European Journal of Pharmacology 700 (2013) 93–101

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Comparative efficacy of 3 soluble epoxide hydrolase inhibitors in rat neuropathic and inflammatory pain models

Karen Wagner, Bora Inceoglu, Hua Dong, Jun Yang, Sung Hee Hwang, Paul Jones, Christophe Morisseau, Bruce D. Hammock*

Department of Entomology and UC Davis Comprehensive Cancer Center, University of California Davis, One Shields Ave, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 8 June 2012
Received in revised form
13 December 2012
Accepted 19 December 2012
Available online 28 December 2012

Keywords:
Nociception
Soluble epoxide hydrolase
Inflammatory pain
Diabetic neuropathic pain
Epoxygenated fatty acid

ABSTRACT

Epoxy-fatty acids have been recognized as important cell signaling molecules with multiple biological effects including anti-nociception. The main degradation pathway of these signaling molecules is via the soluble epoxide hydrolase (sEH) enzyme. Inhibitors of sEH extend the anti-nociceptive effects of fatty acid epoxides. In this study two models of pain with different etiology, streptozocin induced type I diabetic neuropathic pain and lipopolysaccharide induced inflammatory pain were employed to test sEH inhibitors. A dose range of three sEH inhibitors with the same central pharmacophore but varying substituent moieties was used to investigate maximal anti-allodynic effects in these two models of pain. Inhibiting the sEH enzyme in these models successfully blocked pain related behavior in both models. The sEH inhibitors were more potent and more efficacious than celecoxib in reducing both diabetic neuropathic pain and lipopolysaccharide induced inflammatory pain. Because of their ability to block diabetic neuropathic pain sEH inhibition is a promising new approach to treat chronic pain conditions.

1. Introduction

Epoxy-fatty acids are endogenous lipid metabolites with important roles in cellular signaling which is underscored by their tight regulation (Bernstrom et al., 1992; Spector and Norris, 2007). These epoxy-metabolites are formed by cytochrome P450 enzymes acting on parent fatty acids released from cellular membranes by lipases including phospholipase A_2 (Imig, 2012; Spector, 2009; Tomita-Yamaguchi et al., 1990). Epoxy-fatty acids undergo rapid enzymatic degradation by the soluble epoxide hydrolase (sEH, *EPHX2* EC:3.3.2.10). The specific epoxy-fatty acids most frequently investigated are the epoxyeicosatrienoic acids. The epoxyeicosatrienoic acids are arachidonic acid metabolites with multiple biological activities including lowering blood pressure and attenuating inflammation and inflammatory pain (Imig et al., 2002; Inceoglu et al., 2006; Schmelzer et al., 2005). Epoxyeicosatrienoic acids are degraded by sEH into their corresponding diols the dihydroxyeicosatrienoic acids. Inhibitors of sEH have been used to stabilize epoxy-fatty acids, increasing their residence time and lowering dihydroxyeicosatrienoic acid levels both in vitro and in vivo (Spector, 2009). Early sEH inhibitors were successful in vitro but their formulation was problematic for use in vivo. The physical and chemical properties of the inhibitors have been systematically optimized (Morisseau et al., 1999, 2006; Shen and Hammock, 2012) and demonstrate improvements in bioefficacy (Hwang et al., 2007; Tsai et al., 2010). Direct application of epoxy-fatty acids including epoxyeicosatrienoic acids also mediates pain relief in rats (Inceoglu et al., 2006; Morisseau et al., 2010). Recently, results using a PGE_2 induced pain model suggest a mode of action independent of their anti-inflammatory activity (Inceoglu et al., 2011). In addition, results of sEH inhibition in a model of diabetic neuropathic pain support the hypothesis that there is a mode of action independent of anti-inflammation (Inceoglu et al., 2008). These experiments revealed sEH inhibition was anti-hyperalgesic and equipotent to low dose morphine on thermal hyperalgesia in diabetic rats (Inceoglu et al., 2008). However, the extent of sEH inhibitor mediated anti-hyperalgesia and the efficacy of the inhibitors on neuropathic mechanical pain were both unknown. Here, we probed for maximum anti-nociceptive efficacy in two in vivo rat models. The nociceptive assays quantified mechanical allodynia, a pain associated with a stimulus that is normally innocuous and present in both models. Special attention is given to APAU which has investigational new drug status and has the possibility of being used in additional human clinical trials in the near future (Shen and Hammock, 2012). APAU is compared to the selective COX-2 inhibitor celecoxib in both a chronic diabetic neuropathic pain and an acute lipopolysaccharide induced inflammatory pain model. Then dose ranges of three sEH inhibitors including APAU

* Corresponding author. Tel.: +1 530 752 7519; fax: +1 530 752 1537.
E-mail address: bdhammock@ucdavis.edu (B.D. Hammock).

0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.12.015

were compared in both models to test the hypothesis that sEH inhibitors dose dependently reduce both inflammatory and neuropathic allodynia. The sEH inhibitor mediated pain relief was tested with up to a 10 fold increase in dose compared to previous published data and evaluated for time dependent effects. In addition to examining maximum efficacy, these experiments add information about the possible mechanism of action of sEH inhibitors in induced pain states.

2. Materials and methods

All experiments used groups of Sprague-Dawley male rats (250–300 g) purchased from Charles River Laboratories. The rats were allowed to habituate 3 days before the beginning of each experiment and housed under standard conditions (25 °C) in a fixed 12-h light/dark cycle with ad libitum food and water. These experiments were performed in accordance with protocols approved by the University of California Davis Animal Use and Care Committee and with great care to minimize suffering of the animals.

2.1. Chemicals

The sEH inhibitors APAU: 1-(1-acetypiperidin-4-yl)-3-adamantanylurea; *t*-AUCB: *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid; and *t*-TUCB: *trans*-4-[4-(3-trifluoromethoxyphenyl-l-ureido)-cyclohexyloxy]-benzoic acid used for the experiments were synthesized and characterized in house as previously described (Hwang et al., 2007; Jones et al., 2006). The sEH inhibitor APAU is also referred to as AR9281 in published literature (Anandan et al., 2011; Imig and Hammock, 2009). The IC_{50} values for *t*-TUCB on recombinant rat and mouse sEH were determined per previously described methods (Wolf et al., 2006). Doses of sEH inhibitors were formulated in the polyethylene glycol PEG400 for the experiments. The highest doses of the inhibitors were first dissolved in a small amount of DMSO (10% v/v final) to which was added PEG400. Both vehicle concentrations were tested in the nociceptive assay and oxylipin analysis and showed no statistically significant differences (data not shown). Celecoxib was purchased from (Fisher Scientific, USA) and formulated in PEG400 vehicle. Morphine sulfate was purchased (Fisher Scientific, USA) and diluted in saline.

2.2. Diabetic neuropathic pain model

Diabetic neuropathic pain was modeled using the antibiotic drug streptozocin which targets and kills the pancreatic beta islet cells rendering the animals with type I diabetes. The ensuing decrease in nociceptive thresholds develops within five days, persists the lifetime of the animal and was used as the model of diabetic neuropathic pain. Before induction of diabetes rats were acclimated for 1 h and tested for baseline thresholds. Baseline mechanical withdrawal thresholds were established using the von Frey mechanical nociceptive test with an electronic anesthesiometer (IITC, Woodland Hills, CA). Subsequently, streptozocin (55 mg/kg) in saline was injected via tail vein per previously reported methods (Aley and Levine, 2001). After five days the allodynia of diabetic rats was confirmed. Rats that scored 65% or lower of the original pain free baseline were considered allodynic and included the presented groups.

2.3. Lipopolysaccharide induced inflammation model

On each test day the rats were acclimated for 1 h and baseline mechanical withdrawal thresholds were measured with the von Frey anesthesiometer. 1 h after pretreatment 10 μg of lipopolysaccharide (Sigma, St. Louis MO) in 50 μl saline was injected intraplantar in one hind paw of the rats (Kanaan et al., 1996). The mechanical withdrawal threshold of this hind paw was monitored for 6 h following the injection. For oral gavage administration, 3 mg/kg APAU in PEG400 was administered 1 h prior to intraplantar injection of lipopolysaccharide.

2.4. Nociceptive and motor skill bioassays

An electronic von Frey aesthesiometer was used to quantify allodynia baselines on all test days. Rats were placed in clear acrylic chambers on a steel mesh floor. The hind paw of the rat was probed through the mesh with a rigid tip probe connected to an electronic readout pressure meter set to the maximum hold setting. The withdrawal thresholds per rat were measured 3–5 times at 1 min intervals for each time point. For the diabetic neuropathy model, rats were injected s.c. with vehicle, celecoxib or sEH inhibitor and tested at 15 min, 30 min, 1, 2, 3, 4, 5, and 6 h post injection for mechanical withdrawal thresholds. For the inflammatory pain model rats were administered vehicle, celecoxib or sEH inhibitor 60 min prior to intraplantar lipopolysaccharide injection. These procedures were followed for 0.1, 0.3, 1, 3, 10, 30 and 100 mg/kg of APAU and *t*-TUCB and 1, 3, 10, 30 and 100 mg/kg of *t*-AUCB given this inhibitor was inactive at low dose in the assay. The presence of the disease state precluded pretreatment in the diabetic neuropathy model; therefore rats were tested at equivalent time intervals to the inflammatory pain model but immediately after sEH inhibitor administration. The reported scores are the grams of force required to elicit a hind paw withdrawal averaged with standard error of the mean (S.E.M.) per a group of rats tested on the same day under the same conditions. For the diabetic neuropathy model, the baseline scores were normalized to 100 percent to reflect the response to treatments which are reported as % of post diabetic neuropathic baseline. Therefore the diabetic neuropathy model scores can improve to over 100%. For the inflammatory pain model, the baseline scores are considered pain free and assigned 100%. The scores are group averages ± S.E.M. reported as percent of the baseline. Therefore the inflammatory pain model graphs reflect more painful responses as low percent scores with the object of returning toward 100% or more. For the rotorod test the rats were not pre-trained but tested for their baseline scores prior to vehicle or compound. Scores for each rat consisted of three consecutive trials per time point at an accelerating speed from 2–20 rpm on a 7.0 cm shaft diameter Rota-rod Treadmill (MedAssociates Inc., St. Albans, VT). A cutoff endpoint of 180 s was used for successful trials. The scores are the group average ± S.E.M. per time point.

2.5. Oxylipin and blood inhibitor analysis

The inflammatory pain model was used to investigate changes in plasma lipid metabolites after APAU treatment. 3 and 100 mg/kg of APAU were used to analyze sEH substrate and product levels as well as key prostaglandins compared to vehicle controls. For the oxylipin analysis, blood was sampled 2 h post lipopolysaccharide or control intraplantar injection and centrifuged to obtain plasma. The plasma was extracted via solid phase extraction followed by LC/MS analysis as described previously (Yang et al., 2009). APAU concentration was measured using 10 μl of whole blood sampled via tail vein puncture added to 50 μl distilled water. The samples were flash frozen, extracted and analyzed per previous methods (Liu et al., 2009).