Published in final edited form as:

Future Neurol. 2009 March 1; 4(2): 179–199.

Soluble Epoxide Hydrolase Inhibition: Targeting Multiple Mechanisms of Ischemic Brain Injury with a Single Agent

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Summary

Soluble epoxide hydrolase (sEH) is a key enzyme in the metabolic conversion and degradation of P450 eicosanoids called epoxyeicosatrienoic acids (EETs). Genetic variations in the sEH gene, designated EPHX2, are associated with ischemic stroke risk. In experimental studies, sEH inhibition and gene deletion reduce infarct size after focal cerebral ischemia in mice. Although the precise mechanism of protection afforded by sEH inhibition remains under investigation, EETs exhibit a wide array of potentially beneficial actions in stroke, including vasodilation, neuroprotection, promotion of angiogenesis and suppression of platelet aggregation, oxidative stress and post-ischemic inflammation. Herein we argue that by capitalizing on this broad protective profile, sEH inhibition represents a prototype “combination therapy” targeting multiple mechanisms of stroke injury with a single agent.

Keywords

Cerebral ischemia; stroke; epoxyeicosatrienoic acids; EETs; soluble epoxide hydrolase; cerebral blood flow; combination therapy

Treatment options for acute ischemic stroke remain limited owing in part to across-the-board failure in clinical trials of neuroprotective therapies arising from experimental stroke studies. One explanation for these failures lay in the narrow targeting of therapeutics, focusing on a single molecular pathway that impinges upon one element of the complex ischemic cascade. In response to this issue, broader treatment modalities are being sought such as the employment of so-called “cocktail” or combination therapies targeting multiple elements of the ischemic cascade simultaneously [1,2]. An alternative strategy involves the use of a single drug that will individually exert protective effects upon several ischemia-related processes. This approach relies first on the identification of an endogenous molecular signaling system that governs multiple elements of the ischemic cascade, and second upon the ability to regulate this system through a single pharmacological target.

The enzyme soluble epoxide hydrolase (sEH) has recently emerged as a potential therapeutic target in the treatment of ischemic stroke, as highlighted by a series of experimental and genetic

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Disclosures: None.

studies demonstrating protection from cerebral ischemia [3,4] and linking polymorphisms in the human EPHX2 gene, which encodes for sEH, with modified risk of ischemic stroke in a number of human populations [5-7]. These genetic studies have demonstrated that different variations in the EPHX2 gene are linked to either reductions or elevations in stroke risk. Such opposing effects suggest that the gene product sEH may play an important role in the pathogenesis of ischemic stroke. This notion has been validated in experimental studies examining the role of sEH in ischemic brain injury. Below, we review these studies and discuss current knowledge of the biological basis for the protective effects of sEH inhibition in experimental cerebral ischemia, highlighting effects of sEH inhibitors upon multiple elements of the ischemic cascade and their emergence as a prototype single-agent, multi-target therapy in stroke.

Epoxyeicosatrienoic acids (EETs) signaling

Soluble epoxide hydrolase is the enzyme chiefly involved in the metabolism of fatty acid signaling molecules termed epoxyeicosatrienoic acids (EETs) that regulate many aspects of cardiovascular function. EETs are synthesized by cytochrome P450 (CYP) epoxygenases from arachidonic acid (AA). There are four EETs regioisomers corresponding to the four double bonds of AA: 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET (Figure 1) [8]. Additionally, each of these four regioisomers has two stereoisomers (R,S or S,R), resulting in eight chemically distinct enantiomers. EETs act as both paracrine and autocrine factors and are believed to evoke their various cellular actions by multiple signaling pathways including receptor-mediated and intracellular pathways. The biological effects of EETs are terminated primarily by their hydrolysis to the vicinal diols dihydroxyeicosatrienoic acids (DiHETs), by sEH (Figure 1) [9].

Although a specific high-affinity membrane-bound receptor has not been identified for EETs, experimental evidence suggests that such a receptor may exist. A high-affinity binding site for 14,15-EET was identified in human U937 cells [10] and guinea pig mononuclear cells [11]. Certain cellular actions of EETs, including the activation of large conductance calcium-activated potassium (BK) channels by 11,12-EET, are sensitive to inhibition of G protein signaling, suggesting that the putative EETs receptor is a G protein-coupled receptor (GPCR) [12,13]. The receptor-mediated actions of EETs produce a myriad of cellular actions through several signal transduction pathways, including the cAMP/protein kinase A (PKA), phosphatidylinositol 3-kinase (PI3K)-Akt and mitogen-activated protein kinase cascades. These cellular actions and their associated signal transduction pathways have been recently and thoroughly reviewed [9].

In addition to their effects at a putative GPCR, EETs interact directly with a number of ion channels and intracellular proteins, actions that may underlie many of EETs' cellular actions. This includes activation of BK channels, ATP-sensitive potassium (K_{ATP}) channels, the transient receptor potential vanilloid-4 (TRPV4) cation channel and the peroxisome proliferator-activated receptor (PPAR) transcription factors [9].

EETs are important regulators of cardiovascular function

In the peripheral circulation, EETs are potent vasodilators produced by vascular endothelium, signaling in parallel with nitric oxide (NO) as an endothelium-derived hyperpolarizing factor (EDHF) and regulating blood flow in a number of vascular beds including the coronary and renal circulations [14-18]. In addition, EETs exercise a number of non-vasomotor influences upon the cardiovascular system (Figure 1, Table 1). In the kidney, EETs increase salt excretion and participate in the long-term regulation of blood pressure [8]. EETs are also anti-inflammatory, inhibiting signaling through the NF- κ B pathway and reducing endothelial expression of leukocyte adhesion proteins such as VCAM-1 [19,20]. In vascular smooth

muscle, EETs inhibit cell migration through their interaction with the cAMP/PKA pathway [21]. Finally, EETs are anti-thrombotic, inhibiting platelet aggregation and adhesion to the endothelium [22,23], in addition to increasing the expression of the fibrinolytic enzyme tissue plasminogen activator (tPA) [24]. In total, EETs exert a broad spectrum of physiological effects within the cardiovascular system, prompting an increasing appreciation of their role both in cardiovascular regulation as well as disease.

EETs are key regulators of cerebral blood flow

Within the cerebral circulation, EETs are potent vasodilators involved in the regulation of blood flow at several levels of control. In early *in vivo* studies, 5,6-EET dilated rabbit and cat pial arterioles, a response that was indomethacin-sensitive and may involve a 5,6-EET cyclooxygenase (COX) metabolite [15,25]. In isolated cat cerebral arteries, 5,6-EET, 8,9-EET and 11,12-EET exerted a potent dilator effect [26]. In this *in vitro* model, the latter two regioisomers produced greater dilation than 5,6-EET. The vasomotor actions of EETs in cat cerebral arteries were mediated by the opening of BK channels [26]. Administration of the 5,6-, 8,9-, 11,12-EET regioisomers increased BK channel open probability, hyperpolarizing and relaxing the vascular smooth muscle. More recent work implicated the TRPV4 cation channel in the vasomotor actions of EETs in the rat cerebral circulation [27]. In the model proposed by the study authors, the TRPV4, ryanodine and BK channels form a signaling complex in which EETs bind to TRPV4, triggering Ca^{2+} -sparks that evoke the opening of the BK channel and resulting hyperpolarization.

As in the peripheral circulation, the cerebral vascular endothelium produces EETs, as identified in cultured cerebral microvascular endothelial cells by a fluorescence-based bioassay [28]. Although the physiological role of these endothelium-derived EETs remains uncertain, they may mediate the vasoactive effects of such endothelium-dependent agonists as bradykinin (Figure 2) [29].

Non-vascular cells in the brain also release EETs that participate in cerebral blood flow (CBF) regulation. It is now accepted that astrocytes, which extend processes to ensheath both central nervous system (CNS) synapses and the nearby microcirculation, play a key role as mediators of neurovascular coupling in the brain. Through a phenomenon termed ‘functional hyperemia’ that forms the mechanistic basis for functional magnetic resonance imaging (fMRI), local neuronal activity is detected by peri-synaptic astrocytes that produce and release vasoactive metabolites from peri-vascular endfeet, resulting in spatially-restricted elevations in CBF [30]. Brain astrocytes express CYP epoxygenases [31,32] and release EETs when incubated with arachidonic acid [31,33]. Stimulation of primary astrocyte cultures with glutamate increases EETs release from these cells [34].

Evidence from functional studies demonstrates that astrocyte-derived EETs are key mediators of neurovascular coupling (Figure 2). Cortical hyperemia resulting from administration of glutamate [34] or N-methyl-D-aspartate (NMDA) [35] was blocked by the P450 inhibitor miconazole and N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH), a P450 epoxygenase-specific inhibitor that blocks the activity of both CYP2C and CYP2J epoxygenase isoforms [36]. In rat models of functional hyperemia, the administration of miconazole or MS-PPOH inhibited the CBF response to cortical activation by whisker stimulation [37] or electrical forepaw stimulation [32], defining EETs as important mediators of metabolism-blood flow coupling.

More recent findings from our group suggest that in addition to their release from cerebral vascular endothelium and cortical astrocytes, nerve-derived EETs may also take part in the regulation of vascular tone in surface cerebral vessels. We identified the presence of CYP epoxygenases and sEH in parasympathetic and sensory extrinsic perivascular nerves

innervating the cortical surface vasculature and demonstrated a functional role for EETs signaling in the regulation of the cerebral circulation by these vasodilator fibers (Figure 2) [38,39].

EETs exhibit non-vasomotor actions in the cerebral vasculature

EETs additionally exercise non-vasomotor influences upon the cerebral circulation. Among these are the effects of astrocyte-derived EETs in promoting cortical angiogenesis both *in vitro* and *in vivo*. Cerebral microvascular endothelial cells undergo proliferation and exhibit spontaneous tube-like structure formation when co-cultured with cortical astrocytes or exposed to astrocyte conditioned media. This behavior, which is considered an *in vitro* model of angiogenesis, was inhibited by the P450 inhibitor 17-octadecynoic acid (17-ODYA), suggesting that P450 metabolites play a role in cerebral angiogenesis [40]. Exogenous administration of each of the four EETs regioisomers proved mitogenic in both *in vitro* and *in vivo* models of cortical angiogenesis [41]. Although the precise mechanisms governing the regulation of cortical angiogenesis by EETs remain unclear, in the peripheral circulation the angiogenic action of EETs is mediated by activation of the mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3-kinase)-Akt pathways [42-47].

It is noteworthy that recent studies using multiple vascular cell types, including human umbilical vein (HUVEC), porcine coronary artery and bovine retinal endothelial cells demonstrated a role for EETs in hypoxia-induced angiogenesis [48,49]. Exposure of cultured endothelial cells to hypoxic conditions elevated cellular CYP2C epoxygenase protein expression and 11,12-EET production. Hypoxia-evoked increases in endothelial cell proliferation were blocked individually by CYP2C anti-sense oligonucleotide gene knockdown, inhibition of CYP epoxygenase activity with miconazole, MS-PPOH or sulphaphenazole, or by blockade with the putative EETs receptor antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (EEZE) [48,49]. These findings support a key role for EETs signaling in hypoxia-evoked angiogenesis and may have important implications for the mechanisms of protection afforded by endogenous EETs signaling following hypoxic or ischemic insult.

In the cerebral circulation, EETs signaling additionally regulates platelet aggregation. Intravenous administration of 14,15-EET, but not 8,9-EET increased the clot formation latency in an *in vivo* mouse photothrombosis model of platelet aggregation in cerebral surface vessels [22]. In that study, it was noted that 14,15-EET administration correlated with a reduction in circulating thromboxane levels. In an earlier biochemical study utilizing cell-free preparations, 8,9-EET and 14,15-EET inhibited COX activity, reducing both prostaglandin E2 (PGE2) and thromboxane B2 production [50]. These findings were corroborated in intact human platelets, in which both 8,9-EET and 14,15-EET inhibited thromboxane B2 release and platelet aggregation. In a more recent study, 8,9-EET, 11,12-EET and 14,15-EET were observed to hyperpolarize isolated human platelets, with the effect of 11,12-EET being most potent [23]. Furthermore, 11,12-EET inhibited platelet adhesion to endothelial cells, an effect that was sensitive to calcium-sensitive K⁺ (K_{Ca}) channel blockade. The effects of exogenous EETs upon platelet membrane potential and adhesion were mimicked by overexpressing CYP epoxygenase in endothelial cells, suggesting that endothelial EETs may contribute to these effects [23]. In light of previous studies documenting EETs release from platelets [51], it appears likely that the role of EETs in reducing platelet aggregation in the cerebral circulation may involve release both from the endothelium and platelets themselves, and may proceed through membrane potential- and COX-dependent pathways to suppress platelet activation.

Recent evidence also suggests that EETs signaling may suppress the ischemia-evoked inflammatory cytokine response in the brain, supporting an anti-inflammatory role for EETs in the brain circulation [52]. Thus, in addition to the well-known vasodilator functions of EETs,

these lipid signaling molecules exert multiple effects upon the cerebral circulation including pro-angiogenic, anti-thrombotic and anti-inflammatory influences. This wide spectrum of roles argues that EETs are central regulators of cerebrovascular function, exercising a broadly protective influence on the cerebral circulation. As such, EETs are likely modifiers of stroke and other neurovascular disease and consequently, the role of EETs in these processes remain active areas of current research.

EETs metabolism by sEH is a key determinant of EETs bioavailability

Because of the important contribution of EETs to the regulation of cardiovascular function, including its central role in CBF regulation, considerable interest has developed in the modification of tissue EETs levels, both for experimental and therapeutic applications. Direct administration of EETs as a therapeutic compound has proven effective in the treatment of cardiac and cerebral ischemia [4,53]. Although of value in an experimental context, this avenue may prove impractical in human studies, owing in part to the quantities required for the prolonged EETs infusion used experimentally. This requirement for prolonged continuous infusion is likely due to high endogenous sEH activity *in vivo*.

Treatment with the cholesterol-lowering agents fibrates, which can increase CYP epoxygenase expression and activity in some tissues, has been employed experimentally to amplify EETs signaling. In the obese Zucker rat model, chronic treatment with fenofibrate was observed to elevate renal cortical and vascular CYP epoxygenase expression, restoring acetylcholine-evoked vasodilation in the renal vasculature [54]. In a second transgenic rat model of hypertension, fenofibrate similarly increased renal CYP epoxygenase expression and activity [55]. As a therapeutic modality for increasing EETs bioavailability, however, fibrate administration is limited by their non-specific induction of several P450 isoforms, including CYP4A enzymes involved in drug metabolism and production of vasoconstrictor eicosanoid species [55].

A third approach to increasing tissue EETs levels involves inhibition of their metabolism, leading to the accumulation of endogenous EETs. While EETs undergo β -oxidation and chain elongation, sEH represents a major pathway of metabolism for the 8,9-, 11,12- and 14,15-EET regioisomers. 5,6-EET is a comparably poor substrate for sEH, although it in addition to 8,9-EET are metabolized by COX to produce further bioactive metabolites [9,56,57]. A chief route of endogenous EETs metabolism, sEH is now the subject of much attention as a key determinant of biological EETs levels. Indeed, inhibition of sEH activity, either through pharmacological blockade or genetic deletion, increases EETs levels both in blood and tissue [58,59].

Expression of sEH in the brain

Despite extensive investigation of EETs' role in regulating the cerebral circulation, the cell type-specific expression of sEH in the brain has only recently been defined. In the cerebral vasculature, sEH is present both in vascular smooth muscle and endothelial cell layers where it is presumed to regulate the vascular actions of EETs [3,4,38,60]. Extensive glial expression is also observed in humans, restricted primarily to oligodendrocytes in the white matter [38, 60]. These findings are supported by our own group's observations from the mouse and rat where sEH-immunoreactivity is observed in white matter tracks (Figure 3B) but does not co-localize with the astrocyte marker glial fibrillary acidic protein (GFAP, Figure 3B,E-F).

More intriguing, however, is the finding that the most dominant expression of sEH in rodent brain is neither vascular nor glial, but rather neuronal. In brain parenchymal tissue, sEH is markedly expressed in neuronal cell bodies and processes, particularly within the cerebral cortex and striatum (Figure 3C-F) [3]. In the cortical surface vasculature, we have noted that

sEH expression is most prominent in perivascular vasodilator nerve fibers innervating pial arteries (Figure 3A) [38]. Although the significance of this neuronal sEH expression remains unclear, early experimental evidence from our group suggests that expression in perivascular nerves may be involved in neurogenic control of the cerebral vasculature [39], whereas the parenchymal neuronal expression may play a role in non-vascular neuroprotective pathways under pathophysiological conditions, such as cerebral ischemia [61].

Genetic variation in the human EPHX2 gene

Early biochemical studies of sEH reported pronounced intra-individual variability in enzyme activity [62]. Since then, a number of genetic and biochemical studies have identified sEH variants present within human populations and have begun to assess whether this variability contributes to changes in cardiovascular risk. EPHX2 gene sequencing studies identified several polymorphisms within study populations, some of which resulted in non-synonymous amino acid substitutions (Table 2) [63,64]. Biochemical analyses of these variants revealed that some, including the Lys55Arg (substitution of the 55th amino acid Lysine with Arginine), Cys154Tyr and Glu470Gly substitutions resulted in an enzyme with increased epoxide hydrolase activity. Such ‘gain-of-function’ variants might be expected to hydrolyze EETs more efficiently resulting in reduced tissue and circulating EETs levels in individuals harboring these polymorphisms. Conversely, two other variants, the Arg287Gln substitution and the Ser402Arg_{ins} insertion resulted in enzymes with reduced epoxide hydrolase activity and defective protein stability. These ‘loss-of-function’ variants would be expected to increase EETs bioavailability in effected individuals [63-65].

Association of EPHX2 polymorphisms with ischemic stroke risk

With the identification of functionally relevant genetic variants of sEH within the human population (Table 2), a number of epidemiological studies have examined the potential linkage between these polymorphisms and the risk of ischemic stroke. In the earliest study, a positive association was observed within an African American cohort between the Glu470Gly ‘gain-of-function’ sEH variant [63] and the incidence of ischemic stroke [5]. In a second study, the Arg287Gln variant which corresponds with decreased epoxide hydrolase activity and reduced protein stability [63-65] was independently associated with a decreased risk of ischemic stroke in a Chinese population [7]. This latter finding is in agreement with work from our group demonstrating that expression of the human Arg287Gln sEH variant in rat neurons protects them from ischemic damage [61].

These associations between sEH variant activity and ischemic stroke risk correspond well to EETs’ demonstrated role in the regulation of the cerebral vasculature and the function of sEH as the primary mechanism of EETs metabolism in the brain. The presence of loss-of-function sEH variants would be expected to reduce EETs metabolism, thus stabilizing tissue and circulating EETs levels and resulting in reduced cardiovascular and cerebrovascular risk. In contrast to the above findings, however, a second study in a white European cohort reported a positive association between the Arg287Gln variant and the risk of ischemic stroke [6], an effect that was primarily driven by the occurrence of large-vessel stroke. The explanation for the apparently contradictory findings of these studies remains unclear. The findings from the Gschwendtner et al. [6] study are in agreement with recent genetic data linking the Arg287Gln sEH variant with atherosclerotic processes [66]. The study population, however, was of considerably smaller size than the large Atherosclerosis Risk in Communities (ARIC) cohort of the Fornage et al. [5] study and the study failed to account for vascular risk factors such as cigarette smoking.

Association of CYP2C and CYP2J genetic variants with cardiovascular disease and stroke

As with the EPHX2 gene encoding sEH, variants of the human P450 epoxigenase genes CYP2C8, CYP2C9 and CYP2J2 have been identified. Early studies suggested that a polymorphism in the CYP2J2 gene was associated with increased risk of coronary artery disease [67] and early myocardial infarction [68]. More recent studies in larger populations have failed to replicate these findings [69,70]. Genetic variants in the CYP2C8 and CYP2C9 genes have also been linked with an increased risk of heart disease [69] and myocardial infarction [71], however, a subsequent study failed to detect any linkage between CYP2C8 or CYP2C9 genetic variants and the risk of myocardial infarction [70]. While the relationship between genetic variants of human epoxigenases and cardiovascular disease appears to be complex, there is no apparent association between these variants and the risk of ischemic stroke [7,70].

sEH inhibition in the treatment of ischemic stroke

Given EETs' importance as regulators of cardiovascular function and the cerebral vasculature, sEH inhibition (sEH-I) is emerging as a promising therapeutic target for the prevention and treatment of ischemic stroke. The promise of sEH-I derives from the multi-faceted role EETs play in cardiovascular and cerebrovascular regulation. EETs are potent vasodilators [15,72], with anti-inflammatory [20,73], anti-thrombotic [22], fibrinolytic [24], angiogenic [40,41], anti-apoptotic [61,74-77] and anti-oxidant [78] effects (Table 1). Inhibition of sEH thus represents a novel multi-mechanism approach to the treatment of stroke, offering the ability to harness EETs' broad protective profile by pharmacologically targeting a single enzyme.

Modification of cardiovascular risk with sEH inhibition

While treatment options for acute ischemic stroke have not appreciably advanced in recent times, much advantage may be gained by reducing cardiovascular risk factors in those patients demonstrating a heightened risk of stroke as well as those who have previously suffered a stroke. It is noteworthy then that much pre-clinical work has been conducted in animal models exploring the treatment of chronic hypertension with sEH-I. The results and implications of this work have been extensively and recently reviewed elsewhere [79,80]. Briefly, male sEH knockout mice exhibited lower systemic blood pressure compared to wild type animals [81], although a second strain of sEH knockout mice demonstrated no change in systemic blood pressure [82]. In rodent models of spontaneous and angiotensin-induced hypertension, elevated sEH levels are observed in the kidney [83,84]. Pharmacological inhibition of sEH has repeatedly demonstrated efficacy in normalizing blood pressure in these models of hypertension [84-86].

In addition to its effects on the development of hypertension, sEH-I has demonstrated efficacy in retarding the development of atherosclerosis [87-89]. In human populations, polymorphisms in the sEH gene are associated with coronary atherosclerosis [66,90,91]. Treatment with the sEH inhibitor 1-cyclohexyl-3-dodecyl urea (CDU) attenuated proliferation of human aortic vascular smooth muscle cells *in vitro* [87,88]. In an *in vivo* model of accelerated atherogenesis, the apolipoprotein E-knockout (ApoE) mouse, chronic administration of the sEH inhibitor 1-adamantan-3-(5-(2-(2-ethylethoxy)ethoxy)pentyl)urea (AEPu) in drinking water reduced atherosclerotic lesion formation in the descending aorta by more than 50%. This effect was associated with an increased serum EET:DHET ratio, suggesting effective enzymatic inhibition of sEH by this drug [89]. Furthermore, in a separate study, chronic sEH-I improved the compliance of large cerebral arteries in a rodent model of spontaneous hypertension, a finding that may have important implications for ischemic stroke risk and outcome [92]. Based upon the promising results from these and other pre-clinical animal studies, inhibition of sEH appears to hold considerable promise for the treatment of several chronic cardiovascular conditions,

including hypertension and atherosclerosis. Thus, through the modification of cardiovascular risk factors, sEH-I may prove a useful approach for reducing the risk of first-time or recurrent stroke.

Effect of ischemia on EETs synthesis and metabolism

As the key role of EETs signaling in cerebrovascular regulation emerged, EETs' participation in the pathogenesis and treatment of stroke became an active area of research. A substantial body of evidence suggests that the EETs signaling system is regulated by hypoxia or ischemic events. Endothelium from several species and vascular beds respond to hypoxic conditions with increased CYP2C mRNA and protein expression, in addition to EETs production [48, 49]. In cultured human endothelial cells, exposure to hypoxic conditions elevated CYP2C9 promoter activity and CYP2C8 transcript levels, increasing expression of CYP2C protein. The treatment increased endothelial 11,12-EET production and was associated with elevated expression of the transcription factor Hypoxia Inducible Factor (HIF) 1 α [48]. In contrast to these findings, cultured bovine aortic endothelium demonstrated reduced CYP2J expression [78], suggesting that the effects of hypoxia on CYP epoxygenase expression may be isoform-specific. In cultured primary rat cortical astrocytes, hypoxic conditions increased activity at the CYP2C11 promoter in addition to CYP2C11 mRNA and protein expression. These effects were accompanied by increases in HIF 1 α protein expression and binding at the CYP2C11 promoter [76]. In cultured primary rat hippocampal astrocytes, CYP2C11 expression and EETs release was likewise elevated by exposure to hypoxic conditions [93].

Like hypoxia, ischemic conditions appear to up-regulate EETs signaling. In the dog, the onset of experimental cardiac ischemia increased coronary venous outflow of 14,15-EET both during the occlusion and reperfusion periods [94]. This suggests that epoxygenase activity and/or EETs release is elevated acutely in response to ischemic insult. In rat brain, CYP2C11 message and protein up-regulation was evident two and three days following the onset of repeated transient (3×10 min) ischemic insults, respectively [95]. This ischemic 'preconditioning' stimulus protected the brain against subsequent major ischemic injury in a manner that was sensitive to CYP epoxygenase inhibition, suggesting that the EETs signaling pathway represents an ischemia-activated endogenous neuroprotective pathway *in vivo*. Further recent work from our laboratory demonstrated that sEH expression in the mouse brain is reduced in response to ischemic insult [52]. These studies have thus demonstrated the regulation of the EETs signaling system by hypoxia and ischemic conditions, establishing an endogenous cytoprotective role for EETs in hypoxic or ischemic brain injury and suggesting that this pathway is a promising target for the treatment of ischemic stroke.

Treatment of acute ischemic stroke with sEH inhibition

More recent studies have sought to harness the protective effects of cerebrovascular EETs through targeting sEH for the treatment of acute ischemic stroke. Although to date only three studies have directly tested the effects of sEH-I upon stroke outcome *in vivo*, the results have thus far been promising: in each of these three trials, sEH-I has proven to be protective against experimental cerebral ischemia.

The first trial of sEH-I was conducted in a hypertensive model of experimental stroke, the stroke-prone spontaneously hypertensive rat (SHRSP) subjected to permanent MCA occlusion for six hours. In this study, the sEH inhibitor 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA, 25mg/L) was administered chronically in drinking water for six weeks beginning prior to the onset of spontaneous hypertension. sEH-I with AUDA significantly reduced the infarct volume as determined by 2,3,5-triphenyltetrazolium (TTC) staining by 34% [92]. It is interesting to note that in this study, while AUDA administration increased urine epoxide:dihydroxide metabolite ratios suggesting effective inhibition of sEH activity, no effect

upon the development of spontaneous hypertension was observed. AUDA treatment produced plasma drug concentrations of 5ng/ml, levels below those demonstrated to be effective in treating angiotensin II-induced hypertension (15ng/ml). Thus, the apparent protective effects of sEH-I in the context of cerebral ischemia appear independent of blood pressure effects.

More detailed study of sEH-I in the treatment of ischemic stroke has been conducted in a mouse model of focal ischemia/reperfusion injury [3,4]. Mice were subjected to a two-hour occlusion of the proximal MCA, with ischemia confirmed by laser Doppler flowmetry over the ipsilateral cortex. The occlusion was then relieved and reperfusion allowed for 24 hours. In this model, administration of exogenous EETs (1.7µg/kg/hr iv) for 24hr prior to the onset of ischemia reduced infarct volume as determined by TTC staining by 68% compared to vehicle treated (Figure 4B). Pharmacological sEH-I with the sEH inhibitor 12-(3-adamantan-1-yl-uriedo)-dodecanoic acid butyl ester (AUDA-BE, 10mg/kg ip) resulted in a 50% reduction in infarct volume (Figure 4A,C). The protective effect of sEH-I was sensitive to blockade of EETs synthesis with the CYP epoxygenase inhibitor N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH, Figure 4C). These findings demonstrate that under ischemic conditions, sEH-I acts to amplify the endogenous protective EETs signaling pathway. Importantly, sEH-I was protective when AUDA-BE was delivered systemically either 1 hour prior to ischemia onset or at the start of reperfusion [3]. The degree of protection afforded by these two time courses were not different, suggesting that sEH-I is protective in the post-ischemic period and supporting its utility as a clinical treatment for acute ischemic stroke. Corroborating the finding that pharmacological sEH-I improves ischemic outcome, a second study demonstrated that the targeted deletion of the EPHX2 gene in mice also results in a reduced infarct volume (Figure 4D) [4]. In this study, sEH knockout mice had infarct areas reduced more than 50% compared to wild type animals in addition to exhibiting elevated EET:DHET ratios in plasma.

The role of EETs in the ischemic protection by sEH inhibition

Although hydrolysis of EETs to DHET metabolites by sEH is considered the dominant pathway for EETs metabolism in the vasculature, under conditions of sEH-I alternative pathways of EETs metabolism may become physiologically relevant. In cultured porcine endothelial cells radiolabeled with ³H-14,15-EET, 85% of EETs metabolism proceeded through the sEH pathway [56]. Following sEH-I with DCU, this was reduced to 13% while small amounts of β-oxidation and chain elongation products were detected. Interestingly, in cultured human coronary endothelial cells, DHET production accounted for only 15% of radioactivity while β-oxidation and elongation products constituted a combined 28% under control conditions [97]. In this study, it was noted that the β-oxidation product 10,11-epoxy-hexadecadienoic acid (16:2) possessed potent vasodilator and anti-inflammatory properties in the coronary vasculature. Thus, therapeutic inhibition of sEH may result in the shunting of EETs through alternative metabolic pathways, some of which may have physiological effects.

Whether the protective effects of sEH-I against ischemic damage are mediated by EETs *per se* or rather by these bio-active metabolites remains unclear at present. In the heart and the brain, the protective effects of sEH-I against ischemic insult have proven to be sensitive to blockade either with the P450 epoxygenase-specific inhibitor MS-PPOH or the putative EETs receptor antagonist 14,15-EEZE [3,59,61,98]. These data would suggest that if the protective effects of sEH-I are mediated through such 'alternative' metabolites, then they are likely acting at the same molecular target as their parent EETs. As sEH-I progresses as a therapeutic target, further experimental work is necessary to determine the roles of these metabolites under conditions of sEH-I, particularly in defining the un-foreseen side effects of this treatment modality within the vascular system.

Mechanisms of ischemic protection by sEH inhibition: cerebral blood flow effects

EETs are best known for their role as potent vasodilators, including in the cerebral circulation where they are key regulators of CBF [28,30,39]. In light of this, one potential mechanism for the protection from ischemic damage afforded by sEH-I might be through the preservation of brain tissue perfusion during focal ischemia and reperfusion. Inhibition of sEH would be expected to impact upon the levels and actions of EETs produced from any source in the cerebral circulation, including endothelial, astrocytic and neurogenic EETs (Figure 2). This might result in intra-ischemic vasodilation of the occluded vessel, permitting the passage of more residual blood flow into the effected region. Alternatively, the recruitment of collateral blood flow during ischemia might be augmented resulting in improved perfusion either through overlapping watershed regions or through retrograde blood flow within the occluded vascular network. It is also possible that following reperfusion, CBF regulation could be preserved, preventing damage associated with post-ischemic vascular dysregulation in the cerebral circulation.

Initial studies in rodent models of focal cerebral ischemia have begun to shed light on the role preservation of CBF regulation plays in the protection afforded by sEH-I. Chronic sEH-I with AUDA in hypertensive rats did not alter myogenic responses of isolated MCAs, nor were the vasomotor responses to the endothelium-dependent vasodilator bradykinin or the vasoconstrictor serotonin affected [92]. These findings suggest that at least in the hypertensive animal, sEH-I does not alter the vasomotor properties of cerebral arteries.

In the mouse model of transient focal cerebral ischemia, pre-treatment with the sEH inhibitor AUDA-BE did not alter blood flow during the intra-ischemic period (Figure 5A-B) [3]. This finding argues against the preservation of intra-ischemic CBF as the basis for the ischemic protection afforded by pharmacological sEH-I. As of yet, no studies have investigated the effect of sEH-I upon CBF regulation in the reperfusion phase of cerebral ischemia/reperfusion injury. In the mouse, sEH-I initiated at the onset of reperfusion was as effective as pre-ischemia treatment in reducing infarct area [3]. This argues that the critical period for sEH-I is during reperfusion. Whether the effect during the reperfusion period involves a CBF component cannot yet be ruled in or out. Interestingly, sEH knockout (sEHKO) mice, possessing the targeted deletion of the EPHX2 gene, displayed a gradual recovery of ipsilateral CBF during the intra-ischemic interval (Figure 5C-D), as measured by laser Doppler flowmetry and autoradiography [4]. The explanation for the difference between pharmacological sEH-I and sEH gene deletion is unclear at present. One possibility is that the degree of sEH-I achieved by pharmacological blockade was insufficient to elicit the CBF effects of complete sEH inactivation observed with gene deletion. In the brain, EETs signaling and sEH activity are likely present in multiple compartments, including the neuronal (Figure 3) [3, 38, 39, 61], astrocytic [30, 99] and vascular compartments [28]. Differential access of systemically administered sEH inhibitors to these compartments may also explain these disparate results.

A second possibility relates to the fact that sEH is a bi-functional protein, possessing a C-terminus hydrolase domain in addition to an N-terminus lipid phosphatase domain [100]. The physiological function of this phosphatase domain remains unclear, and while a discussion of the implications of the bi-functional nature of sEH is beyond the scope of the present review, it is noteworthy that sEHKO mice lack both sEH functional domains, whereas pharmacological inhibition of sEH is presumed to inhibit primarily the hydrolase domain [100]. Thus the discrepant effect of pharmacological sEH inhibition and EPHX2 gene deletion on intra-ischemic perfusion may reflect an unappreciated contribution of the N-terminus phosphatase domain to CBF regulation.

Mechanisms of ischemic protection by sEH inhibition: cytoprotective effects

The absence of an effect upon intra-ischemic CBF by pharmacological sEH-I [3] suggests that this intervention may exert protective effects in a blood flow-independent manner. Indeed, in addition to their well known vasomotor actions in the cerebral circulation, EETs have demonstrated direct cytoprotective effects in a number of cell types, including astrocytes [76] and neurons [61] subjected to simulated ischemic conditions.

The cytoprotective effects of EETs in cortical astrocytes were initially investigated in the context of the phenomenon of hypoxic or ischemic preconditioning [76,95]. *In vivo* ischemic preconditioning proved to be blood flow-independent, and was associated with an up-regulation of the astrocytic CYP epoxygenase CYP2C11. In rat primary cortical astrocytes, hypoxic preconditioning was found to promote CYP2C11 expression through activation of hypoxia-inducible factor-1 α (HIF-1 α) and was cytoprotective in astrocytes exposed to simulated ischemia through oxygen and glucose deprivation (OGD) [95]. This effect involved EETs signaling, as it could be blocked through inhibition of EETs synthesis with the CYP epoxygenase inhibitor MS-PPOH. Direct administration of all four EETs regioisomers additionally proved protective to cortical astrocytes in the OGD model [76]. Thus, EETs protect cortical astrocytes from ischemic damage in a blood flow-independent manner, both as part of an endogenous cytoprotective pathway and also when exogenously administered.

EETs are likewise protective against ischemic insult in rat primary cortical neurons [61]. In this study, rat neurons were transduced with human sEH variants including those that have been linked with increased or decreased ischemic stroke risk. Strikingly, the Arg287Gln variant that exhibits reduced epoxide hydrolase activity and protein stability and is linked with reduced stroke risk in humans (Table 1) also proved protective against OGD in rat neurons *in vitro* [61]. Among many sEH variants, including the human wild type protein, direct administration of EETs or pharmacological sEH-I protected neurons from OGD-evoked cell death. Thus, sEH-I, through its interaction with EETs signaling pathways, appears to exert cytoprotective effects in cortical neurons within a blood flow-independent model of ischemic cell death.

The specific signaling mechanisms by which EETs exert their direct protective effects in cortical astrocytes and neurons remain unclear. Recent evidence suggests that EETs activate calcium-permeable TRPV4 channels [27,101]. Activation of such channels has been implicated in neuronal calcium overload in response to ischemic conditions and thus might not be expected to support direct neuroprotection [102]. In cerebrovascular smooth muscle, however, EETs-evoked activation of TRPV4 channels was associated with local calcium signaling events termed ‘sparks’ that result in BK channel activation and membrane hyperpolarization [27]. Whether this or other molecular interactions underpin the direct cytoprotective effects of EETs in neurons or astrocytes remains unknown and is a subject warranting further study as sEH-I emerges as a potential therapeutic avenue in the treatment of ischemic stroke.

The role of EETs as cytoprotective agents has been more extensively explored in the vascular endothelium. Direct administration of EETs to cultured endothelial cells provides protection from apoptosis induced by serum deprivation, Fas-inactivation or tumor necrosis factor- α (TNF- α) administration [74,77]. The cellular signaling cascades involved in these anti-apoptotic effects have begun to be defined and have been recently reviewed [75]. EETs exert protective effects via activation of the anti-apoptotic phosphatidylinositol-3'-OH (PI3) kinase/AKT pathway. Similarly, activation of the mitogen-activated protein kinase (MAPK) signaling cascade has been implicated in EETs-mediated cardioprotection. Whether these signaling pathways, or others, are involved in the cytoprotective effects of EETs and sEH-I in cortical astrocytes and neurons remains an important issue to be addressed in this field. What is apparent based upon the studies that have been conducted *in vitro* is that EETs in the brain can exert

blood flow-independent anti-apoptotic effects in several components of the neurovascular unit: neurons [61], astrocytes [76], and vascular endothelium [74,75,77]. This ability to directly target multiple components of the neurovascular unit for cytoprotection may underlie a part or all of the ischemic protection afforded by sEH-I.

Mechanisms of ischemic protection by sEH inhibition: anti-inflammatory effects

The activation of CNS inflammatory pathways in response to ischemic insult represents a major contributing factor to ischemic brain injury [103]. Inflammatory cytokines are released by ischemic brain cells, causing the recruitment of leukocytes into the CNS, activation of latent microglial cells, and eventual breakdown of the blood brain barrier. This vicious inflammatory cycle promotes cell death in the brain and expansion the tissue infarct. A recent study from our group demonstrated that inactivation of sEH may alter the brain inflammatory response to ischemic insult [52]. In response to ischemia/reperfusion injury, mice with the targeted deletion of the EPHX2 gene exhibited markedly lower levels of ischemia-evoked pro-inflammatory cytokine expression in the ipsilateral hemisphere than wild type controls during the initial post-ischemic period. In addition to these effects observed in the brain, EETs are broadly recognized to exert anti-inflammatory actions within the vascular endothelium, reducing expression of the leukocyte adhesion proteins V-CAM, I-CAM and E-selectin [9,104]. These effects are mediated by the EETs-evoked suppression of NF- κ B signaling. Although the influence of EETs upon cerebral endothelial adhesion molecule expression has not yet been investigated, it is plausible that EETs signaling in the brain may influence both the recruitment of inflammatory cells into the CNS as well as blood brain barrier integrity. Thus, either through its effects upon CNS inflammatory cytokine production or cerebral endothelium-leukocyte dynamics, the protective role of sEH-I in ischemia/reperfusion injury may be the result of the potentiation of the anti-inflammatory actions of endogenous EETs.

Other potential effects of sEH inhibition

The cellular effects of EETs have been explored in a myriad of cell and tissue systems. Their effects have been described variously as vasodilatory [15,72], anti-apoptotic [61,74-77], anti-inflammatory [20,73], angiogenic [40,41], fibrinolytic [24], anti-thrombotic [22] and anti-oxidant [78] (Table 1). Early pre-clinical studies in animal stroke models have provided evidence that the former three effects may contribute to the protective results of sEH-I in the treatment of acute ischemic stroke [3,4,52,61,76]. A role for the other cellular actions of EETs in the ischemic protection afforded by sEH-I cannot be ruled out.

The relative importance of these protective actions has not yet been defined and likely changes throughout the evolution of ischemic injury. The early minutes of cerebral ischemia are characterized by the development of excitotoxicity and occurrence of peri-infarct depolarizations. Beginning hours and persisting for days following the ischemic injury is the recruitment of an inflammatory response, followed by delayed apoptosis [105]. In the ischemic phase of the injury, the fibrinolytic, anti-thrombotic and vasoactive effects of EETs may contribute to the maintenance of local and collateral blood flow, thus reducing the metabolic and excitotoxic load and potentially attenuating recruitment of at risk tissue into the infarct core. Throughout this early period of the ischemic injury, EETs may likewise reduce apoptotic signaling and thus inhibit programmed death of penumbral cells later in the ischemic cascade. During reperfusion and early recovery, the anti-inflammatory actions of EETs may emerge into significance, blunting the recruitment of inflammatory cells into the damaged tissue. The pro-angiogenic effects of EETs likely participate late in the recovery and remodeling phase of the ischemic injury. Further studies are needed to probe the effects of sEH-I upon these cytoprotective pathways and to elucidate their specific contribution to the protective effects of sEH-I throughout cerebral ischemia/reperfusion injury. It is important to note, however, that although these differing protective effects of EETs are likely to wax and wane through the

course of ischemia/reperfusion injury, sEH-I offers a unique single pharmacological target capable of amplifying them each in turn.

Development of therapeutically useful sEH inhibitors

As interest in sEH as a therapeutic target in the treatment of cardiovascular disease and stroke has emerged, significant progress has been made in the design and synthesis of increasingly potent and bioavailable small molecule inhibitors of sEH activity, as has been reviewed recently [79]. Early work identified dicyclohexylurea (DCU), a common synthetic byproduct, as a potent inhibitor of sEH activity ($IC_{50} = 52nM$). Crystallographic evidence suggests that this and related compounds act to inhibit sEH by binding to the enzyme active site with high affinity, stabilizing the transition state. As a pharmacological agent, DCU proved problematic due to its high lipophilicity, appropriate only for early laboratory studies in animal models of disease.

The development of high-throughput screening assays for sEH activity and mass spectroscopic analysis of drug metabolite levels in the blood has since facilitated the rapid refinement of the initial DCU pharmacophore [79]. A second group commonly used sEH inhibitors were based on the compound 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) and its esters, replacing the DCU cyclohexyl groups with an adamantyl and long chain carboxylic acid or ester group. AUDA demonstrated high potency compared to DCU ($IC_{50}=3nM$) and despite a modest bioavailability, could be formulated to be administered in drinking water which proved to be protective against experimental ischemic stroke in hypertensive rats [92]. Its butyl ester form AUDA-BE ($IC_{50} = 7nM$) displayed improved bioavailability compared to AUDA and could be administered ip to improve stroke outcome in a mouse model of ischemia/reperfusion injury [3].

More recent refinements in sEH inhibitor structure have been aimed at improving the oral bioavailability of these compounds and reducing the rate of systemic clearance. A raft of potent lead compounds have emerged exhibiting excellent oral bioavailability (area under curve, AUC/ IC_{50}) and long elimination half-lives in blood [79]. These compounds may serve as lead structures for the pharmaceutical industry in the development of sEH inhibitors, usable in patients for the treatment of cardiovascular disease and acute ischemic stroke.

Conclusions

Acute ischemic stroke remains a major contributor to mortality and disability in the industrialized world. Despite decades of committed basic science research, therapeutic options for this condition remain extremely limited [1,2]. The evolution of cerebral ischemic injury and infarction is complex, involving the dynamic interplay of many cell types, including neuronal, glial, vascular and inflammatory cells, regulated through a myriad of signaling pathways. It is increasingly evident that therapeutic approaches that target these various players in the ischemic cascade, either through a cocktail approach or via a single drug targeting multiple mechanisms, hold considerable promise for the treatment of acute ischemic stroke.

Epoxyeicosatrienoic acids are broadly cytoprotective signaling molecules, exerting vasodilator, angiogenic, anti-apoptotic, anti-inflammatory, anti-thrombotic and anti-oxidant effects in many tissues (Table 1). In the cerebral circulation EETs mediate several levels of cerebral blood flow regulation, from local metabolic coupling to centrally driven neural control [28,30,37,39]. EETs exert direct, blood flow-independent, cytoprotective effects upon several individual components of the neurovascular unit: neurons [61], astrocytes [76] and vascular endothelium [75]. The pharmacological inhibition of soluble epoxide hydrolase, the chief breakdown enzyme for EETs, provides a novel multi-target therapeutic avenue to harness this powerful endogenous protective signaling pathway, impacting multiple cell types and systems involved in the evolution of ischemic injury. Early pre-clinical studies in animal models have

proven encouraging, demonstrating in all cases that inactivation of sEH reduces ischemic damage to experimental acute ischemic stroke [3,4,92]. Ongoing research from our group and others will continue to elucidate both the mechanisms underlying the ischemic protection afforded by sEH inhibition, while evaluating the translational potential of this multi-mechanism therapeutic approach for the treatment of human stroke.

Future Perspectives

It is clear from the above discussion that sEH-I is a novel and promising therapeutic target for the treatment of acute ischemic stroke, offering the opportunity to pursue multiple mechanisms of the ischemic cascade through action at a single pharmacological target. Early studies in rodent models of focal cerebral ischemia have provided proof of concept by demonstrating that sEH inactivation, either via pharmacological blockade or EPHX2 gene disruption, afford significant protection against ischemic damage *in vivo* [3,4]. Because the protective effects of sEH inhibition are generally considered to involve the activation of endogenous EETs signaling pathways, the development of this therapeutic avenue depends in large part upon wider trends in the field of epoxyeicosanoid signaling. Two important themes emerging in this field will likely shape our conception of sEH inhibition in the context of ischemic stroke treatment and how it may eventually translate clinically. These themes are the striking capacity both for specificity and promiscuity in the EETs signaling pathway, as well as this system's dynamic relationship with other members of the arachidonic acid cascade.

For the sake of simplicity, EETs signaling is often described as a single effector pathway that impacts upon multiple elements of vascular and non-vascular cell function. However, there are eight chemically distinct EETs enantiomers that may exhibit specific affinity for the various molecular targets thus far defined for EETs, including BK channel, TRPV4 cation channel, or peroxisome proliferator-activated nuclear receptors (PPARs) [9]. The wide spectrum of EETs' cellular actions, including the cytoprotective effects detailed on Table 1, may reflect the action of discrete EETs enantiomers serving as ligands for distinct receptors and their associated signal transduction pathways.

The identification of specific EETs binding partners and their respective affinities for the EETs enantiomers has been a persistent dilemma in the epoxyeicosanoid field, including the failure to identify a putative GPCR(s) thought to underpin many cellular actions of EETs. Interestingly, a recent study provided evidence that EETs represent a novel endogenous competitive antagonist at the thromboxane receptor and that at least some of EETs' vascular effects are related to the antagonism of thromboxane A2 signaling [106]. This provocative hypothesis, if confirmed, would have important implications on the mechanisms of ischemic protection afforded by sEH-I, specifically regarding the potential contribution of blood flow and platelet dynamics to these observed protective effects.

The specificity apparent in the EETs signaling pathway may prove important to the refinement of sEH inhibition in the treatment of ischemic stroke. One of the benefits of sEH as a therapeutic target, which forms the basis of its identification as a prototype multiple-mechanism therapy, is its inherent non-specificity. Because it is the enzyme chiefly responsible for the metabolism of EETs, sEH inhibition will serve to increase endogenous levels of all of these compounds, amplifying the myriad protective cellular actions associated with them. In particular, sEH-I would be expected to exert its greatest effects in tissues and under conditions where EETs production is greatest. Importantly, hypoxic and ischemic conditions are known regulators of the EETs signaling pathway, increasing CYP epoxygenase expression and activity [49,76, 95]. Thus, sEH-I may have a more profound therapeutic action in hypoxic or ischemic tissue relative to healthy tissue; a useful characteristic in a prospective anti-stroke therapy. As understanding of the specific contributions of the individual EETs enantiomers and their

associated receptors develops, further fine tuning of stroke therapy and the targeting of specific components of the ischemic cascade may become possible.

A second source of complexity in the cardiovascular and CNS EETs signaling pathway lay in the cell type localization and metabolite specificity of CYP epoxygenase enzymes. Different tissues, including the brain, heart, and their associated vasculatures express CYP epoxygenase enzymes in a cell type-specific pattern [8]. For example, rat astrocytes express CYP2C11, the homologue to human CYP2C8 [31,32], while rat trigeminal neurons express only CYP2J3 and CYP 2J4, the homologues to human CYP2J2 [39]. Each of these isoforms produces a distinct profile of EETs regio- and stereoisomers. Human CYP2C8 produces only 11,12- and 14,15-EET with a 4:1 R,S:S,R stereo-selectivity [107] while human CYP2J2 produces all four EETs regioisomers in roughly equal proportion with a 3:1 R,S:S,R stereo-selectivity [108]. In the CNS, EETs may originate from several distinct cellular compartments, including neurons [39], astrocytes [31,32] and endothelium [28]. Each of these compartments potentially involves distinct profiles of EETs enantiomers, interactions with discrete molecular targets, downstream signaling pathways, and associated cellular effects.

While pharmacokinetic data suggests that the sEH inhibitor AUDA-butyl ester (AUDA-BE) can cross the blood brain barrier [3], it remains unclear which EETs signaling compartments in the brain account for the ischemic protection afforded by sEH inhibition. It is noteworthy that sEH knockout mice undergoing experimental focal cerebral ischemia exhibited improved intra-ischemic CBF and reduced brain pro-inflammatory cytokine production [4,52]. In contrast, wild type mice undergoing pharmacological sEH inhibition did not exhibit these effects [3,52], although they were protected from ischemic damage to a similar degree. One explanation for these findings is that EPHX2 gene disruption affected all EETs signaling compartments whereas pharmacological sEH inhibition gained incomplete access to one or more of these compartments. As the respective roles these signaling compartments in the ischemic cascade are defined experimentally, it may become possible to differentially target them with sEH inhibition or another related therapy. By altering the formulations of sEH inhibitors, it should prove possible to specifically inhibit vascular sEH or perhaps increase access of the compound into the neuronal compartment. More ambitiously, cell type-specific gene knockdown may in the future permit the fine targeting of specific EETs signaling compartments through emerging small interfering (siRNA) or short hairpin RNA (shRNA) technologies.

One final intriguing perspective concerning the targeting of sEH as a therapeutic avenue in the treatment of acute ischemic stroke relates to the dynamic interplay between the P450 epoxygenase pathway and other pathways of arachidonic acid metabolism. The P450, COX and lipoxygenase (LOX) metabolic pathways utilize arachidonic acid as substrates for the synthesis of various eicosanoid metabolites, including the vasoconstrictor P450 hydroxylase metabolite 20-hydroxyeicosatetraenoic acid (20-HETE) and pro-inflammatory COX metabolite prostaglandin E2 (PGE2). Within subcellular domains where the AA pool is held in common, interventions that alter the rate of AA metabolism by one pathway are likely to have a reciprocal effect in the parallel pathway. Alternatively, metabolites of one pathway may exercise regulation over components of other pathways.

20-HETE is a potent vasoconstrictor produced from arachidonic acid in cerebrovascular smooth muscle by P450 ω -hydroxylase enzymes [8]. Following stroke, plasma 20-HETE levels increase while inhibition of 20-HETE synthesis reduces infarct volume and improves functional outcome in rat models of focal ischemia [109,110]. While such an approach has not yet been tested experimentally, sEH and P450 hydroxylase inhibition may act synergistically to reduce stroke damage. In addition to blocking the direct vasoconstrictor and cytotoxic effects of 20-HETE, P450 hydroxylase inhibition would be expected to shunt arachidonic acid into

other metabolic pathways, increasing flux through the P450 epoxygenase pathway. This effect, in combination with the inhibition of EETs metabolism by sEH, might further increase endogenous levels of vasoactive and cytoprotective EETs.

A second provocative example of the integrated regulation of distinct eicosanoid pathways is presented in a recent review from Dr. Bruce Hammock's group [111]. The authors demonstrate that prophylactic treatment of mice with the sEH inhibitor AUDA-BE reduces COX-2 protein induction in the liver following inflammatory challenge with lipopolysaccharide (LPS). Similarly, following LPS challenge, plasma levels of PGE₂ were reduced by sEH-I. Co-administration of AUDA-BE with COX-2 inhibitors celecoxib or rofecoxib had a synergistic effect upon plasma PGE₂ levels that is likely the result of reduced COX-2 expression and activity. Interestingly, this same combination therapy elevated plasma EETs levels to nearly double that observed with sEH-I alone [111]. The authors suggest that while sEH-I supports and stabilizes endogenous EETs levels, concomitant inhibition of the COX pathway may serve to 'shunt' AA into the CYP epoxygenase metabolic pathway, thus further elevating EETs levels. Such a reciprocal synergism between COX and sEH inhibition, resulting in changes in prostaglandin and EETs levels may be of great utility in the context of acute ischemic stroke. Through the effect of sEH-I upon COX-2 expression and activity, the inflammatory component of the ischemic cascade may be reduced. Through the effect of COX inhibition upon endogenous EETs levels, the protective effects of sEH-I may be amplified or perhaps achieved at lower (and potentially safer) therapeutic dosages. These effects, in light of the recent evidence that EETs are endogenous competitive antagonists at the thromboxane receptor [106], argue that sEH inhibition may hold therapeutic promise not simply as a stand-alone stroke therapy, but also as a potential member of an effective combination therapy.

As the mechanistic basis for the ischemic protection afforded by soluble epoxide hydrolase inhibition is defined experimentally and sEH inhibitors continue to advance towards clinical application, we have good reason to be optimistic for the prospects of this therapy as a multiple-mechanism single-target avenue for the treatment of acute ischemic stroke.

Executive Summary

Multi-mechanism therapeutic approach for ischemic stroke

- Stroke treatments based on pre-clinical animal models have proven largely unsuccessful in human clinical trials. One explanation for these failures is the narrow mechanistic targeting of emergent therapies that cannot account for multi-system evolution of ischemic injury in humans.
- A multi-mechanism approach, targeting multiple elements of the ischemic cascade through the action of a single pharmacological agent, may prove more effective.

Epoxyeicosatrienoic acids (EETs) signaling

- EETs are synthesized from arachidonic acid by P450 epoxygenases, producing four regioisomers: 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET. Each regioisomer represents two stereoisomers, making eight chemically distinct EETs enantiomers.
- EETs are potent vasodilators in the cerebral circulation, regulating the endothelial, metabolic and neurogenic control of the cerebral vasculature.
- EETs exert direct cytoprotective effects upon several components of the neurovascular unit, including cortical neurons, astrocytes and vascular endothelium.
- EETs are angiogenic, anti-inflammatory and anti-thrombotic in the brain circulation.

- Soluble epoxide hydrolase (sEH) is the primary pathway of endogenous EETs metabolism, critically regulating bioactive EETs levels. Pharmacological sEH inhibition increases endogenous EETs levels in both tissue and blood.

Genetic variation in EPHX2 gene and ischemic stroke risk

- Genetic variants of the sEH gene EPHX2 have been identified in humans associated with ischemic stroke risk.

Soluble epoxide hydrolase (sEH) inhibition in the treatment of ischemic stroke

- Pharmacological inhibition of sEH or targeted sEH gene deletion is protective against ischemic damage in rodent models of focal cerebral ischemia.
- sEH gene deletion is associated with preservation of intra-ischemic cerebral blood flow in the brain, suggesting that effects upon cerebral blood flow participate in the protective effects of sEH inactivation.
- sEH inhibition directly protects cultured cortical neurons from simulated ischemic conditions, arguing that at least a portion of the beneficial effects of sEH blockade involves the blood flow-independent cytoprotective effects of EETs.
- Pharmacological sEH inhibition reduces the pro-inflammatory response to cerebral ischemic insult, suggesting that the anti-inflammatory effects of EETs contribute to the protective effect of sEH inhibition in ischemic stroke.

Development of therapeutically useful sEH inhibitors

- Potent and selective sEH inhibitors are currently under development, many displaying promising pharmacokinetic profiles.

Future perspectives

- sEH is a promising multi-mechanism therapeutic target for the treatment of stroke. Through this single pharmacological target, sEH inhibitors can modulate both circulating and tissue EETs levels, harnessing these endogenous agents' broad protective profile and modifying many distinct injury/recovery pathways involved in development of ischemic stroke.

Bibliography

1. Fisher M. Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke* 2003;34:1539–1546. [PubMed: 12750546]
2. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol* 2006;59:467–477. [PubMed: 16453316]
3. Zhang W, Koerner IP, Noppens R, Grafe M, Tsai H, Morisseau C, Luria A, Hammock BD, Falck JR, Alkayed NJ. Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J Cereb Blood Flow Metab* 2007;27:1931–1940. [PubMed: 17440491]
4. Zhang W, Otsuka T, Sugo N, Ardeshiri A, Alhadid YK, Iliff JJ, DeBarber AE, Koop DR, Alkayed NJ. Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. *Stroke* 2008;39:2073–2078. [PubMed: 18369166]
5. Fornage M, Lee CR, Doris PA, Bray MS, Heiss G, Zeldin DC, Boerwinkle E. The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. *Hum Mol Genet* 2005;14:2829–2837. [PubMed: 16115816]
6. Gschwendtner A, Ripke S, Freilinger T, Lichtner P, Müller-Myhsok B, Wichmann H, Meitinger T, Dichgans M. Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with an increased risk of ischemic stroke in white Europeans. *Stroke* 2008;39:1593–1596. [PubMed: 18323494]

7. Zhang L, Ding H, Yan J, Hui R, Wang W, Kissling GE, Zeldin DC, Wang DW. Genetic variation in cytochrome P450 2J2 and soluble epoxide hydrolase and risk of ischemic stroke in a Chinese population. *Pharmacogenet Genomics* 2008;18:45–51. [PubMed: 18216721]
8. Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002;82:131–185. [PubMed: 11773611]
9. Spector AA, Norris AW. Action of epoxyeicosatrienoic acids on cellular function. *Am J Physiol, Cell Physiol* 2007;292:C996–1012. [PubMed: 16987999]
10. Wong PY, Lai PS, Shen SY, Belosludtsev YY, Falck JR. Post-receptor signal transduction and regulation of 14(R),15(S)-epoxyeicosatrienoic acid (14,15-EET) binding in U-937 cells. *J Lipid Mediat Cell Signal* 1997;16:155–169. [PubMed: 9246605]
11. Wong PY, Lin KT, Yan YT, Ahern D, Iles J, Shen SY, Bhatt RK, Falck JR. 14(R),15(S)-epoxyeicosatrienoic acid (14(R),15(S)-EET) receptor in guinea pig mononuclear cell membranes. *J Lipid Mediat* 1993;6:199–208. [PubMed: 8395243]
12. Fukao M, Mason HS, Kenyon JL, Horowitz B, Keef KD. Regulation of BK(Ca) channels expressed in human embryonic kidney 293 cells by epoxyeicosatrienoic acid. *Mol Pharmacol* 2001;59:16–23. [PubMed: 11125019]
13. Li PL, Campbell WB. Epoxyeicosatrienoic acids activate K⁺ channels in coronary smooth muscle through a guanine nucleotide binding protein. *Circ Res* 1997;80:877–884. [PubMed: 9168791]
14. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 1996;78:415–423. [PubMed: 8593700]
15. Ellis EF, Police RJ, Yancey L, McKinney JS, Amruthesh SC. Dilatation of cerebral arterioles by cytochrome P-450 metabolites of arachidonic acid. *Am J Physiol* 1990;259:H1171–7. [PubMed: 2121049]
16. Proctor KG, Falck JR, Capdevila J. Intestinal vasodilation by epoxyeicosatrienoic acids: arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. *Circ Res* 1987;60:50–59. [PubMed: 3105909]
17. Stephenson AH, Sprague RS, Weintraub NL, McMurdo L, Lonigro AJ. Inhibition of cytochrome P-450 attenuates hypoxemia of acute lung injury in dogs. *Am J Physiol* 1996;270:H1355–62. [PubMed: 8967376]
18. Zou AP, Fleming JT, Falck JR, Jacobs ER, Gebremedhin D, Harder DR, Roman RJ. Stereospecific effects of epoxyeicosatrienoic acids on renal vascular tone and K⁽⁺⁾-channel activity. *Am J Physiol* 1996;270:F822–32. [PubMed: 8928844]
19. Fleming I, Michaelis UR, Bredenkötter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res* 2001;88:44–51. [PubMed: 11139472]
20. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 1999;285:1276–1279. [PubMed: 10455056]
21. Sun J, Sui X, Bradbury JA, Zeldin DC, Conte MS, Liao JK. Inhibition of vascular smooth muscle cell migration by cytochrome p450 epoxygenase-derived eicosanoids. *Circ Res* 2002;90:1020–1027. [PubMed: 12016269]
22. Heizer ML, McKinney JS, Ellis EF. 14,15-Epoxyeicosatrienoic acid inhibits platelet aggregation in mouse cerebral arterioles. *Stroke* 1991;22:1389–1393. [PubMed: 1750047]
23. Krötz F, Riexinger T, Buerkle MA, Nithipatikom K, Gloe T, Sohn H, Campbell WB, Pohl U. Membrane-potential-dependent inhibition of platelet adhesion to endothelial cells by epoxyeicosatrienoic acids. *Arterioscler Thromb Vasc Biol* 2004;24:595–600. [PubMed: 14715644]
24. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, Liao JK. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J Biol Chem* 2001;276:15983–15989. [PubMed: 11279071]
25. Amruthesh SC, Falck JR, Ellis EF. Brain synthesis and cerebrovascular action of epoxygenase metabolites of arachidonic acid. *J Neurochem* 1992;58:503–510. [PubMed: 1729396]

26. Gebremedhin D, Ma YH, Falck JR, Roman RJ, VanRollins M, Harder DR. Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. *Am J Physiol* 1992;263:H519–25. [PubMed: 1510149]
27. Earley S, Heppner TJ, Nelson MT, Brayden JE. TRPV4 forms a novel Ca²⁺ signaling complex with ryanodine receptors and BKCa channels. *Circ Res* 2005;97:1270–1279. [PubMed: 16269659]
28. Medhora M, Narayanan J, Harder D. Dual regulation of the cerebral microvasculature by epoxyeicosatrienoic acids. *Trends Cardiovasc Med* 2001;11:38–42. [PubMed: 11413051]
29. Gebremedhin D, Harder DR, Pratt PF, Campbell WB. Bioassay of an endothelium-derived hyperpolarizing factor from bovine coronary arteries: role of a cytochrome P450 metabolite. *J Vasc Res* 1998;35:274–284. [PubMed: 9701712]
30. Koehler RC, Gebremedhin D, Harder DR. Role of astrocytes in cerebrovascular regulation. *J Appl Physiol* 2006;100:307–317. [PubMed: 16357084]
31. Alkayed NJ, Birks EK, Hudetz AG, Roman RJ, Henderson L, Harder DR. Inhibition of brain P-450 arachidonic acid epoxygenase decreases baseline cerebral blood flow. *Am J Physiol* 1996;271:H1541–6. [PubMed: 8897950]
32. Peng X, Zhang C, Alkayed NJ, Harder DR, Koehler RC. Dependency of cortical functional hyperemia to forepaw stimulation on epoxygenase and nitric oxide synthase activities in rats. *J Cereb Blood Flow Metab* 2004;24:509–517. [PubMed: 15129182]
33. Amruthesh SC, Boerschel MF, McKinney JS, Willoughby KA, Ellis EF. Metabolism of arachidonic acid to epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, and prostaglandins in cultured rat hippocampal astrocytes. *J Neurochem* 1993;61:150–159. [PubMed: 8515261]
34. Alkayed NJ, Birks EK, Narayanan J, Petrie KA, Kohler-Cabot AE, Harder DR. Role of P-450 arachidonic acid epoxygenase in the response of cerebral blood flow to glutamate in rats. *Stroke* 1997;28:1066–1072. [PubMed: 9158651]
35. Bhardwaj A, Northington FJ, Carhuapoma JR, Falck JR, Harder DR, Traystman RJ, Koehler RC. P-450 epoxygenase and NO synthase inhibitors reduce cerebral blood flow response to N-methyl-D-aspartate. *Am J Physiol Heart Circ Physiol* 2000;279:H1616–24. [PubMed: 11009448]
36. Wang MH, Brand-Schieber E, Zand BA, Nguyen X, Falck JR, Balu N, Schwartzman ML. Cytochrome P450-derived arachidonic acid metabolism in the rat kidney: characterization of selective inhibitors. *J Pharmacol Exp Ther* 1998;284:966–973. [PubMed: 9495856]
37. Peng X, Carhuapoma JR, Bhardwaj A, Alkayed NJ, Falck JR, Harder DR, Traystman RJ, Koehler RC. Suppression of cortical functional hyperemia to vibrissal stimulation in the rat by epoxygenase inhibitors. *Am J Physiol Heart Circ Physiol* 2002;283:H2029–37. [PubMed: 12384482]
38. Iliiff JJ, Close LN, Selden NR, Alkayed NJ. A novel role for P450 eicosanoids in the neurogenic control of cerebral blood flow in the rat. *Exp Physiol* 2007;92:653–658. [PubMed: 17639071]
39. Iliiff JJ, Wang R, Zeldin DC, Alkayed NJ. Epoxyeicosanoids as mediators of neurogenic vasodilation in cerebral vessels. *Am J Physiol Heart Circ Physiol* (Under review). 2008
40. Munzenmaier DH, Harder DR. Cerebral microvascular endothelial cell tube formation: role of astrocytic epoxyeicosatrienoic acid release. *Am J Physiol Heart Circ Physiol* 2000;278:H1163–7. [PubMed: 10749710]
41. Zhang C, Harder DR. Cerebral capillary endothelial cell mitogenesis and morphogenesis induced by astrocytic epoxyeicosatrienoic Acid. *Stroke* 2002;33:2957–2964. [PubMed: 12468797]
42. Medhora M, Daniels J, Munday K, Fisslthaler B, Busse R, Jacobs ER, Harder DR. Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2003;284:H215–24. [PubMed: 12388259]
43. Michaelis UR, Fisslthaler B, Medhora M, Harder D, Fleming I, Busse R. Cytochrome P450 2C9-derived epoxyeicosatrienoic acids induce angiogenesis via cross-talk with the epidermal growth factor receptor (EGFR). *FASEB J* 2003;17:770–772. [PubMed: 12586744]
44. Potente M, Fisslthaler B, Busse R, Fleming I. 11,12-Epoxyeicosatrienoic acid-induced inhibition of FOXO factors promotes endothelial proliferation by down-regulating p27Kip1. *J Biol Chem* 2003;278:29619–29625. [PubMed: 12773534]
45. Pozzi A, Macias-Perez I, Abair T, Wei S, Su Y, Zent R, Falck JR, Capdevila JH. Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. *J Biol Chem* 2005;280:27138–27146. [PubMed: 15917237]

46. Wang Y, Wei X, Xiao X, Hui R, Card JW, Carey MA, Wang DW, Zeldin DC. Arachidonic acid epoxygenase metabolites stimulate endothelial cell growth and angiogenesis via mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways. *J Pharmacol Exp Ther* 2005;314:522–532. [PubMed: 15840765]
47. Fleming I. Epoxyeicosatrienoic acids, cell signaling and angiogenesis. *Prostaglandins Other Lipid Mediat* 2007;82:60–67. [PubMed: 17164133]
48. Michaelis UR, Fisslthaler B, Barbosa-Sicard E, Falck JR, Fleming I, Busse R. Cytochrome P450 epoxygenases 2C8 and 2C9 are implicated in hypoxia-induced endothelial cell migration and angiogenesis. *J Cell Sci* 2005;118:5489–5498. [PubMed: 16291720]
49. Michaelis UR, Xia N, Barbosa-Sicard E, Falck JR, Fleming I. Role of cytochrome P450 2C epoxygenases in hypoxia-induced cell migration and angiogenesis in retinal endothelial cells. *Invest Ophthalmol Vis Sci* 2008;49:1242–1247. [PubMed: 18326754]
50. Fitzpatrick FA, Ennis MD, Baze ME, Wynalda MA, McGee JE, Liggett WF. Inhibition of cyclooxygenase activity and platelet aggregation by epoxyeicosatrienoic acids. Influence of stereochemistry. *J Biol Chem* 1986;261:15334–15338. [PubMed: 3095326]
51. Zhu Y, Schieber EB, McGiff JC, Balazy M. Identification of arachidonate P-450 metabolites in human platelet phospholipids. *Hypertension* 1995;25:854–859. [PubMed: 7721444]
52. Koerner IP, Zhang W, Cheng J, Parker S, Hurn PD, Alkayed NJ. Soluble epoxide hydrolase: regulation by estrogen and role in the inflammatory response to cerebral ischemia. *Front Biosci* 2008;13:2833–2841. [PubMed: 17981757]
53. Gross GJ, Gauthier KM, Moore J, Falck JR, Hammock BD, Campbell WB, Nithipatikom K. Effects of the selective EET antagonist, 14,15-EEZE, on cardioprotection produced by exogenous or endogenous EETs in the canine heart. *Am J Physiol Heart Circ Physiol* 2008;294:H2838–44. [PubMed: 18441205]
54. Zhao X, Quigley JE, Yuan J, Wang M, Zhou Y, Imig JD. PPAR- α activator fenofibrate increases renal CYP-derived eicosanoid synthesis and improves endothelial dilator function in obese Zucker rats. *Am J Physiol Heart Circ Physiol* 2006;290:H2187–95. [PubMed: 16501022]
55. Muller DN, Theuer J, Shagdarsuren E, Kaergel E, Honeck H, Park J, Markovic M, Barbosa-Sicard E, Dechend R, Wellner M, Kirsch T, Fiebeler A, Rothe M, Haller H, Luft FC, Schunck W. A peroxisome proliferator-activated receptor- α activator induces renal CYP2C23 activity and protects from angiotensin II-induced renal injury. *Am J Pathol* 2004;164:521–532. [PubMed: 14742258]
56. Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, Thompson DA, Hammock BD, Spector AA. Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. *J Biol Chem* 2001;276:14867–14874. [PubMed: 11278979]
57. Kroetz DL, Zeldin DC. Cytochrome P450 pathways of arachidonic acid metabolism. *Curr Opin Lipidol* 2002;13:273–283. [PubMed: 12045397]
58. Ghosh S, Chiang P, Wahlstrom JL, Fujiwara H, Selbo JG, Roberds SL. Oral delivery of 1,3-dicyclohexylurea nanosuspension enhances exposure and lowers blood pressure in hypertensive rats. *Basic Clin Pharmacol Toxicol* 2008;102:453–458. [PubMed: 18312493]
59. Seubert JM, Sinal CJ, Graves J, DeGraff LM, Bradbury JA, Lee CR, Goralski K, Carey MA, Luria A, Newman JW, Hammock BD, Falck JR, Roberts H, Rockman HA, Murphy E, Zeldin DC. Role of soluble epoxide hydrolase in posts ischemic recovery of heart contractile function. *Circ Res* 2006;99:442–450. [PubMed: 16857962]
60. Sura P, Sura R, Enayetallah AE, Grant DF. Distribution and expression of soluble epoxide hydrolase in human brain. *J Histochem Cytochem* 2008;56:551–559. [PubMed: 18319271]
61. Koerner IP, Jacks R, DeBarber AE, Koop D, Mao P, Grant DF, Alkayed NJ. Polymorphisms in the human soluble epoxide hydrolase gene EPHX2 linked to neuronal survival after ischemic injury. *J Neurosci* 2007;27:4642–4649. [PubMed: 17460077]
62. Norris KK, DeAngelo TM, Vesell ES. Genetic and environmental factors that regulate cytosolic epoxide hydrolase activity in normal human lymphocytes. *J Clin Invest* 1989;84:1749–1756. [PubMed: 2592558]

63. Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, Bradbury JA, Enayetallah AE, Zeldin DC, Grant DF. Polymorphisms in human soluble epoxide hydrolase. *Mol Pharmacol* 2003;64:482–490. [PubMed: 12869654]
64. Sandberg M, Hassett C, Adman ET, Meijer J, Omiecinski CJ. Identification and functional characterization of human soluble epoxide hydrolase genetic polymorphisms. *J Biol Chem* 2000;275:28873–28881. [PubMed: 10862610]
65. Srivastava PK, Sharma VK, Kalonia DS, Grant DF. Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure. *Arch Biochem Biophys* 2004;427:164–169. [PubMed: 15196990]
66. Fornage M, Boerwinkle E, Doris PA, Jacobs D, Liu K, Wong ND. Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: The Coronary Artery Risk Development in Young Adults (CARDIA) study. *Circulation* 2004;109:335–339. [PubMed: 14732757]
67. Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, Node K, Börgel J, Mügge A, Lindpaintner K, Huesing A, Maisch B, Zeldin DC, Liao JK. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxigenase CYP2J2. *Circulation* 2004;110:2132–2136. [PubMed: 15466638]
68. Liu P, Li Y, Chao T, Wu H, Lin L, Tsai L, Chen J. Synergistic effect of cytochrome P450 epoxigenase CYP2J2*7 polymorphism with smoking on the onset of premature myocardial infarction. *Atherosclerosis* 2007;195:199–206. [PubMed: 17126841]
69. Lee CR, North KE, Bray MS, Couper DJ, Heiss G, Zeldin DC. CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study. *Pharmacogenet Genomics* 2007;17:349–358. [PubMed: 17429317]
70. Marcianti KD, Totah RA, Heckbert SR, Smith NL, Lemaitre RN, Lumley T, Rice KM, Hindorf LA, Bis JC, Hartman B, Psaty BM. Common variation in cytochrome P450 epoxigenase genes and the risk of incident nonfatal myocardial infarction and ischemic stroke. *Pharmacogenet Genomics* 2008;18:535–543. [PubMed: 18496133]
71. Yasar U, Bennet AM, Eliasson E, Lundgren S, Wiman B, De Faire U, Rane A. Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. *Pharmacogenetics* 2003;13:715–720. [PubMed: 14646690]
72. Ellis EF, Amruthesh SC, Police RJ, Yancey LM. Brain synthesis and cerebrovascular action of cytochrome P-450/monooxygenase metabolites of arachidonic acid. *Adv Prostaglandin Thromboxane Leukot Res* 1991;21A:201–204. [PubMed: 1825540]
73. Fleming I. Cytochrome P450 epoxigenases as EDHF synthase(s). *Pharmacol Res* 2004;49:525–533. [PubMed: 15026030]
74. Yang S, Lin L, Chen J, Lee CR, Seubert JM, Wang Y, Wang H, Chao Z, Tao D, Gong J, Lu Z, Wang DW, Zeldin DC. Cytochrome P-450 epoxigenases protect endothelial cells from apoptosis induced by tumor necrosis factor- α via MAPK and PI3K/Akt signaling pathways. *Am J Physiol Heart Circ Physiol* 2007;293:H142–51. [PubMed: 17322420]
75. Medhora M, Dhanasekaran A, Gruenloh SK, Dunn LK, Gabrilovich M, Falck JR, Harder DR, Jacobs ER, Pratt PF. Emerging mechanisms for growth and protection of the vasculature by cytochrome P450-derived products of arachidonic acid and other eicosanoids. *Prostaglandins Other Lipid Mediat* 2007;82:19–29. [PubMed: 17164129]
76. Liu M, Alkayed NJ. Hypoxic preconditioning and tolerance via hypoxia inducible factor (HIF) 1 α -linked induction of P450 2C11 epoxigenase in astrocytes. *J Cereb Blood Flow Metab* 2005;25:939–948. [PubMed: 15729289]
77. Dhanasekaran A, Al-Saghir R, Lopez B, Zhu D, Gutterman DD, Jacobs ER, Medhora M. Protective effects of epoxyeicosatrienoic acids on human endothelial cells from the pulmonary and coronary vasculature. *Am J Physiol Heart Circ Physiol* 2006;291:H517–31. [PubMed: 16617127]
78. Yang B, Graham L, Dikalov S, Mason RP, Falck JR, Liao JK, Zeldin DC. Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. *Mol Pharmacol* 2001;60:310–320. [PubMed: 11455018]
79. Chiamvimonvat N, Ho C, Tsai H, Hammock BD. The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J Cardiovasc Pharmacol* 2007;50:225–237. [PubMed: 17878749]

80. Imig JD. Cardiovascular therapeutic aspects of soluble epoxide hydrolase inhibitors. *Cardiovasc Drug Rev* 2006;24:169–188. [PubMed: 16961727]
81. Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, Gonzalez FJ. Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* 2000;275:40504–40510. [PubMed: 11001943]
82. Luria A, Weldon SM, Kabcenell AK, Ingraham RH, Matera D, Jiang H, Gill R, Morisseau C, Newman JW, Hammock BD. Compensatory mechanism for homeostatic blood pressure regulation in Ephx2 gene-disrupted mice. *J Biol Chem* 2007;282:2891–2898. [PubMed: 17135253]
83. Seubert JM, Xu F, Graves JP, Collins JB, Sieber SO, Paules RS, Kroetz DL, Zeldin DC. Differential renal gene expression in prehypertensive and hypertensive spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 2005;289:F552–61. [PubMed: 15798089]
84. Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* 2002;39:690–694. [PubMed: 11882632]
85. Jung O, Brandes RP, Kim I, Schweda F, Schmidt R, Hammock BD, Busse R, Fleming I. Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension* 2005;45:759–765. [PubMed: 15699457]
86. Imig JD, Zhao X, Zaharis CZ, Olearczyk JJ, Pollock DM, Newman JW, Kim I, Watanabe T, Hammock BD. An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* 2005;46:975–981. [PubMed: 16157792]
87. Davis BB, Thompson DA, Howard LL, Morisseau C, Hammock BD, Weiss RH. Inhibitors of soluble epoxide hydrolase attenuate vascular smooth muscle cell proliferation. *Proc Natl Acad Sci USA* 2002;99:2222–2227. [PubMed: 11842228]
88. Davis BB, Morisseau C, Newman JW, Pedersen TL, Hammock BD, Weiss RH. Attenuation of vascular smooth muscle cell proliferation by 1-cyclohexyl-3-dodecyl urea is independent of soluble epoxide hydrolase inhibition. *J Pharmacol Exp Ther* 2006;316:815–821. [PubMed: 16221742]
89. Ulu A, Davis BB, Tsai H, Kim I, Morisseau C, Inceoglu B, Fiehn O, Hammock BD, Weiss RH. Soluble Epoxide Hydrolase Inhibitors Reduce the Development of Atherosclerosis in Apolipoprotein E-Knockout Mouse Model. *J Cardiovasc Pharmacol*. 2008
90. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, Couper DJ, Heiss G, Zeldin DC. Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Hum Mol Genet* 2006;15:1640–1649. [PubMed: 16595607]
91. Wei Q, Doris PA, Pollizotto MV, Boerwinkle E, Jacobs DRJ, Siscovick DS, Fornage M. Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking. *Atherosclerosis* 2007;190:26–34. [PubMed: 16545818]
92. 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* 2005;46:842–848. [PubMed: 16306811]
93. Yamaura K, Gebremedhin D, Zhang C, Narayanan J, Hoefert K, Jacobs ER, Koehler RC, Harder DR. Contribution of epoxyeicosatrienoic acids to the hypoxia-induced activation of Ca²⁺-activated K⁺ channel current in cultured rat hippocampal astrocytes. *Neuroscience* 2006;143:703–716. [PubMed: 17027168]
94. Nithipatikom K, DiCamelli RF, Kohler S, Gumina RJ, Falck JR, Campbell WB, Gross GJ. Determination of cytochrome P450 metabolites of arachidonic acid in coronary venous plasma during ischemia and reperfusion in dogs. *Anal Biochem* 2001;292:115–124. [PubMed: 11319825]
95. Alkayed NJ, Goyagi T, Joh H, Klaus J, Harder DR, Traystman RJ, Hurn PD. Neuroprotection and P450 2C11 upregulation after experimental transient ischemic attack. *Stroke* 2002;33:1677–1684. [PubMed: 12053011]
96. 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* 2002;283:H2306–14. [PubMed: 12388281]

97. Motoki A, Merkel MJ, Packwood WH, Cao Z, Liu L, Iliff J, Alkayed NJ, Van Winkle DM. Soluble epoxide hydrolase inhibition and gene deletion are protective against myocardial ischemia-reperfusion injury in vivo. *Am J Physiol Heart Circ Physiol* 2008;295:H2128–34. [PubMed: 18835921]
98. Alkayed NJ, Narayanan J, Gebremedhin D, Medhora M, Roman RJ, Harder DR. Molecular characterization of an arachidonic acid epoxigenase in rat brain astrocytes. *Stroke* 1996;27:971–979. [PubMed: 8623121]
99. Newman JW, Morisseau C, Harris TR, Hammock BD. The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. *Proc Natl Acad Sci USA* 2003;100:1558–1563. [PubMed: 12574510]
100. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 2003;424:434–438. [PubMed: 12879072]
101. Lipski J, Park TIH, Li D, Lee SCW, Trevarton AJ, Chung KKH, Freestone PS, Bai J. Involvement of TRP-like channels in the acute ischemic response of hippocampal CA1 neurons in brain slices. *Brain Res* 2006;1077:187–199. [PubMed: 16483552]
102. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol* 2007;184:53–68. [PubMed: 17188755]
103. Larsen BT, Campbell WB, Gutterman DD. Beyond vasodilatation: non-vasomotor roles of epoxyeicosatrienoic acids in the cardiovascular system. *Trends Pharmacol Sci* 2007;28:32–38. [PubMed: 17150260]
104. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999;22:391–397. [PubMed: 10441299]
105. 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*. 2008
106. Daikh BE, Lasker JM, Raucy JL, Koop DR. Regio- and stereoselective epoxidation of arachidonic acid by human cytochromes P450 2C8 and 2C9. *J Pharmacol Exp Ther* 1994;271:1427–1433. [PubMed: 7996455]
107. Wu S, Moomaw CR, Tomer KB, Falck JR, Zeldin DC. Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxigenase highly expressed in heart. *J Biol Chem* 1996;271:3460–3468. [PubMed: 8631948]
108. Omura T, Tanaka Y, Miyata N, Koizumi C, Sakurai T, Fukasawa M, Hachiuma K, Minagawa T, Susumu T, Yoshida S, Nakaike S, Okuyama S, Harder DR, Roman RJ. Effect of a new inhibitor of the synthesis of 20-HETE on cerebral ischemia reperfusion injury. *Stroke* 2006;37:1307–1313. [PubMed: 16601220]
109. Poloyac SM, Zhang Y, Bies RR, Kochanek PM, Graham SH. Protective effect of the 20-HETE inhibitor HET0016 on brain damage after temporary focal ischemia. *J Cereb Blood Flow Metab* 2006;26:1551–1561. [PubMed: 16570075]
110. 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 Mediat* 2007;82:42–49. [PubMed: 17164131]
111. Loot AE, Popp R, Fisslthaler B, Vriens J, Nilius B, Fleming I. Role of cytochrome P450-dependent transient receptor potential V4 activation in flow-induced vasodilatation. *Cardiovasc Res*. 2008

Reference Annotations

112. **Spector AA, Norris AW. Action of epoxyeicosatrienoic acids on cellular function. *Am J Physiol Cell Physiol* 2007;292:C996–1012. [PubMed: 16987999]A recent and detailed review of cellular mechanisms of EETs signaling. An excellent place to start for a basic science overview of the epoxyeicosanoid field.
113. **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*. 2008A provocative article detailing evidence

that EETs act as novel endogenous competitive antagonist at the thromboxane receptor and that this action accounts in part for EETs vascular actions.

114. *Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002;82:131–185. [PubMed: 11773611]An exhaustive and scholarly review of P450 eicosanoid signaling. Covers both P450 epoxigenase and P450 hydroxylase metabolites and their respective roles in all elements of cardiovascular function.
115. *Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 1996;78:415–423. [PubMed: 8593700] A seminal paper in epoxyeicosanoid signaling, detailing the characterization of EETs as candidate endothelium-derived hyperpolarizing factors.
116. *Ellis EF, Police RJ, Yancey L, McKinney JS, Amruthesh SC. Dilation of cerebral arterioles by cytochrome P-450 metabolites of arachidonic acid. *Am J Physiol* 1990;259:H1171–7. [PubMed: 2121049]Article that first described a functional role for EETs in the regulation of the cerebral vasculature.
117. *Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, Thompson DA, Hammock BD, Spector AA. Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. *J Biol Chem* 2001;276:14867–14874. [PubMed: 11278979]Detailed analysis of routes of EETs metabolism in endothelial cells, identifying sEH as an enzyme chiefly responsible EETs breakdown. Also identified a role for sEH in regulating EETs incorporation in membrane phospholipids.
118. **Chiamvimonvat N, Ho C, Tsai H, Hammock BD. The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J Cardiovasc Pharmacol* 2007;50:225–237. [PubMed: 17878749]A recent review detailing the emergence of sEH inhibition as a therapeutic avenue for the treatment of hypertension.

Figure 1. Pathways of EETs synthesis, metabolism and action

Epoxyeicosatrienoic acids (EETs) are synthesized from arachidonic acid (AA) by cytochrome P450 epoxigenase enzymes of the 2C (CYP2C) and 2J (CYP2J) subfamilies to form four regioisomers corresponding to the four AA double bonds: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (shown). Known primarily as potent vasodilators, EETs act through several signaling pathways, including a putative G protein-coupled receptor (GPCR), to regulate many elements of vascular physiology. The biological activity of EETs is terminated through their metabolism by soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs), a process that serves as a key regulator of tissue EETs levels.

Figure 2. Vasomotor regulation of the cerebral vasculature by EETs

Astrocytic EETs are key mediators of neurovascular coupling. Local neuronal activity (Glutamate, Glut) is detected by metabotropic glutamate receptors (mGluRs) on peri-synaptic astrocyte processes. Vasodilator EETs are synthesized by P450 epoxygenases (P450) and released from astrocytic endfeet abutting the cerebral microcirculation, linking changes in neuronal activity to local regulation of CBF. **Endothelial EETs** contribute to the endothelium (Endo)-dependent modulation of vasomotor tone by agonists such as bradykinin. Recent evidence suggests that **Neurogenic EETs** may be involved in the regulation of CBF by perivascular nerves innervating the cortical surface vasculature. These include parasympathetic vasodilator fibers originating in the sphenopalatine (SPG, or pterygopalatine) and otic ganglia (OG), as well as vasodilator sensory afferents projecting to the trigeminal ganglia (TG). Vascular smooth muscle, VSM; soluble epoxide hydrolase, sEH; calcitonin gene-related peptide, CGRP; nitric oxide, NO; vasoactive intestinal peptide, VIP.

Figure 3. Soluble epoxide hydrolase expression within cerebrovascular nerve fibers and cortical neurons

Immunofluorescence labeling of whole-mount rat middle cerebral arteries (MCA, A), striatal (B) and cortical (C-F) brain slices. (A) Cortical surface arteries such as the MCA are densely innervated by sEH-immunoreactive (green) nerve bundles and individual fibers. Vessel margins are indicated in white. (B) White matter tracks in the striatum display sEH-immunoreactivity (green), though sEH does not co-localize with the astrocyte marker glial fibrillary acidic protein (GFAP, red). (C) Cortical neurons labeled with the neuronal marker NeuN (red) exhibit strong sEH-immunoreactivity (green) throughout all cortical layers. High magnification inset shown in (D). (E) In contrast, sEH (green) does not appear to be present

in GFAP-expressing cortical astrocytes (red). High magnification inset shown in (F). Scale bars –200µm: B, C, E; 100µm: D, F.

Figure 4. Soluble epoxide hydrolase inhibition reduces infarct volume in a mouse model of ischemia-reperfusion injury

Mice were subjected to 2 hours of unilateral transient middle cerebral artery occlusion (MCAO), followed by 24 hours of reperfusion. (A) Infarct volume was determined by 2,3,5-triphenyltetrazolium staining. (B) Intravenous administration of 14,15-EET (1 μ g) for 24 hours prior to ischemia onset reduced infarct volume by 70% (n=4 per group, *P<0.05). (C) Pharmacological inhibition of sEH with AUDA-BE (10mg/kg ip) at the time of reperfusion reduced infarct volume by 50% (n=5 per group, *P<0.05). This effect was blocked by the specific CYP epoxygenase inhibitor MS-PPOH, indicating that the protective effect of sEH inhibition involves EETs biosynthesis. (D) Genetic deletion of sEH (sEHKO) reduced infarct volume by 55% compared to wild type (WT) animals (n=5 per group, *P<0.05). These findings suggest that sEH inhibition is protective in animal models of acute ischemic stroke, and that this protection involves the EETs signaling system. (A,B,D) From: Zhang W et al. *Stroke*. 2008 Jul;39(7):2073-8. Used by permission. (C) From: Zhang W et al. *J Cereb Blood Flow Metab*. 2007 Dec;27(12):1931-40. Used by permission.

Figure 5. Differing effects of sEH inactivation upon intra-ischemic blood flow

In mice subjected to 2 hours of transient middle cerebral artery occlusion (MCAO), intra-ischemic changes in cerebral blood flow (CBF) were monitored by laser Doppler flowmetry (A, C). At the end of 2-hour occlusion, CBF was quantitatively assessed by iodoantipyrine autoradiography (B,D). (A) Pharmacological inhibition of sEH (10mg/kg ip 30min prior to MCAO) did not alter cortical perfusion during the ischemic period (n=5 animals per group). (B) End-ischemic CBF was not altered by AUDA-BE treatment (n=5 animals per group). This suggests that the ischemic protection afforded by sEH inhibition is blood-flow independent. (C) sEH knockout (sEHKO) animals exhibited a progressive recovery of CBF during the intra-ischemic period (n=5 animals per group, *P<0.05). (D) This finding was confirmed by autoradiography carried out at the end of the 2 hour ischemic period (n=5 animals per group, P<0.05). This suggests that the ischemic protection afforded by sEH gene deletion may be dependent upon changes in CBF. (A,B) From: Zhang W et al. *J Cereb Blood Flow Metab.* 2007 Dec;27(12):1931-40. Used by permission. (C,D) From: Zhang W et al. *Stroke.* 2008 Jul; 39(7):2073-8. Used by permission.

Table 1
Protective Effects of Epoxyeicosatrienoic Acids (EETs)

Effect	Tissue	Mechanism	References
Vasodilation	B, K, M, H, L	BK, GPCR, TRPV4	[12-18,25,26, 112]
Anti-Inflammation	B, H	NF- κ B inactivation	[19,20]
Anti-Smooth Muscle Migration	Ao	cAMP-PKA	[21]
Anti-Platelet Aggregation	B, HUVEC	BK, COX	[22,23,50]
Fibrinolysis	Ao	GPCR	[24]
Angiogenesis	B,H, L, HUVEC, Ao, Ret	MAPK, PI3 kinase-Akt, cAMP-PKA	[40-49]
Anti-Apoptosis	B, H, L, Ao	PI3 kinase-Akt	[61,74-77]
Anti-Oxidant	Ao		[78]

H, Heart; K, Kidney; M, Mesentery; B, Brain; L, Lung; Ao, Aorta; HUVEC, Human umbilical vein endothelial cells; Ret, Retina; BK, large-conductance Ca^{2+} -sensitive K^{+} channel; GPCR, G protein-coupled receptor; TRPV4, transient receptor potential vanilloid 4 channel; cAMP-PKA, cyclic AMP – protein kinase A; COX, cyclooxygenase; MAPK, mitogen activated protein kinase; PI3 kinase, phosphatidylinositol 3-kinase;

Table 2

EPHX2 gene polymorphisms present in human populations

Variant	Frequency	Hydrolase Activity	Protein Stability	Neuronal Cell Death	Ischemic Stroke Risk
Lys55Arg	10%	Increased	NC	NC	
Arg103Cys	4%	NC	NC	NC	
Cys154Tyr	3% *	Increased	NC	NC	
Arg287Gln	7%, 8% *	Reduced	Reduced	Reduced	Reduced/Increased [#]
Val422Ala	1%	NC	NC	NC	
Glu470Gly	<1%	Increased [*]	NC	NC	Increased
Arg403 _{ins}	16% *	Reduced [*]	Reduced	NC	

Lys55Arg, Lysine substitution for Arginine at sEH residue 55. NC, no change. Frequency, Hydrolase Activity and Protein Stability values from Przybyla-Zawislak et al. (2003)

* Sandberg et al. (2000). Neuronal Cell Death values from Koerner et al. (2007). Ischemic Stroke Risk values from Fornage et al. (2005)

[#] Gschwendtner et al. (2008).