontal lobe of depressed mice. In possible future research, the incorporation of the EET antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (EEZE) could demonstrate whether the effects of TPPU were mediated by the effect of 14,15-EET or to a lesser degree other regioisomers of EETs [111]. However, it is possible that the action of regioisomers of EETs or EDPs may also be affected by 14,15-EEZE depending on the target protein for these EpFAs. Thus, much work is needed to test which regioisomers of any or all the EpFA classes could be the most efficacious against induced symptoms of depression.

### Alzheimer's Disease

Neurodegeneration in Alzheimer's disease (AD) is defined by the aggregation of amyloid- $\beta$  (A $\beta$ ) caused by cleavage of amyloid precursor protein (APP) primarily by  $\beta$ - and  $\gamma$ -secretases [112], accumulation of neurofibrillary tangles (NFTs) caused by tau phosphorylation, and mitochondrial dysfunction [113]. A $\beta$  aggregation is thought to exert neurotoxicity through mitochondrial complex IV inhibition or through Toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) dependent microglial activation [114]. Risk genes for AD such as apolipoprotein E4 (ApoE4) can exacerbate the inflammatory effect of A $\beta$  aggregates in microglia. However, in astrocytes ApoE4 may dampen the activation TLR, suggesting a cell type specific effect of ApoE in the brain [50, 115, 116]. Data examining the effect of sEH inhibition as therapeutic agents in an AD model is forthcoming, however published experiments with a flavonoid pinocembrin seems to have a therapeutic effect on APP/PS1 AD mice through a mechanism that may involve sEH inhibition (IC<sub>50</sub>, 2.58  $\mu$ M) [117, 118]. While four-month-old APP/PS1 mice orally administered with pinocembrin 5 days a week showed no improvement in A $\beta$  plaque

formation, they have shown increased acetylcholine levels, improved BBB integrity, reduced RAGE dependent p38 mitogen-activated protein kinases (MAPK) activation, and reduced inflammatory markers including: GFAP, Iba-1, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 [117]. It is possible that anti-inflammatory effect of pinocembrin did not have an impact on the A $\beta$  plaque clearance since astrocytic activation is vital for A $\beta$  clearance [117]. In addition, Yao et al. 2016 has shown that pretreatment of HEK293 cells transfected with human tau gene prior to H<sub>2</sub>O<sub>2</sub> treatment with TPPU significantly diminished the H<sub>2</sub>O<sub>2</sub> induced tau hyperphosphorylation at Thr231 and Ser396 [119]. While the authors claim the effect may be mediated by the PI3K/AKT pathway activation, more research is needed to determine the mechanism underlying the effect of the sEH inhibitor TPPU. Regardless, the ability of TPPU to prevent tau hyperphosphorylation provides a clear indication that sEH inhibition may delay or mitigate AD pathology *in vivo*.

**Effect of A $\beta$  on Production of EETs**—Brain CYP450 levels change depending on various factors including glutamate availability or inflammation [120, 121] and the inflammatory conditions in AD may affect the endogenous levels of EETs in the CNS. The oxylipin profile in an AD brain remains to be elucidated, however, when isolated rat brain microsomes along with primary astrocyte and hippocampal neuronal cultures are treated with A $\beta$ 42, CYP450 activity seem to decrease as total EET and DHET levels diminish [122]. In contrast, CYP450 metabolites including EETs are increased in the hippocampus of hAPP transgenic mice overexpressing human APP, though this can be attributed to the MEK/MAPK dependent PLA2 activation in response to A $\beta$  as opposed to increase in CYP450 activity [123]. Mutating PLA2 mitigates hyperactivity, anxiety-related behavior, and premature mortality observed in hAPP mice, indicating that ARA metabolism dysregulation may contribute to neurodegeneration in hAPP mice. More research is necessary to determine how sEH inhibition would affect the level of EETs and other EpFAs including their amides in the dysregulated state.

**PUFAs and Hippocampal function**—Proper hippocampal development is disrupted in AD pathogenesis [124], and epoxyeicosanoids such as EETs, DHA derived EDPs, and other EpFAs may serve as therapeutic tools to maintain hippocampal function. For example, 14,15-EET – but not 14,15-DHET – facilitates TPRV4 mediated calcium flux in primary rat hippocampal culture, promoting neurite outgrowth and axon maturation [125]. In addition, incubation of prefrontal cortex slice with 14,15-EETs or AUDA for 10 minutes enhances long-term potentiation and glutamate transmission, suggesting that EETs may play a role in synapse formation in a healthy brain [126]. Furthermore, the level of oxylipins earlier in the developmental stages can modulate hippocampal neurodevelopment by affecting the differentiation of neural stem cells (NSCs) [127]. *In vitro* application of selected individual regioisomers on NSCs indicate that application of ARA derived 11,12-EET to NSCs promote glial differentiation of NSCs, while the application of DHA derived 16,17-EpDPE promotes neural differentiation of NSCs [127]. Finally, the antiinflammatory effect of EETs or EDPs may affect neural differentiation of NSCs as well, as inflammatory cytokines in the CNS such as IL-6 can disrupt hippocampal neurogenesis [128]. While the effect of EpFAs on hippocampal neurogenesis needs further investigation, sEH inhibition is a promising avenue for stimulating neurogenesis and synaptogenesis in the hippocampus of AD patients.

Both an advantage and a caution stemming from these observations is that sEH inhibition will stabilize all EpFA so far tested, with relative stabilization depending on substrate concentration and structure [14].

## CELL AND MOLECULAR MECHANISMS

Many studies indicate that the mechanisms of the action of EETs and other EpFA are diverse, involving PPAR $\alpha/\gamma$ , TRPV4, Na<sup>+</sup>, BK<sub>Ca</sub>, L-type Ca<sup>2+</sup> channel, and thromboxane receptor agonism among others; however, it is unclear what the primary mechanism of EETs could be in neuroinflammatory disorders [23, 129]. Potential mechanisms underlying EETs or sEHs are summarized in Table 2. EETs mediated suppression of inflammation involves reduced activation of NF- $\kappa$ B, reduced phosphorylation of p38, and suppression of I $\kappa$ B $\alpha$  degradation [74]. Node et al. 1999 has demonstrated that TNF- $\alpha$  induced nuclear translocation of NF- $\kappa$ B in endothelial cells is blocked in the presence of 11,12-EET, and that both 11,12-EET and 14,15-EET prevents the degradation of I $\kappa$ B- $\alpha$  though inhibition of I $\kappa$ B kinase (IKK) activity [73]. Phosphorylation of I $\kappa$ B- $\alpha$  and nuclear translocation of NF- $\kappa$ B decreases with sEH inhibition in murine hearts from a transverse aortic constriction (TAC) model and cardiomyocytes, supporting the idea that IKK inhibition mediates the effect of EETs [130] While the regulation of IKK and I $\kappa$ B $\alpha$  is multifaceted, enzyme complex PP2A is implicated in inhibiting the kinase activity of IKK $\beta$ , the IKK subunit that mediates the canonical NF- $\kappa$ B pathway activation by proinflammatory stimuli [131, 132]. Since 11,12-EET, sEH KO, and *l*-AUCB treatment have been shown to upregulate PP2A activity in the cardiovascular system [133–135], PP2A activation may underlie the inhibition of IKK activity by EETs. However, more research is needed to elucidate how EETs and other EpFAs regulate the activities of IKK and NF- $\kappa$ B. While there are EpFA responsive GPCRs such as the newly identified GPR132 that is activated by EETs [136], there is evidence suggesting that GPR132 expression correlates with increasing inflammatory markers such as CCL-2, MMP-9, and COX-2 in breast cancer lesions [137]. Thus, its involvement in the anti-inflammatory effect of EETs may not be probable. Alternatively, EETs can exert their anti-apoptotic effect through ameliorating the mitochondrial damage. For example, sEH inhibition and exogenous EET administration are shown to protect mitochondrial membrane potential from dissipating under laser-induced oxidative stress, as well as to preserve mitochondrial functional activity under starvation conditions [138, 139]. EETs are also shown to exert cardioprotective effects through activating the mitochondrial K<sub>ATP</sub> channels, though more research is needed to determine its impact on mitochondrial health [140]. Preserving mitochondrial health through EETs could mitigate reactive oxygen species (ROS) production and release and the ER Stress response, ultimately contributing to the attenuation of multiple cellular damages associated with neuroinflammatory disorders [141–143].

Inceoglu et al. 2008 previously described that, in addition to mitigating LPS induced COX-2 mRNA expression, EETs can induce spinal steroidogenic acute regulatory protein (StARD1) mRNA expression possibly through the cAMP signaling pathway [144, 145]. Such increase in StARD1 can contribute to reduction in inflammatory response through increased mitochondrial production of 25-hydroxycholesterol, 27-hydroxycholesterol, and 5-cholesten-3 $\beta$ ,25diol 3-sulfonate (25HC3S) [145, 146], which is thought to exert its anti-inflammatory and proliferative effects through PPAR- $\gamma$  binding [147]. This effect was

observed by Chen et al. 2018 as well, where stroke induced by injection of type IV collagenase into rat thalamus, a model for central pain, caused significant decrease in the StAR mRNA expression in the perilesion site of thalamus. In addition, an increase in paw sensitivity to mechanical stimuli was rescued by daily microinjection of 0.1 ug 14,15-EET for 3 days using an implanted canula [148]. However, since EETs are also known to activate PPAR- $\gamma$  [149], it is unclear the magnitude by which the EETs mediated induction of StAR affects PPAR activity.

### ER Stress in Neuroinflammation

Altering the ER stress pathway may be part of the therapeutic mechanism of EpFAs against neuroinflammatory disorders, since the inflammatory and ER stress pathways have shared molecular components. For example, Hu et al., 2006 demonstrated that an increase in proinflammatory cytokines such as TNF- $\alpha$  causes degradation of I $\kappa$ B $\alpha$  through association of IKK with ER stress sensor inositol-requiring enzyme (IRE1 $\alpha$ ) and TNF receptor associated factor (TRAF2) in the human breast cancer cell line MCF-7 [150]. This activation of IRE1 $\alpha$  and TRAF2 is sufficient to induce NF- $\kappa$ B independent of TNF $\alpha$ , because ER stressors tunicamycin and thapsigargin activate NF- $\kappa$ B even when cells are treated with TNFR1-siRNA to block TNF signaling [150]. EETs are known to affect the phosphorylation state of these ER stress proteins. For example, Bettiab et al., 2013 found that ER stress markers associated with consumption of high fat diet such as phosphorylation of IRE1 and ATF6 is significantly diminished in sEH KO mice. In addition, Inceoglu et al. 2015 has also observed a reduction in ER stress proteins in sciatic nerve upon systemic administration of TPPU in a tunicamycin rat model [151]. Similar results were obtained *in vitro* with sEH inhibition in HepG2 cells treated with thapsigargin [152]. Involvement of EETs in ER stress was also observed in human bronchial epithelial cells treated with cigarette smoke extracts, where the loss of viability and increased phosphorylation of ER stress proteins was reversed with pretreatment of 14,15-EET but not with 14,15-EEZE co-administration [153].

While the mechanisms of action of EETs on the ER stress pathway remain to be elucidated, there are several points of evidence. EETs may act partly through the AMPK-AKT pathway, as exposure of neonatal myocytes and HL-1 cardiac muscle cells to 14,15-EETs upon hypoxia/reoxygenation treatment results in an increased phosphorylation of AKT and increased PIP<sub>3</sub> concentration, indicating PI3K activity [154]. Activation of AKT can prevent ER-stress induced cell death through upregulating inhibitors of apoptotic protein (IAP) family proteins cIAP-2 and XIAP [155], or through upregulation of mitochondrial Bcl-2, [156, 157] though the evidence of direct interaction has not yet been elucidated.

Regardless, the observed reduction in ER stress protein phosphorylation upon sEH inhibition indicates an integral role of EETs and other EpFAs against ER dysfunction. ER stress is a hallmark of many neuroinflammatory disorders including PD and AD [158–160], induced by number of factors including high glucose levels and exposure to ROS generating mitochondrial toxins [161, 162]. Clarifying the nature of involvement of EETs on ER stress is vital to our understanding of the mechanism of EETs and sEH inhibition.

## CYP450 Derived Endocannabinoids: Beyond EETs

The primary focus of many researchers investigating the mechanism underlying sEH inhibition is on EETs. However, increasingly the literature refers broadly to EpFA because the term EETs is reserved for the monoepoxides of arachidonic acid. Inhibition of sEH can also involve the endocannabinoid (ECB) system, which has an anti-inflammatory component that is investigated vigorously to combat neuroinflammation [163, 164]. For example, Lastres-Becker et al. 2005 has shown that cannabinoid (CB) receptor agonists such as <sup>9</sup> THC are neuroprotective against 6-hydroxydopamine induce dopaminergic cell death *in vivo* [163]. However, using a general CB agonist such as HU210 is not optimal as a long-term treatment because CB<sub>1</sub> receptor agonism may produce undesirable side effects such as impaired working memory [165]. A CB<sub>2</sub> agonist JWH-015 has a profound effect on IFN- $\gamma$  induced CD40 expression as well as LPS induced TNF- $\alpha$  secretion in primary murine microglial culture, suggesting that CB<sub>2</sub> agonism may be a potent tool to combat neuroinflammatory conditions [166].

Inceoglu et al. 2007 has previously demonstrated that 5,6-EET can bind to CB<sub>2</sub> receptors, implicating a potential mechanism by which EETs and EET analogues could exert their effects on cells [167]. EET analogues are present in the ECB system; for example, anandamide (AEA) can be metabolized by the orphan enzyme CYP4X1 to one of 4 regioisomers of epoxyeicosatrienoic acid ethanolamide (EET-EA), whereas 2-arachidonoylglycerol (2-AG) can be metabolized to 14,15- and 11,12- epoxyeicosatrienoic glycerols (EET-G) by the CYP2J family [168, 169]. This can potentially shift the affinity of these ECBs to primarily favor CB<sub>2</sub> binding over CB<sub>1</sub> as well as improve their resistance to degradation. Illustrating this point, Snider et al. 2009 has shown that 5,6-EET-EA can be formed from AEA by microglia, and it has several orders of magnitude greater affinity for CB<sub>2</sub> receptor compared to AEA. The 5,6-EET-EA affinity for CB<sub>1</sub> receptor is diminished compared to AEA but it has an increased half-life from 14.3 minutes to 32.2 minutes when incubated in a mouse brain homogenate [170]. Similarly, epoxidation of EPA ethanolamide (EPEA) at the 17,18- position increases its potency against CB<sub>2</sub> receptors while lessening its potency for CB<sub>1</sub> receptors [171]. This effect is not observed for DHA derived 19,20-EDP-EA [171], possibly indicating that the epoxidation of eicosanoid ethanolamides in particular shifts their CB receptor affinity to favor CB<sub>2</sub> receptors. Because EET-EAs are ligands for sEH analogous to EETs, combination of sEH inhibition with fatty acid amide hydrolase (FAAH) inhibitors or monoglyceride lipase (MGL) inhibitors can potentially increase CB<sub>2</sub> selective EET-EAs [172]. In fact, Sasso et al., 2016 demonstrates that sEH and FAAH dual inhibition has a synergistic antinociceptive effect against carrageenan induced inflammatory pain and neuropathic pain, likely due to increased EET-EAs that possess significantly greater CB<sub>2</sub> affinity [173]. Finally, pretreating BV-2 microglia with 17,18-EEQ-EA and 19,20-EEQ-EA significantly reduces LPS induced IL-6 production and nitrite production through a CB mediated mechanism [171]. Further research may find that the dual inhibition of sEH and FAAH and/or MGL could increase the concentration of these CB<sub>2</sub> activating EpFAs, mitigating neuroinflammation beyond the effect of sEH inhibition alone.

## CONCLUSION

Neuroinflammation is a necessary process for debris clearance and proper neurodevelopment. However, it is evident that sEH and exogenous administration of EETs can ameliorate damage associated with inflammatory disease models such as PD, depression, stroke, and epilepsy. They act through mechanisms that can involve PPARs, GPCRs, receptor tyrosine kinase (RTK) phosphorylation, and modulation of ion channels. Through these mechanisms, EpFAs and sEH inhibition may diminish intracellular ROS levels and ER stress mediated apoptosis, neuronal DAMP and cytokine release, and subsequent activation of glial cells such as astrocytes and microglia. Preventing glial activation mitigates the induction of inflammatory cytokines, chemokines, and cell adhesion molecules, in addition to averting disruption of glutamate/GABA homeostasis, all of which exacerbate neurodegeneration. Finally, further research may yield positive results for other neuroinflammatory disorders such as amyotrophic lateral sclerosis (ALS) because an ALS hallmark is increased ROS attributed to superoxide dismutase 1 (SOD1) mutation, mitochondrial and ER calcium dysregulation, downregulation of astrocytic EAAT2 and potential glutamate excitotoxicity [174] that could potentially be mitigated through sEH inhibition.

Wide spanning function of sEH against all classes of EpFA complicates the task of elucidating specific conclusions about the effect of EETs. By testing EDPs and EEQs in the same model systems, using sEH KO in conjunction with sEH inhibitors, utilizing more than one sEH inhibitor, and distinguishing the effects of all four regioisomers of EETs, we can develop a more accurate view of the effect of EpFAs against neuroinflammatory diseases. In addition, while the discovery of a putative EET binding GPCR such as GPR132 is helpful in clarifying the mechanism underlying EETs, further exploration of a more selective GPCR for EETs regioisomers could shine light upon the functional distinction between EET regioisomers. In conclusion, sEH inhibition has been shown to be therapeutic against a wide array of disease models with neuroinflammation as a common pathology and neuronal loss possibly through multiple anti-inflammatory and pro-survival signaling pathways. Investigating the effect of sEH inhibitors against other neuroinflammatory diseases such as ALS or AD is therefore a promising avenue for further discoveries in both basic science as well as disease diagnosis.

## Acknowledgments

This work was supported by the National Institute of Environmental Health Sciences (NIEHS) Grant R01 ES002710, NIEHS Superfund Research Program P42 ES004699, NIEHS T32 ES007059 (J.A.), and AMED JP18dm0107119 (K.H.) The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## References

- [1]. Nebert DW, Dalton TP The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 6(12) (2006) 947–60. [PubMed: 17128211]
- [2]. Capdevila JH, Falck JR The CYP P450 arachidonic acid monooxygenases: from cell signaling to blood pressure regulation. *Biochem Biophys Res Commun* 285(3) (2001) 571–6. [PubMed: 11453630]

- [3]. Konkel A, Schunck WH Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim Biophys Acta* 1814(1) (2011) 210–22. [PubMed: 20869469]
- [4]. Funk CD Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294(5548) (2001) 1871–5. [PubMed: 11729303]
- [5]. Nasrallah R, Clark J, Hebert RL Prostaglandins in the kidney: developments since Y2K. *Clin Sci (Lond)* 113(7) (2007) 297–311. [PubMed: 17760567]
- [6]. Sarau HM, Ames RS, Chambers J, Ellis C, Elshourbagy N, Foley JJ, Schmidt DB, Muccitelli RM, Jenkins O, Murdock PR, Herrity NC, Halsey W, Sathe G, Muir AI, Nuthulaganti P, Dytko GM, Buckley PT, Wilson S, Bergsma DJ, Hay DW Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol Pharmacol* 56(3) (1999) 657–63. [PubMed: 10462554]
- [7]. Samii A, Etmnan M, Wiens MO, Jafari S NSAID use and the risk of Parkinson's disease: systematic review and meta-analysis of observational studies. *Drugs Aging* 26(9) (2009) 769–79. [PubMed: 19728750]
- [8]. Vlad SC, Miller DR, Kowall NW, Felson DT Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology* 70(19) (2008) 1672–7. [PubMed: 18458226]
- [9]. Jatana M, Giri S, Ansari MA, Elango C, Singh AK, Singh I, Khan M Inhibition of NF-kappaB activation by 5-lipoxygenase inhibitors protects brain against injury in a rat model of focal cerebral ischemia. *J Neuroinflammation* 3 (2006) 12. [PubMed: 16689995]
- [10]. Firuzi O, Zhuo J, Chinnici CM, Wisniewski T, Pratico D 5-Lipoxygenase gene disruption reduces amyloid-beta pathology in a mouse model of Alzheimer's disease. *FASEB J* 22(4) (2008) 1169–78. [PubMed: 17998412]
- [11]. Kang KH, Liou HH, Hour MJ, Liou HC, Fu WM Protection of dopaminergic neurons by 5-lipoxygenase inhibitor. *Neuropharmacology* 73 (2013) 380–7. [PubMed: 23800665]
- [12]. Spector AA, Fang X, Snyder GD, Weintraub NL Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog Lipid Res* 43(1) (2004) 55–90. [PubMed: 14636671]
- [13]. Fang X, Weintraub NL, Stoll LL, Spector AA Epoxyeicosatrienoic acids increase intracellular calcium concentration in vascular smooth muscle cells. *Hypertension* 34(6) (1999) 1242–6. [PubMed: 10601125]
- [14]. Morisseau C, Inceoglu B, Schmelzer K, Tsai HJ, Jinks SL, Hegedus CM, Hammock BD Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. *J Lipid Res* 51(12) (2010) 3481–90. [PubMed: 20664072]
- [15]. Zhang G, Panigrahy D, Mahakian LM, Yang J, Liu JY, Stephen Lee KS, Wettersten HI, Ulu A, Hu X, Tam S, Hwang SH, Ingham ES, Kieran MW, Weiss RH, Ferrara KW, Hammock BD Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. *Proc Natl Acad Sci U S A* 110(16) (2013) 6530–5. [PubMed: 23553837]
- [16]. Kroetz DL, Xu F Regulation and inhibition of arachidonic acid omega-hydroxylases and 20-HETE formation. *Annu Rev Pharmacol Toxicol* 45 (2005) 413–38. [PubMed: 15822183]
- [17]. Han X, Zhao X, Lan X, Li Q, Gao Y, Liu X, Wan J, Yang Z, Chen X, Zang W, Guo AM, Falck JR, Koehler RC, Wang J 20-HETE synthesis inhibition promotes cerebral protection after intracerebral hemorrhage without inhibiting angiogenesis. *J Cereb Blood Flow Metab* (2018) 271678X18762645.
- [18]. Williams JM, Murphy S, Burke M, Roman RJ 20-hydroxyeicosatetraenoic acid: a new target for the treatment of hypertension. *J Cardiovasc Pharmacol* 56(4) (2010) 336–44. [PubMed: 20930591]
- [19]. Hu S, Kim H Activation of K<sup>+</sup> channel in vascular smooth muscles by cytochrome P450 metabolites of arachidonic acid. *European Journal of Pharmacology* 230(2) (1993) 215–221. [PubMed: 8422904]
- [20]. Earley S, Heppner TJ, Nelson MT, Brayden JE TRPV4 forms a novel Ca<sup>2+</sup> signaling complex with ryanodine receptors and BKCa channels. *Circ Res* 97(12) (2005) 1270–9. [PubMed: 16269659]
- [21]. Satoh T, Cohen HT, Katz AI Intracellular signaling in the regulation of renal Na-K-ATPase. II. Role of eicosanoids. *J Clin Invest* 91(2) (1993) 409–15. [PubMed: 8381820]



- [22]. Carroll MA, Doumad AB, Li J, Cheng MK, Falck JR, McGiff JC Adenosine2A receptor vasodilation of rat preglomerular microvessels is mediated by EETs that activate the cAMP/PKA pathway. *Am J Physiol Renal Physiol* 291(1) (2006) F155–61. [PubMed: 16478979]
- [23]. Behm DJ, Ogbonna A, Wu C, Burns-Kurtis CL, Douglas SA Epoxyeicosatrienoic acids function as selective, endogenous antagonists of native thromboxane receptors: identification of a novel mechanism of vasodilation. *J Pharmacol Exp Ther* 328(1) (2009) 231–9. [PubMed: 18836067]
- [24]. Fitzpatrick FA, Soberman R Regulated formation of eicosanoids. *J Clin Invest* 107(11) (2001) 1347–51. [PubMed: 11390414]
- [25]. Spector AA Arachidonic acid cytochrome P450 epoxygenase pathway. *J Lipid Res* 50 Suppl (2009) S52–6. [PubMed: 18952572]
- [26]. Spector AA, Kim HY Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. *Biochim Biophys Acta* 1851(4) (2015) 356–65. [PubMed: 25093613]
- [27]. Graves JP, Edin ML, Bradbury JA, Gruzdev A, Cheng J, Lih FB, Masinde TA, Qu W, Clayton NP, Morrison JP, Tomer KB, Zeldin DC Characterization of four new mouse cytochrome P450 enzymes of the CYP2J subfamily. *Drug Metab Dispos* 41(4) (2013) 763–73. [PubMed: 23315644]
- [28]. Chacos N, Capdevila J, Falck JR, Manna S, Martin-Wixtrom C, Gill SS, Hammock BD, Estabrook RW The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase. *Arch Biochem Biophys* 223(2) (1983) 639–48. [PubMed: 6859878]
- [29]. Fang X, Weintraub NL, Oltman CL, Stoll LL, Kaduce TL, Harmon S, Dellsperger KC, Morisseau C, Hammock BD, Spector AA Human coronary endothelial cells convert 14,15-EET to a biologically active chain-shortened epoxide. *Am J Physiol Heart Circ Physiol* 283(6) (2002) H2306–14. [PubMed: 12388281]
- [30]. Liu JY, Lin YP, Qiu H, Morisseau C, Rose TE, Hwang SH, Chiamvimonvat N, Hammock BD Substituted phenyl groups improve the pharmacokinetic profile and anti-inflammatory effect of urea-based soluble epoxide hydrolase inhibitors in murine models. *Eur J Pharm Sci* 48(4–5) (2013) 619–27. [PubMed: 23291046]
- [31]. Ostermann AI, Herbers J, Willenberg I, Chen R, Hwang SH, Greite R, Morisseau C, Gueler F, Hammock BD, Schebb NH Oral treatment of rodents with soluble epoxide hydrolase inhibitor 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU): Resulting drug levels and modulation of oxylipin pattern. *Prostaglandins & other lipid mediators* 121(Pt A) (2015) 131–7. [PubMed: 26117215]
- [32]. Kundu S, Roome T, Bhattacharjee A, Carnevale KA, Yakubenko VP, Zhang R, Hwang SH, Hammock BD, Cathcart MK Metabolic products of soluble epoxide hydrolase are essential for monocyte chemotaxis to MCP-1 in vitro and in vivo. *J Lipid Res* 54(2) (2013) 436–47. [PubMed: 23160182]
- [33]. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat Med* 3(5) (1997) 562–6. [PubMed: 9142128]
- [34]. Sisemore MF, Zheng J, Yang JC, Thompson DA, Plopper CG, Cortopassi GA, Hammock BD Cellular characterization of leukotoxin diol-induced mitochondrial dysfunction. *Arch Biochem Biophys* 392(1) (2001) 32–7. [PubMed: 11469791]
- [35]. Wood JT, Williams JS, Pandarinathan L, Janero DR, Lammi-Keefe CJ, Makriyannis A Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. *J Lipid Res* 51(6) (2010) 1416–23. [PubMed: 20071693]
- [36]. Brenna JT, Diau GY The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids* 77(5–6) (2007) 247–50. [PubMed: 18023566]
- [37]. Fischer R, Konkel A, Mehling H, Blossey K, Gapelyuk A, Wessel N, von Schacky C, Dechend R, Muller DN, Rothe M, Luft FC, Weylandt K, Schunck WH Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway. *J Lipid Res* 55(6) (2014) 1150–64. [PubMed: 24634501]

- [38]. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am J Clin Nutr* 93(5) (2011) 950–62. [PubMed: 21367944]
- [39]. Pinot F, Grant DF, Spearow JL, Parker AG, Hammock BD Differential regulation of soluble epoxide hydrolase by clofibrate and sexual hormones in the liver and kidneys of mice. *Biochem Pharmacol* 50(4) (1995) 501–8. [PubMed: 7646556]
- [40]. De Taeye BM, Morisseau C, Coyle J, Covington JW, Luria A, Yang J, Murphy SB, Friedman DB, Hammock BB, Vaughan DE Expression and regulation of soluble epoxide hydrolase in adipose tissue. *Obesity (Silver Spring)* 18(3) (2010) 489–98. [PubMed: 19644452]
- [41]. Ren Q, Ma M, Ishima T, Morisseau C, Yang J, Wagner KM, Zhang J.-c., Yang C, Yao W, Dong C Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress. *Proceedings of the National Academy of Sciences* 113(13) (2016) E1944–E1952.
- [42]. Ren Q, Ma M, Yang J, Nonaka R, Yamaguchi A, Ishikawa K.-i., Kobayashi K, Murayama S, Hwang SH, Saiki S Soluble epoxide hydrolase plays a key role in the pathogenesis of Parkinson's disease. *Proceedings of the National Academy of Sciences* 115(25) (2018) E5815–E5823.
- [43]. Marowsky A, Burgener J, Falck JR, Fritschy JM, Arand M Distribution of soluble and microsomal epoxide hydrolase in the mouse brain and its contribution to cerebral epoxyeicosatrienoic acid metabolism. *Neuroscience* 163(2) (2009) 646–61. [PubMed: 19540314]
- [44]. Sura P, Sura R, Enayetallah AE, Grant DF Distribution and expression of soluble epoxide hydrolase in human brain. *J Histochem Cytochem* 56(6) (2008) 551–9. [PubMed: 18319271]
- [45]. Hung TH, Shyue SK, Wu CH, Chen CC, Lin CC, Chang CF, Chen SF Deletion or inhibition of soluble epoxide hydrolase protects against brain damage and reduces microglia-mediated neuroinflammation in traumatic brain injury. *Oncotarget* 8(61) (2017) 103236–103260. [PubMed: 29262558]
- [46]. Gilda JE, Ghosh R, Cheah JX, West TM, Bodine SC, Gomes AV Western Blotting Inaccuracies with Unverified Antibodies: Need for a Western Blotting Minimal Reporting Standard (WBMRS). *PLoS One* 10(8) (2015) e0135392. [PubMed: 26287535]
- [47]. Rawal S, Morisseau C, Hammock BD, Shivachar AC Differential subcellular distribution and colocalization of the microsomal and soluble epoxide hydrolases in cultured neonatal rat brain cortical astrocytes. *J Neurosci Res* 87(1) (2009) 218–27. [PubMed: 18711743]
- [48]. Morisseau C, Beetham JK, Pinot F, Debernard S, Newman JW, Hammock BD Cress and potato soluble epoxide hydrolases: purification, biochemical characterization, and comparison to mammalian enzymes. *Arch Biochem Biophys* 378(2) (2000) 321–32. [PubMed: 10860549]
- [49]. Zhang Y, Hong G, Lee KS, Hammock BD, Gebremedhin D, Harder DR, Koehler RC, Sapirstein A Inhibition of soluble epoxide hydrolase augments astrocyte release of vascular endothelial growth factor and neuronal recovery after oxygen-glucose deprivation. *J Neurochem* 140(5) (2017) 814–825. [PubMed: 28002622]
- [50]. Guo S, Wang S, Kim WJ, Lee SR, Frosch MP, Bacskai BJ, Greenberg SM, Lo EH Effects of apoE isoforms on beta-amyloid-induced matrix metalloproteinase-9 in rat astrocytes. *Brain Res* 1111(1) (2006) 222–6. [PubMed: 16919608]
- [51]. Jin X, Yamashita T Microglia in central nervous system repair after injury. *J Biochem* 159(5) (2016) 491–6. [PubMed: 26861995]
- [52]. Ma M, Ren Q, Yang J, Zhang K, Xiong Z, Ishima T, Pu Y, Hwang SH, Toyoshima M, Iwayama Y, Hisano Y, Yoshikawa T, Hammock BD, Hashimoto K Key role of soluble epoxide hydrolase in the neurodevelopmental disorders of offspring after maternal immune activation. *Proc Natl Acad Sci U S A* 116(14) (2019) 7083–7088. [PubMed: 30890645]
- [53]. Yushmanov VE, Kharlamov A, Yanovski B, LaVerde G, Boada FE, Jones SC Correlated sodium and potassium imbalances within the ischemic core in experimental stroke: a <sup>23</sup>Na MRI and histochemical imaging study. *Brain Res* 1527 (2013) 199–208. [PubMed: 23792152]
- [54]. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF Pathogenic mechanisms following ischemic stroke. *Neurol Sci* 38(7) (2017) 1167–1186. [PubMed: 28417216]

- [55]. Gan Y, Liu Q, Wu W, Yin JX, Bai XF, Shen R, Wang Y, Chen J, La Cava A, PoursineLaurent J, Yokoyama W, Shi FD Ischemic neurons recruit natural killer cells that accelerate brain infarction. *Proc Natl Acad Sci U S A* 111(7) (2014) 2704–9. [PubMed: 24550298]
- [56]. del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol* 10(1) (2000) 95–112. [PubMed: 10668900]
- [57]. Tu R, Armstrong J, Lee KSS, Hammock BD, Sapirstein A, Koehler RC Soluble epoxide hydrolase inhibition decreases reperfusion injury after focal cerebral ischemia. *Sci Rep* 8(1) (2018) 5279. [PubMed: 29588470]
- [58]. Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, Morisseau C, Luria A, Hammock BD, Falck JR, Alkayed NJ Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J Cereb Blood Flow Metab* 27(12) (2007) 1931–40. [PubMed: 17440491]
- [59]. Dorrance AM, Rupp N, Pollock DM, Newman JW, Hammock BD, Imig JD An epoxide hydrolase inhibitor, 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA), reduces ischemic cerebral infarct size in stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 46(6) (2005) 842–8. [PubMed: 16306811]
- [60]. Simpkins AN, Rudic RD, Schreihofner DA, Roy S, Manhiani M, Tsai HJ, Hammock BD, Imig JD Soluble epoxide inhibition is protective against cerebral ischemia via vascular and neural protection. *Am J Pathol* 174(6) (2009) 2086–95. [PubMed: 19435785]
- [61]. Shaik JS, Ahmad M, Li W, Rose ME, Foley LM, Hitchens TK, Graham SH, Hwang SH, Hammock BD, Poloyac SM Soluble epoxide hydrolase inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid is neuroprotective in rat model of ischemic stroke. *Am J Physiol Heart Circ Physiol* 305(11) (2013) H1605–13. [PubMed: 24043255]
- [62]. Muralikrishna Adibhatla R, Hatcher JF Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic Biol Med* 40(3) (2006) 376–87. [PubMed: 16443152]
- [63]. Lee HJ, Kim KS, Park IH, Kim SU Human neural stem cells over-expressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model. *PLoS One* 2(1) (2007) e156. [PubMed: 17225860]
- [64]. Jin KL, Mao XO, Greenberg DA Vascular endothelial growth factor rescues HN33 neural cells from death induced by serum withdrawal. *J Mol Neurosci* 14(3) (2000) 197–203. [PubMed: 10984196]
- [65]. Xu DY, Davis BB, Wang ZH, Zhao SP, Wasti B, Liu ZL, Li N, Morisseau C, Chiamvimonvat N, Hammock BD A potent soluble epoxide hydrolase inhibitor, t-AUCB, acts through PPARgamma to modulate the function of endothelial progenitor cells from patients with acute myocardial infarction. *Int J Cardiol* 167(4) (2013) 1298–304. [PubMed: 22525341]
- [66]. Hasan AU, Ohmori K, Konishi K, Igarashi J, Hashimoto T, Kamitori K, Yamaguchi F, Tsukamoto I, Uyama T, Ishihara Y, Noma T, Tokuda M, Kohno M Eicosapentaenoic acid upregulates VEGF-A through both GPR120 and PPARgamma mediated pathways in 3T3-L1 adipocytes. *Mol Cell Endocrinol* 406 (2015) 10–8. [PubMed: 25697344]
- [67]. Chang LH, Lin HC, Huang SS, Chen IC, Chu KW, Chih CL, Liang YW, Lee YC, Chen YY, Lee YH, Lee IH Blockade of soluble epoxide hydrolase attenuates post-ischemic neuronal hyperexcitation and confers resilience against stroke with TrkB activation. *Sci Rep* 8(1) (2018) 118. [PubMed: 29311641]
- [68]. Tao X, West AE, Chen WG, Corfas G, Greenberg ME A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron* 33(3) (2002) 383–95. [PubMed: 11832226]
- [69]. Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res* 1067(1) (2006) 1–12. [PubMed: 16376315]
- [70]. Chamorro A, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R The immunology of acute stroke. *Nat Rev Neurol* 8(7) (2012) 401–10. [PubMed: 22664787]
- [71]. Jordan J, Segura T, Brea D, Galindo MF, Castillo J Inflammation as therapeutic objective in stroke. *Curr Pharm Des* 14(33) (2008) 3549–64. [PubMed: 19075732]

- [72]. Minami M, Katayama T, Satoh M Brain cytokines and chemokines: roles in ischemic injury and pain. *J Pharmacol Sci* 100(5) (2006) 461–70. [PubMed: 16682788]
- [73]. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK Antiinflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285(5431) (1999) 1276–9. [PubMed: 10455056]
- [74]. Jiang JX, Zhang SJ, Liu YN, Lin XX, Sun YH, Shen HJ, Yan XF, Xie QM EETs alleviate ox-LDL-induced inflammation by inhibiting LOX-1 receptor expression in rat pulmonary arterial endothelial cells. *Eur J Pharmacol* 727 (2014) 43–51. [PubMed: 24486707]
- [75]. Liu QH, Liu X, Wen Z, Hondowicz B, King L, Monroe J, Freedman BD Distinct calcium channels regulate responses of primary B lymphocytes to B cell receptor engagement and mechanical stimuli. *J Immunol* 174(1) (2005) 68–79. [PubMed: 15611229]
- [76]. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424(6947) (2003) 434–8. [PubMed: 12879072]
- [77]. Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. *Circ Res* 99(9) (2006) 988–95. [PubMed: 17008604]
- [78]. Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MI, Heppner TJ, Hill-Eubanks DC, Nelson MT Elementary Ca<sup>2+</sup> signals through endothelial TRPV4 channels regulate vascular function. *Science* 336(6081) (2012) 597–601. [PubMed: 22556255]
- [79]. Bystrom J, Wray JA, Sugden MC, Holness MJ, Swales KE, Warner TD, Edin ML, Zeldin DC, Gilroy DW, Bishop-Bailey D Endogenous epoxygenases are modulators of monocyte/macrophage activity. *PLoS One* 6(10) (2011) e26591. [PubMed: 22028915]
- [80]. Gilroy DW, Edin ML, De Maeyer RP, Bystrom J, Newson J, Lih FB, Stables M, Zeldin DC, Bishop-Bailey D CYP450-derived oxylipins mediate inflammatory resolution. *Proc Natl Acad Sci U S A* 113(23) (2016) E3240–9. [PubMed: 27226306]
- [81]. DiSabato DJ, Quan N, Godbout JP Neuroinflammation: the devil is in the details. *J Neurochem* 139 Suppl 2 (2016) 136–153. [PubMed: 26990767]
- [82]. Hirsch EC, Hunot S Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol* 8(4) (2009) 382–97. [PubMed: 19296921]
- [83]. Teismann P, Vila M, Choi DK, Tieu K, Wu DC, Jackson-Lewis V, Przedborski S COX-2 and neurodegeneration in Parkinson's disease. *Ann N Y Acad Sci* 991 (2003) 272–7. [PubMed: 12846993]
- [84]. Russo I, Bubacco L, Greggio E LRRK2 and neuroinflammation: partners in crime in Parkinson's disease? *J Neuroinflammation* 11 (2014) 52. [PubMed: 24655756]
- [85]. Pan-Montojo F, Anichtchik O, Dening Y, Knels L, Pursche S, Jung R, Jackson S, Gille G, Spillantini MG, Reichmann H, Funk RH Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS One* 5(1) (2010) e8762. [PubMed: 20098733]
- [86]. Qin X, Wu Q, Lin L, Sun A, Liu S, Li X, Cao X, Gao T, Luo P, Zhu X Soluble epoxide hydrolase deficiency or inhibition attenuates MPTP-induced parkinsonism. *Molecular neurobiology* 52(1) (2015) 187–195. [PubMed: 25128026]
- [87]. Olearczyk JJ, Field MB, Kim IH, Morisseau C, Hammock BD, Imig JD Substituted adamantyl-urea inhibitors of the soluble epoxide hydrolase dilate mesenteric resistance vessels. *J Pharmacol Exp Ther* 318(3) (2006) 1307–14. [PubMed: 16772540]
- [88]. Sherer TB, Betarbet R, Kim JH, Greenamyre JT Selective microglial activation in the rat rotenone model of Parkinson's disease. *Neurosci Lett* 341(2) (2003) 87–90. [PubMed: 12686372]
- [89]. Emmrich JV, Hornik TC, Neher JJ, Brown GC Rotenone induces neuronal death by microglial phagocytosis of neurons. *FEBS J* 280(20) (2013) 5030–8. [PubMed: 23789887]
- [90]. Wang T, Pei Z, Zhang W, Liu B, Langenbach R, Lee C, Wilson B, Reece JM, Miller DS, Hong JS MPP<sup>+</sup>-induced COX-2 activation and subsequent dopaminergic neurodegeneration. *FASEB J* 19(9) (2005) 1134–6. [PubMed: 15845609]

- [91]. Lakkappa N, Krishnamurthy PT, M DP, Hammock BD, Hwang SH Soluble epoxide hydrolase inhibitor, APAU, protects dopaminergic neurons against rotenone induced neurotoxicity: Implications for Parkinson's disease. *Neurotoxicology* 70 (2019) 135–145. [PubMed: 30472438]
- [92]. Goldberg JA, Guzman JN, Estep CM, Ilijic E, Kondapalli J, Sanchez-Padilla J, Surmeier DJ Calcium entry induces mitochondrial oxidant stress in vagal neurons at risk in Parkinson's disease. *Nat Neurosci* 15(10) (2012) 1414–21. [PubMed: 22941107]
- [93]. Chen J, Capdevila JH, Zeldin DC, Rosenberg RL Inhibition of cardiac L-type calcium channels by epoxyeicosatrienoic acids. *Mol Pharmacol* 55(2) (1999) 288–95. [PubMed: 9927620]
- [94]. Ilijic E, Guzman JN, Surmeier DJ The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. *Neurobiol Dis* 43(2) (2011) 364–71. [PubMed: 21515375]
- [95]. Vezzani A, French J, Bartfai T, Baram TZ The role of inflammation in epilepsy. *Nat Rev Neurol* 7(1) (2011) 31–40. [PubMed: 21135885]
- [96]. Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis* 29(1) (2008) 142–60. [PubMed: 17931873]
- [97]. Mittelman A, Puccio C, Gafney E, Coombe N, Singh B, Wood D, Nadler P, Ahmed T, Arlin Z A phase I pharmacokinetic study of recombinant human tumor necrosis factor administered by a 5-day continuous infusion. *Invest New Drugs* 10(3) (1992) 183–90. [PubMed: 1428728]
- [98]. Shandra AA, Godlevsky LS, Vastyanov RS, Oleinik AA, Konovalenko VL, Rapoport EN, Korobka NN The role of TNF-alpha in amygdala kindled rats. *Neurosci Res* 42(2) (2002) 147–53. [PubMed: 11849734]
- [99]. Hung YW, Hung SW, Wu YC, Wong LK, Lai MT, Shih YH, Lee TS, Lin YY Soluble epoxide hydrolase activity regulates inflammatory responses and seizure generation in two mouse models of temporal lobe epilepsy. *Brain Behav Immun* 43 (2015) 118–29. [PubMed: 25135858]
- [100]. Inceoglu B, Zolkowska D, Yoo HJ, Wagner KM, Yang J, Hackett E, Hwang SH, Lee KS, Rogawski MA, Morisseau C, Hammock BD Epoxy fatty acids and inhibition of the soluble epoxide hydrolase selectively modulate GABA mediated neurotransmission to delay onset of seizures. *PLoS One* 8(12) (2013) e80922. [PubMed: 24349022]
- [101]. Mule NK, Orjuela Leon AC, Falck JR, Arand M, Marowsky A 11,12 -Epoxyeicosatrienoic acid (11,12 EET) reduces excitability and excitatory transmission in the hippocampus. *Neuropharmacology* 123 (2017) 310–321. [PubMed: 28526610]
- [102]. Wu HF, Chen YJ, Wu SZ, Lee CW, Chen IT, Lee YC, Huang CC, Hsing CH, Tang CW, Lin HC Soluble Epoxide Hydrolase Inhibitor and 14,15-Epoxyeicosatrienoic Acid-Facilitated Long-Term Potentiation through cAMP and CaMKII in the Hippocampus. *Neural Plast* 2017 (2017) 3467805.
- [103]. Garcia-Bueno B, Caso JR, Perez-Nievas BG, Lorenzo P, Leza JC Effects of peroxisome proliferator-activated receptor gamma agonists on brain glucose and glutamate transporters after stress in rats. *Neuropsychopharmacology* 32(6) (2007) 1251–60. [PubMed: 17119541]
- [104]. Bridges RJ, Esslinger CS The excitatory amino acid transporters: pharmacological insights on substrate and inhibitor specificity of the EAAT subtypes. *Pharmacol Ther* 107(3) (2005) 271–85. [PubMed: 16112332]
- [105]. Simon NM, McNamara K, Chow CW, Maser RS, Papakostas GI, Pollack MH, Nierenberg AA, Fava M, Wong KK A detailed examination of cytokine abnormalities in Major Depressive Disorder. *Eur Neuropsychopharmacol* 18(3) (2008) 230–3. [PubMed: 17681762]
- [106]. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lancot KL A meta-analysis of cytokines in major depression. *Biol Psychiatry* 67(5) (2010) 446–57. [PubMed: 20015486]
- [107]. Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, Miyazaki C, Alexander N, Hotopf M, Cleare AJ, Norris S, Cassidy E, Aitchison KJ, Miller AH, Pariante CM Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Mol Psychiatry* 14(12) (2009) 1095–104. [PubMed: 18458677]
- [108]. Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirmics K Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry* 16(7) (2011) 751–62. [PubMed: 20479761]

- [109]. Dale E, Bang-Andersen B, Sanchez C Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. *Biochem Pharmacol* 95(2) (2015) 81–97. [PubMed: 25813654]
- [110]. Wu Q, Cai H, Song J, Chang Q The effects of sEH inhibitor on depression-like behavior and neurogenesis in male mice. *J Neurosci Res* 95(12) (2017) 2483–2492. [PubMed: 28699310]
- [111]. Gauthier KM, Deeter C, Krishna UM, Reddy YK, Bondlela M, Falck JR, Campbell WB 14,15-Epoxyeicosa-5(Z)-enoic acid: a selective epoxyeicosatrienoic acid antagonist that inhibits endothelium-dependent hyperpolarization and relaxation in coronary arteries. *Circ Res* 90(9) (2002) 1028–36. [PubMed: 12016270]
- [112]. Nunan J, Small DH Regulation of APP cleavage by alpha-, beta- and gamma-secretases. *FEBS Lett* 483(1) (2000) 6–10. [PubMed: 11033346]
- [113]. Ittner LM, Gotz J Amyloid-beta and tau--a toxic pas de deux in Alzheimer's disease. *Nat Rev Neurosci* 12(2) (2011) 65–72.
- [114]. Minter MR, Taylor JM, Crack PJ The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. *J Neurochem* 136(3) (2016) 457–74. [PubMed: 26509334]
- [115]. Egensperger R, Kosel S, von Eitzen U, Graeber MB Microglial activation in Alzheimer disease: Association with APOE genotype. *Brain Pathol* 8(3) (1998) 439–47. [PubMed: 9669695]
- [116]. Maezawa I, Maeda N, Montine TJ, Montine KS Apolipoprotein E-specific innate immune response in astrocytes from targeted replacement mice. *J Neuroinflammation* 3 (2006) 10. [PubMed: 16603079]
- [117]. Liu R, Li JZ, Song JK, Zhou D, Huang C, Bai XY, Xie T, Zhang X, Li YJ, Wu CX, Zhang L, Li L, Zhang TT, Du GH Pinocembrin improves cognition and protects the neurovascular unit in Alzheimer related deficits. *Neurobiol Aging* 35(6) (2014) 1275–85. [PubMed: 24468471]
- [118]. Wang SB, Pang XB, Gao M, Fang LH, Du GH Pinocembrin protects rats against cerebral ischemic damage through soluble epoxide hydrolase and epoxyeicosatrienoic acids. *Chin J Nat Med* 11(3) (2013) 207–13. [PubMed: 23725831]
- [119]. Yao ES, Tang Y, Liu XH, Wang MH TPPU protects tau from H<sub>2</sub>O<sub>2</sub>-induced hyperphosphorylation in HEK293/tau cells by regulating PI3K/AKT/GSK-3beta pathway. *J Huazhong Univ Sci Technolog Med Sci* 36(6) (2016) 785–790. [PubMed: 27924507]
- [120]. Liu M, Zhu Q, Wu J, Yu X, Hu M, Xie X, Yang Z, Yang J, Feng YQ, Yue J Glutamate affects the production of epoxyeicosanoids within the brain: The up-regulation of brain CYP2J through the MAPK-CREB signaling pathway. *Toxicology* 381 (2017) 31–38. [PubMed: 28237619]
- [121]. Li Y, Wu J, Yu X, Na S, Li K, Yang Z, Xie X, Yang J, Yue J The Protective Role of Brain CYP2J in Parkinson's Disease Models. *Oxid Med Cell Longev* 2018 (2018) 2917981.
- [122]. Sarkar P, Narayanan J, Harder DR Differential effect of amyloid beta on the cytochrome P450 epoxygenase activity in rat brain. *Neuroscience* 194 (2011) 241–9. [PubMed: 21843605]
- [123]. Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, Halabisky B, Cisse M, Scarce-Levie K, Cheng IH, Gan L, Palop JJ, Bonventre JV, Mucke L Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci* 11(11) (2008) 1311–8. [PubMed: 18931664]
- [124]. Rodriguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, Oddo S, Verkhratsky A Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. *PLoS One* 3(8) (2008) e2935. [PubMed: 18698410]
- [125]. Oguro A, Inoue T, Kudoh SN, Imaoka S 14,15-epoxyeicosatrienoic acid produced by cytochrome P450s enhances neurite outgrowth of PC12 and rat hippocampal neuronal cells. *Pharmacol Res Perspect* 6(5) (2018) e00428. [PubMed: 30237892]
- [126]. Wu HF, Yen HJ, Huang CC, Lee YC, Wu SZ, Lee TS, Lin HC Soluble epoxide hydrolase inhibitor enhances synaptic neurotransmission and plasticity in mouse prefrontal cortex. *J Biomed Sci* 22 (2015) 94. [PubMed: 26494028]
- [127]. Sakayori N, Kikkawa T, Tokuda H, Kiryu E, Yoshizaki K, Kawashima H, Yamada T, Arai H, Kang JX, Katagiri H, Shibata H, Innis SM, Arita M, Osumi N Maternal dietary imbalance between omega-6 and omega-3 polyunsaturated fatty acids impairs neocortical development via epoxy metabolites. *Stem Cells* 34(2) (2016) 470–82. [PubMed: 26580686]

- [128]. Vallieres L, Campbell IL, Gage FH, Sawchenko PE Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci* 22(2) (2002) 486–92. [PubMed: 11784794]
- [129]. Spector AA, Norris AW Action of epoxyeicosatrienoic acids on cellular function. *Am J Physiol Cell Physiol* 292(3) (2007) C996–1012. [PubMed: 16987999]
- [130]. Xu D, Li N, He Y, Timofeyev V, Lu L, Tsai HJ, Kim IH, Tuteja D, Mateo RK, Singapuri A, Davis BB, Low R, Hammock BD, Chiamvimonvat N Prevention and reversal of cardiac hypertrophy by soluble epoxide hydrolase inhibitors. *Proc Natl Acad Sci U S A* 103(49) (2006) 18733–8. [PubMed: 17130447]
- [131]. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M A cytokine-responsive I $\kappa$ B kinase that activates the transcription factor NF- $\kappa$ B. *Nature* 388(6642) (1997) 548–54. [PubMed: 9252186]
- [132]. Israel A The IKK complex, a central regulator of NF- $\kappa$ B activation. *Cold Spring Harb Perspect Biol* 2(3) (2010) a000158. [PubMed: 20300203]
- [133]. Dimitropoulou C, West L, Field MB, White RE, Reddy LM, Falck JR, Imig JD Protein phosphatase 2A and Ca<sup>2+</sup>-activated K<sup>+</sup> channels contribute to 11,12-epoxyeicosatrienoic acid analog mediated mesenteric arterial relaxation. *Prostaglandins & other lipid mediators* 83(1–2) (2007) 50–61. [PubMed: 17259072]
- [134]. Imig JD, Dimitropoulou C, Reddy DS, White RE, Falck JR Afferent arteriolar dilation to 11, 12-EET analogs involves PP2A activity and Ca<sup>2+</sup>-activated K<sup>+</sup> Channels. *Microcirculation* 15(2) (2008) 137–50. [PubMed: 18260004]
- [135]. Chaudhary KR, Zordoky BN, Edin ML, Alsaleh N, El-Kadi AO, Zeldin DC, Seubert JM Differential effects of soluble epoxide hydrolase inhibition and CYP2J2 overexpression on postischemic cardiac function in aged mice. *Prostaglandins & other lipid mediators* 104–105 (2013) 8–17.
- [136]. Lahvic JL, Ammerman M, Li P, Blair MC, Stillman ER, Fast EM, Robertson AL, Christodoulou C, Perlin JR, Yang S, Chiang N, Norris PC, Daily ML, Redfield SE, Chan IT, Chatrizeh M, Chase ME, Weis O, Zhou Y, Serhan CN, Zon LI Specific oxylipins enhance vertebrate hematopoiesis via the receptor GPR132. *Proc Natl Acad Sci U S A* 115(37) (2018) 9252–9257. [PubMed: 30139917]
- [137]. Cheng WY, Huynh H, Chen P, Pena-Llopis S, Wan Y Macrophage PPAR $\gamma$  inhibits Gpr132 to mediate the anti-tumor effects of rosiglitazone. *Elife* 5 (2016).
- [138]. Katragadda D, Batchu SN, Cho WJ, Chaudhary KR, Falck JR, Seubert JM Epoxyeicosatrienoic acids limit damage to mitochondrial function following stress in cardiac cells. *J Mol Cell Cardiol* 46(6) (2009) 867–75. [PubMed: 19285984]
- [139]. Samokhvalov V, Alsaleh N, El-Sikhry HE, Jamieson KL, Chen CB, Lopaschuk DG, Carter C, Light PE, Manne R, Falck JR, Seubert JM Epoxyeicosatrienoic acids protect cardiac cells during starvation by modulating an autophagic response. *Cell Death Dis* 4 (2013) e885. [PubMed: 24157879]
- [140]. Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, Gooch R, Foley J, Newman J, Mao L, Rockman HA, Hammock BD, Murphy E, Zeldin DC Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K<sup>+</sup> channels and p42/p44 MAPK pathway. *Circ Res* 95(5) (2004) 506–14. [PubMed: 15256482]
- [141]. Akao M, Ohler A, O'Rourke B, Marban E Mitochondrial ATP-sensitive potassium channels inhibit apoptosis induced by oxidative stress in cardiac cells. *Circ Res* 88(12) (2001) 1267–75. [PubMed: 11420303]
- [142]. Inceoglu B, Beltaieb A, Haj FG, Gomes AV, Hammock BD Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors. *Prostaglandins & other lipid mediators* 133 (2017) 68–78. [PubMed: 28847566]
- [143]. Witte ME, Geurts JJ, de Vries HE, van der Valk P, van Horssen J Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration? *Mitochondrion* 10(5) (2010) 411–8. [PubMed: 20573557]

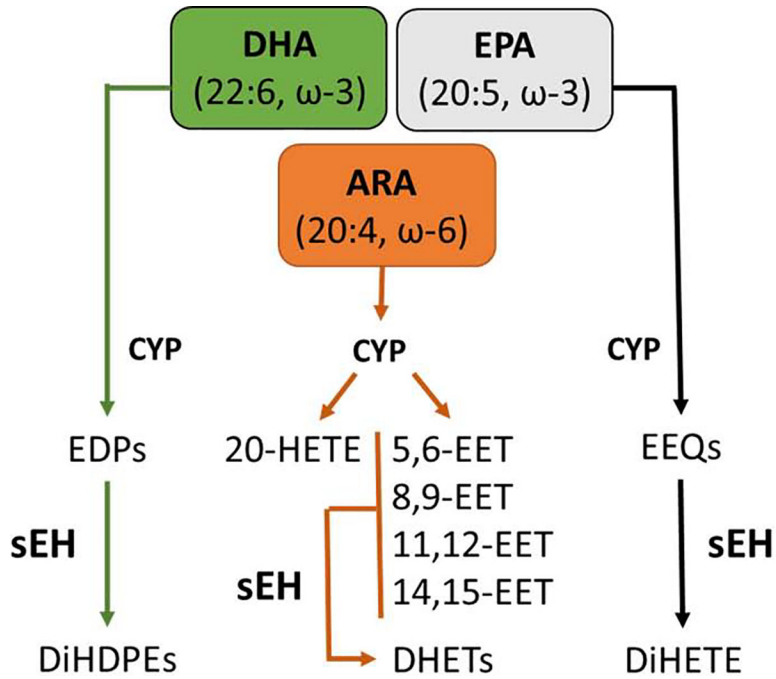
- [144]. Lavoie HA, King SR Transcriptional regulation of steroidogenic genes: STARD1, CYP11A1 and HSD3B. *Exp Biol Med* (Maywood) 234(8) (2009) 880–907. [PubMed: 19491374]
- [145]. Inceoglu B, Jinks SL, Ulu A, Hegedus CM, Georgi K, Schmelzer KR, Wagner K, Jones PD, Morisseau C, Hammock BD Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways. *Proc Natl Acad Sci U S A* 105(48) (2008) 18901–6. [PubMed: 19028872]
- [146]. Ren S, Hylemon P, Zhang ZP, Rodriguez-Agudo D, Marques D, Li X, Zhou H, Gil G, Pandak WM Identification of a novel sulfonated oxysterol, 5-cholesten-3beta,25-diol 3-sulfonate, in hepatocyte nuclei and mitochondria. *J Lipid Res* 47(5) (2006) 1081–90. [PubMed: 16505492]
- [147]. Ren S, Ning Y Sulfation of 25-hydroxycholesterol regulates lipid metabolism, inflammatory responses, and cell proliferation. *Am J Physiol Endocrinol Metab* 306(2) (2014) E123–30. [PubMed: 24302009]
- [148]. Chen X, Li Z, Zhang B, Hu R, Li J, Feng M, Yao W, Zhang C, Wan L, Zhang Y Alleviation of Mechanical Allodynia by 14,15-Epoxyeicosatrienoic Acid in a Central Poststroke Pain Model: Possible Role of Allopregnanolone and delta-Subunit-Containing Gamma-Aminobutyric Acid A Receptors. *J Pain* (2018).
- [149]. Liu Y, Zhang Y, Schmelzer K, Lee TS, Fang X, Zhu Y, Spector AA, Gill S, Morisseau C, Hammock BD, Shyy JY The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc Natl Acad Sci U S A* 102(46) (2005) 16747–52. [PubMed: 16267130]
- [150]. Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* 26(8) (2006) 3071–84. [PubMed: 16581782]
- [151]. Inceoglu B, Bettaieb A, Trindade da Silva CA, Lee KS, Haj FG, Hammock BD Endoplasmic reticulum stress in the peripheral nervous system is a significant driver of neuropathic pain. *Proc Natl Acad Sci U S A* 112(29) (2015) 9082–7. [PubMed: 26150506]
- [152]. Bettaieb A, Nagata N, AbouBechara D, Chahed S, Morisseau C, Hammock BD, Haj FG Soluble epoxide hydrolase deficiency or inhibition attenuates diet-induced endoplasmic reticulum stress in liver and adipose tissue. *J Biol Chem* 288(20) (2013) 14189–99. [PubMed: 23576437]
- [153]. Yu G, Zeng X, Wang H, Hou Q, Tan C, Xu Q, Wang H 14,15-epoxyeicosatrienoic Acid suppresses cigarette smoke extract-induced apoptosis in lung epithelial cells by inhibiting endoplasmic reticulum stress. *Cell Physiol Biochem* 36(2) (2015) 474–86. [PubMed: 25968975]
- [154]. Dhanasekaran A, Gruenloh SK, Buonaccorsi JN, Zhang R, Gross GJ, Falck JR, Patel PK, Jacobs ER, Medhora M Multiple antiapoptotic targets of the PI3K/Akt survival pathway are activated by epoxyeicosatrienoic acids to protect cardiomyocytes from hypoxia/anoxia. *Am J Physiol Heart Circ Physiol* 294(2) (2008) H724–35. [PubMed: 18055514]
- [155]. Hu P, Han Z, Couvillon AD, Exton JH Critical role of endogenous Akt/IAPs and MEK1/ERK pathways in counteracting endoplasmic reticulum stress-induced cell death. *J Biol Chem* 279(47) (2004) 49420–9. [PubMed: 15339911]
- [156]. Geng HX, Li RP, Li YG, Wang XQ, Zhang L, Deng JB, Wang L, Deng JX 14,15-EET Suppresses Neuronal Apoptosis in Ischemia-Reperfusion Through the Mitochondrial Pathway. *Neurochem Res* 42(10) (2017) 2841–2849. [PubMed: 28508993]
- [157]. Rodriguez D, Rojas-Rivera D, Hetz C Integrating stress signals at the endoplasmic reticulum: The BCL-2 protein family rheostat. *Biochim Biophys Acta* 1813(4) (2011) 564–74. [PubMed: 21122809]
- [158]. Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D, Greene LA Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J Neurosci* 22(24) (2002) 10690–8. [PubMed: 12486162]
- [159]. Celardo I, Costa AC, Lehmann S, Jones C, Wood N, Mencacci NE, Mallucci GR, Loh SH, Martins LM Mitofusin-mediated ER stress triggers neurodegeneration in pink1/parkin models of Parkinson's disease. *Cell Death Dis* 7(6) (2016) e2271. [PubMed: 27336715]



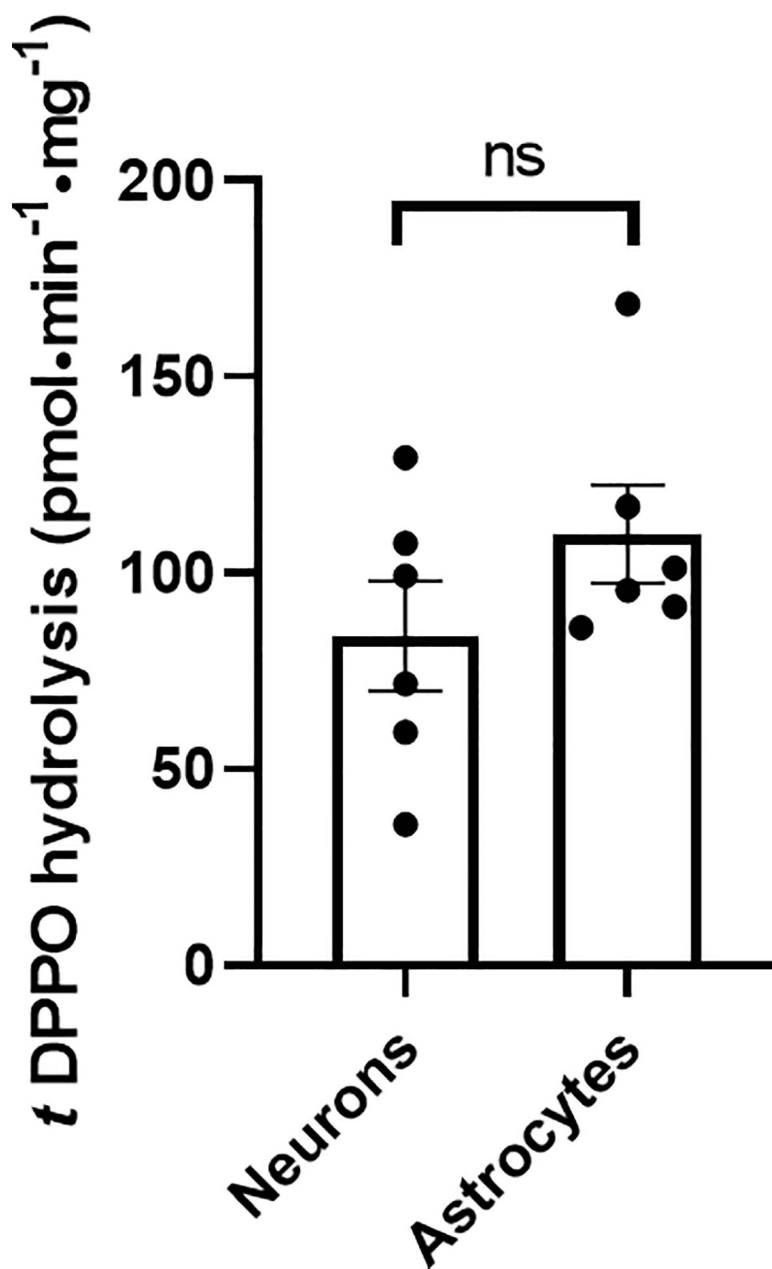
- [160]. Katayama T, Imaizumi K, Manabe T, Hitomi J, Kudo T, Tohyama M Induction of neuronal death by ER stress in Alzheimer's disease. *J Chem Neuroanat* 28(1–2) (2004) 67–78. [PubMed: 15363492]
- [161]. Selvaraj S, Sun Y, Watt JA, Wang S, Lei S, Birnbaumer L, Singh BB Neurotoxin-induced ER stress in mouse dopaminergic neurons involves downregulation of TRPC1 and inhibition of AKT/mTOR signaling. *J Clin Invest* 122(4) (2012) 1354–67. [PubMed: 22446186]
- [162]. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306(5695) (2004) 457–61. [PubMed: 15486293]
- [163]. Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R, Fernandez-Ruiz J Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiol Dis* 19(1–2) (2005) 96–107. [PubMed: 15837565]
- [164]. Hill MN, Gorzalka BB Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur Neuropsychopharmacol* 15(6) (2005) 593–9. [PubMed: 15916883]
- [165]. Wise LE, Thorpe AJ, Lichtman AH Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology* 34(9) (2009) 2072–80. [PubMed: 19322169]
- [166]. Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y, Klein T, Fernandez F, Tan J, Shytle RD Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* 2 (2005) 29. [PubMed: 16343349]
- [167]. Inceoglu B, Schmelzer KR, Morisseau C, Jinks SL, Hammock BD Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). *Prostaglandins & other lipid mediators* 82(1–4) (2007) 42–9. [PubMed: 17164131]
- [168]. McDougle DR, Kambalyal A, Meling DD, Das A Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxidase. *J Pharmacol Exp Ther* 351(3) (2014) 616–27. [PubMed: 25277139]
- [169]. Stark K, Dostalek M, Guengerich FP Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide. *FEBS J* 275(14) (2008) 3706–17. [PubMed: 18549450]
- [170]. Snider NT, Nast JA, Tesmer LA, Hollenberg PF A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist. *Mol Pharmacol* 75(4) (2009) 965–72. [PubMed: 19171674]
- [171]. McDougle DR, Watson JE, Abdeen AA, Adili R, Caputo MP, Krapf JE, Johnson RW, Kilian KA, Holinstat M, Das A Anti-inflammatory omega-3 endocannabinoid epoxides. *Proc Natl Acad Sci U S A* 114(30) (2017) E6034–E6043. [PubMed: 28687674]
- [172]. Rand AA, Helmer PO, Inceoglu B, Hammock BD, Morisseau C LC-MS/MS Analysis of the Epoxides and Diols Derived from the Endocannabinoid Arachidonoyl Ethanolamide. *Methods Mol Biol* 1730 (2018) 123–133. [PubMed: 29363071]
- [173]. Sasso O, Wagner K, Morisseau C, Inceoglu B, Hammock BD, Piomelli D Peripheral FAAH and soluble epoxide hydrolase inhibitors are synergistically antinociceptive. *Pharmacol Res* 97 (2015) 7–15. [PubMed: 25882247]
- [174]. Kawamata H, Manfredi G Mitochondrial dysfunction and intracellular calcium dysregulation in ALS. *Mech Ageing Dev* 131(7–8) (2010) 517–26. [PubMed: 20493207]

**Highlights**

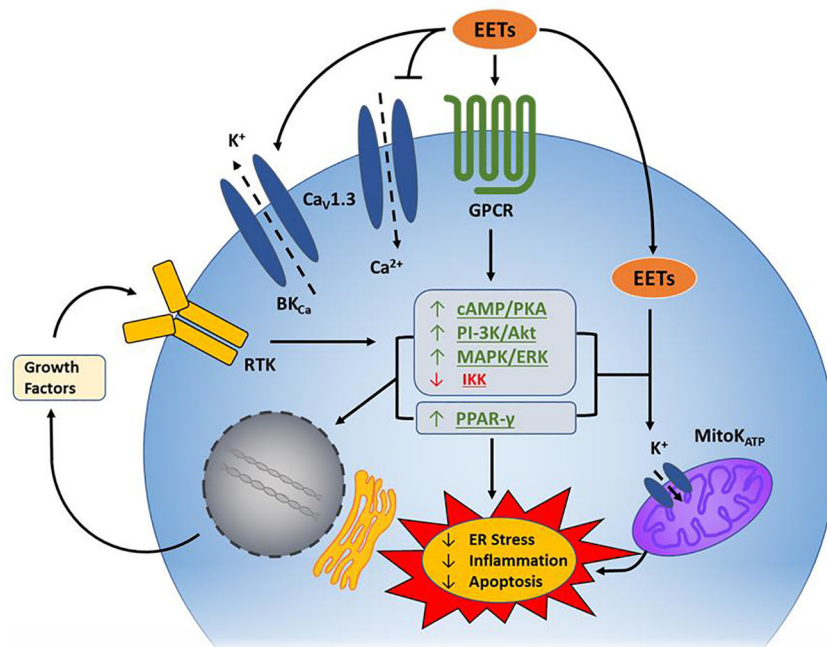
- Soluble epoxide hydrolase inhibition and epoxy fatty acids are neuroprotective
- The mechanisms are diverse, involving attenuation of ER and mitochondrial stress
- EpFAs and sEH inhibition may be therapeutic against neuroinflammatory diseases



**Figure 1.** Polyunsaturated fatty acids (PUFAs) including arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DHA), can all be metabolized by cytochrome P450 to produce epoxyeicosatrienoic acids (EETs), epoxyeicosatetraenoic acids (EEQs), and epoxydocosapentaenoic acids (EDPs), respectively. All regioisomers of these antiinflammatory metabolites can be hydrolyzed by soluble epoxide hydrolase (sEH) into corresponding diols. As such, sEH inhibition can bring about a significant change in the epoxide to diol ratio of DHA and EPA metabolites in addition to ARA derived metabolites. The diols are more polar, more easily excreted, and appear less beneficially active than their corresponding epoxides, and in some cases are pro-inflammatory. These PUFAs may alternatively be metabolized by cyclooxygenase (COX), and lipoxygenase (LOX) independently, or in some cases sequentially.



**Figure 2.** Enzymatic activity of sEH, measured as a rate of [<sup>3</sup>H] *trans*-1,3-diphenylpropene oxide (*t*DPPO) hydrolysis using [<sup>3</sup>H] *t*DPPO at a final concentration of 50  $\mu$ M. Activity was measured in primary rat cortical astrocytes and neurons harvested from neonatal rat pups. The sEH activity of primary neurons were comparable to that of astrocytes, indicating that sEH inhibitors may act directly on rat neurons in addition to glia to prevent neural loss (bars represent  $\pm 1$  SEM). Two tailed t-test, 10 df, ns.



**Figure 3.**

Schematic representation of how epoxyeicosatrienoic acids (EETs) may exert their anti-inflammatory and anti-apoptotic effects. EETs are hypothesized to suppress the activation of NF- $\kappa$ B and ER stress pathways through various means including IKK inhibition and RTK signaling. Increased cAMP could facilitate neural survival through transcription of proteins such as brain derived neurotrophic factor (BDNF), which can amplify through a positive feedback loop, mitigating apoptosis. EETs may also act through regulating ion channels such as Ca<sub>v</sub>1.3 and mitochondrial K<sub>ATP</sub> channels, preventing apoptosis. EET regioisomers have differential affinity for activating various pathways, with cell type and receptor specific effects on inflammation and vasculature remodeling.

Table 1.

Studies describing the therapeutic effect of sEH inhibition against neuroinflammatory disorders. Inhibition of sEH is therapeutic in cases of PD, depression, and stroke, while effects on AD and epilepsy are not yet fully elucidated.

Disorder	Model	Therapeutic Effect	Behavioral Improvement	Treatment	Species	Reference
PD	MPTP, Paraquat	Improved TH+ neuron and primary cultured neuron viability	Yes	sEH KO; AUDA (5 mg/kg; i.g.) pre- and post-MPTP; 14,15- and 11,12-EET lateral ventricle injection	Mice	Qin et al. 2015 (86)
	MPTP	Improved DA content and TH+ cell viability; reduced activated microglia		sEH KO; TPPU (0.3, 1.0, or 3.0 mg/kg; p.o.)	Mice	Ren et al. 2018 (42)
	Rotenone	Reduced dopaminergic neuron viability and inflammatory markers, increased redox gene expression	Yes	APAU (50, 100, 250 $\mu$ M; DW)	Drosophila	Lakkappa et al. 2019 (91)
Epilepsy	Pilocarpine, amygdala kindling	Reduced inflammatory cytokines, reduced number and duration of seizures with AUDA	Yes	sEH KO; AUDA (10 mg/kg; i.n.) once daily 30 min prior; TPPU (0.5, 1 and 1.5 mg/kg; i.n.)	Mice	Hung et al. 2015 (99)
	Picrotoxin, Pentyleneetetrazol	Increased PTZ seizure threshold, delayed seizure onset, reduced tonic seizure	Yes	sEH KO; EpFAs (0.5 $\mu$ g; i.c.v.); TUPS/TPPU (0.1 or 1 $\mu$ g; i.c.v.)	Mice	Inceoglu et al. 2013 (100)
Depression	Social defeat stress, LPS	Reduced depression-like behavior and inflammatory cytokine	Yes	sEH KO; TPPU (0.3, 1.0, or 3.0 mg/kg; p.o.)	Mice	Ren et al. 2016 (102)
	Social defeat	Reduced depression-like behavior and increased neurogenesis	Yes	TPPU (0.01 – 0.1 mg/kg; i.p.)	Mice	Wu et al. 2017 (103)
Stroke	MCAO	Reduced infarct size and neural death, decreased inflammatory cytokines	Yes	TPPU (1 mg/kg; i.p) after 90 min	Rats	Tu et al. 2018 (57)
	MCAO	Reduced infarct size	-	AUDA-BE (10 mg/kg; i.p.) 30 min prior and at reperfusion	Mice	Zhang et al. 2007 (58)
	MCAO	Reduced infarct size	-	AUDA (25 mg/L; DW) 6 weeks	Rats (SHSRP)	Dorrance et al. 2005 (59)
	MCAO	Reduced infarct size, improved vasculature	-	AUDA (50 mg/L; DW) 6 weeks; $\pm$ AUCB (50 mg/L; DW) 5 weeks	Rats (SHSRP; WKY)	Simpkins et al. 2009 (60)
	MCAO	Reduced infarct size, improved vasculature	Yes	$\pm$ AUCB (0.9 mg/kg; i.v.)	Rats	Shaik et al. 2013 (61)
	MCAO	Reduced infarct size, reduced glial activation, increased pTrkB	Yes	sEH KO; AUDA (10 mg/kg; i.p.) 7 consecutive days post-MCAO	Mice	Chang et al. 2018 (67)
Other	TBI	Reduced contusion volume, hemispheric enlargement ratio, neural death, neutrophil infiltration, microglial activation, and inflammatory cytokine/chemokine	Yes	sEH KO; AUDA (10 $\mu$ M in 0.5 $\mu$ L); intracerebroventricular injection 30 min prior	Mice	Hung et al. 2017 (45)

Disorder	Model	Therapeutic Effect	Behavioral Improvement	Treatment	Species	Reference
	Zymosan	inflammatory; improved BBB integrity Alteration in monocyte recruitment, chemokine expression, and cytokine expression	-	sEH KO; SKF525A (30mg/kg; i.p.)	Mice	Gilroy et al. 2016 (80)

Table 2.

Potential mechanisms of the therapeutic effect of EETs and/or sEH inhibition. While CYP metabolites of EPA or DHA are also known to have therapeutic effects, their mechanisms are not included in this table.

Model	Treatment	Mechanism	Species/Cell Type	Reference
H <sub>2</sub> O <sub>2</sub>	TPPU (0, 0.1, 1, 10 μmol/L)	Decreased tau phosphorylation, increased phosphorylation of Akt and GSK-3β	HEK293 transfected with tau	Yao et al. 2016 (119)
Hypoxia	EETs (1 μM)	Increased activity of p38 and phosphorylation of Akt	Myocytes	Dhanasekaran et al. 2008 (154)
OGD	TPPU (100 μM); <i>±</i> AUCB (100 μM)	Increased expression of VEGF and phosphorylation of Akt	Primary rat neurons and astrocytes	Zhang et al. 2017 (49)
-	<i>±</i> AUCB (1 – 100 μM)	Increased expression of VEGF	Endothelial progenitor cells	Xu et al. 2013(65)
Social defeat, LPS	sEH KO; TPPU (0.3, 1.0, or 3.0 mg/kg, p.o.)	Increased mature BDNF and p-TrkB	Mice	Ren et al. 2016 (102)
Social defeat	TPPU (0.01 – 0.1 mg/kg, i.p.)	Increased BDNF expression and therapeutic effect through Trk	Mice	Wu et al. 2017 (103)
MCAO	sEH KO; AUDA (10 mg/kg, i.p.)	Increased mature BDNF and p-TrkB	Mice	Chang et al. 2018 (67)
-	5,6-EET	Activation of NSCC	Mice B cells	Liu et al. 2005 (75)
-	11,12-FET (2 μM)	Activation of GIRK	Mice hippocampal slices	Mule et al. 2017 (101)
-	EETs and HETEs	Activation of TRPV4	PC12 cells	Oguro et al. 2018 (125)
-	11,12-EET (20, 125 nM); 14,15-EET	Inhibition of L-type Ca <sup>2+</sup> channel	Porcine cardiac muscle cell membrane	Chen et al. 1998 (93)
-	CYP2J2	Activation of mitoK <sub>ATP</sub> and increased phospho-p42/p44 MAPK	Human cardiomyocytes	Seubert et al. 2004 (140)
Streptozocin	TPPU (10 mg/kg, i.p.)	Reduced expression of ER stress markers	Rats	Inceoglu et al. 2015 (151)
Thapsigargin, palmitate, high fat diet	sEH KO; TUPS (10 μg/ml, DW); EpFAs	Reduced expression of ER stress markers	Mice, HEPG2 cells, 3T3-L1 cells	Bettaieb et al. 2013 (152)
MPTP	sEH KO; TPPU (0.3, 1.0, or 3.0 mg/kg; p.o.)	Reduced expression of ER stress markers	Mice	Ren et al. 2018 (42)
Cigarette smoke extract	14,15-EET (1 μM)	Reduced expression of ER stress markers	Human bronchial endothelial cells	Yu et al. 2015 (153)
-	EETs and Linoleic epoxides	Increased β-arrestin recruitment by GPR132	-	Lahvic et al. 2018 (136)
Cytokine Stimulation	14,15- and 11,12-EET (1 – 100 nM)	Reduction in adhesion molecules, regulation of NF-κB through inhibition of IKK activity	Human endothelial cells	Node et al. 1999 (73)
ox-LDL	14,15- and 11,12-EET (1 μM)	Regulation of NF-κB	Pulmonary artery endothelial cells	Jiang et al. 2014 (74)
Phloretin, amygdala kindling	sEH KO, AUDA (10 mg/kg, i.n.); TPPU (0.5, 1 and 1.5 mg/kg, i.n.)	Regulation of NF-κB	Mice	Hung et al. 2015 (99)



Model	Treatment	Mechanism	Species/Cell Type	Reference
IL-1 $\beta$	8,9-EET and 11,12-EET (1 $\mu$ M), CYP2J2	Regulation of NF- $\kappa$ B, involvement of PPAR- $\alpha$ .	HEK293 cells	Bystrom et al. 2011 (79)
Ascending aortic constriction	AEPU (0.1 mg/ml, DW) 3 weeks; AUDA	Increased expression and decreased phosphorylation of I $\kappa$ B $\alpha$ , regulation of NF- $\kappa$ B	Mice	Xu et al. 2006 (130)
LPS	AEPU (0.1, 1, 3 $\mu$ g); TPAU (0.1 – 10 mg/kg); EETs (5 $\mu$ g)	Increased StAR expression	Rats	Inceoglu et al. 2008 (145)
-	AUDA (1 $\mu$ M); EETs (5 – 100 $\mu$ M)	Binding and regulation of PPAR- $\gamma$	Endothelial cells	Liu et al. 2005 (149)