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Peripheral FAAH and soluble epoxide hydrolase inhibitors are synergistically antinociceptive

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ABSTRACT

We need better medicines to control acute and chronic pain. Fatty acid amide hydrolase (FAAH) and soluble epoxide hydrolase (sEH) catalyze the deactivating hydrolysis of two classes of bioactive lipid mediators – fatty acid ethanolamides (FAEs) and epoxidized fatty acids (EpFAs), respectively – which are biogenetically distinct but share the ability to attenuate pain responses and inflammation. In these experiments, we evaluated the antihyperalgesic activity of small-molecule inhibitors of FAAH and sEH, administered alone or in combination, in two pain models: carrageenan-induced hyperalgesia in mice and streptozocin-induced allodynia in rats. When administered separately, the sEH inhibitor 1-trifluoromethoxyphenyl-3-(1-propionylpiperidine-4-yl)urea (TPPU) and the peripherally restricted FAAH inhibitor URB937 were highly active in the two models. The combination TPPU plus URB937 was markedly synergistic, as assessed using isobolographic analyses. The results of these experiments reveal the existence of a possible functional crosstalk between FAEs and EpFAs in regulating pain responses. Additionally, the results suggest that combinations of sEH and FAAH inhibitors might be exploited therapeutically to achieve greater analgesic efficacy.

1. Introduction

Various bioactive lipid mediators regulate nociceptive pain and inflammation in peripheral tissues by interacting with receptor systems on primary sensory neurons and neighboring host–defense cells, such as macrophages, mast cells and keratinocytes [1]. In this context, two classes of long-chain fatty acid derivatives have been studied with particular attention: the fatty acid ethanolamides (FAE) [1] and the epoxidized fatty acids (EpFAs) [2,3]. These lipids demonstrate marked analgesic and anti-inflammatory properties but are subject to rapid deactivating metabolism, with the primary route for both being enzymatic hydrolysis [1–3]. The FAEs are degraded primarily by fatty acid amide hydrolase (FAAH) while the EpFAs are metabolized by soluble epoxide hydrolase (sEH). Both enzymes are hydrolases, but they are in different protein families. Because of the roles of the FAE and EpFAs in pain modulation [1] FAAH and sEH have been intensely studied to develop pharmacological inhibitors that are able to sustain endogenous levels of these analgesic and anti-inflammatory mediators.

FAAH is an intracellular serine hydrolase that belongs to the amide signature hydrolase superfamily of enzymes [4,5]. Preferred FAAH substrates include endogenous agonists of cannabinoid receptors, such as anandamide (arachidonoylethanolamide) [6,7], and type-α peroxisome proliferator-activated receptors (PPAR-α), such as oleoylethanolamide (OEA) [8] and palmitoylethanolamide (PEA) [9]. sEH is a bifunctional enzyme with an N-terminal phosphatase activity, whose physiological function is unclear, and a C-terminal hydrolase activity that catalyzes the conversion of EpFAs into dihydroxy fatty acids [10]. The epoxide hydrolase domain of sEH belongs to the α/β-hydrolase fold family of proteins [11]. Most of the activity of sEH is attributed to this domain, because EpFAs are known to activate large-conductance potassium (BK) channels [12], nuclear receptors (*e.g.* PPAR-α and PPAR-γ) [13,14], transient receptor potential (TRP) channels [15–17] as well as to inhibit the pro-inflammatory transcription factor NF-κB [18,19].

Abbreviations: BK, large-conductance potassium channels; CB, cannabinoid receptor; CL, confidence limits; ED_{50}, median effective dose; EpFA, epoxidized fatty acids, FAAH, fatty-acid amide hydrolase; FAE, fatty acid ethanolamides; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPARα and PPARγ, peroxisome proliferator-activated receptors; sEH, soluble epoxide hydrolase.

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While there is evidence for the existence of EpFA receptors [20,21], the identities of these putative receptors remain undefined [22]. By contrast, the endocannabinoid anandamide is a known agonist of two G protein-coupled receptors, namely the CB_1 and CB_2 cannabinoid receptors [23]. Interestingly, the epoxyeicosatrienoic acids (EETs) weakly displace radioligand binding at CB_2, but not CB_1 receptors [24]. The significance of this weak CB_2 agonism remains to be determined, but it is of interest due to the suggested role of CB_2 receptors in peripheral pain modulation [25]. Little is known about the interaction of EpFAs derived from n-3 polyunsaturated fatty acids (*e.g.*, docosahexaenoic acid and eicosapentaenoic acid) with the endocannabinoid system. However, EETs and n-3 EpFAs are robustly antihyperalgesic in acute and chronic models of pain [26,27]. The fact that EETs, as a subset, do not significantly activate the endocannabinoid receptor system suggests EpFAs and FAEs act through complementary rather than overlapping mechanisms. Preclinical studies have documented the anti-inflammatory and anti-hyperalgesic effects of FAAH [1] and sEH inhibitors [3]. In the present study, we asked whether these two classes of agents act in an additive or super-additive (synergistic) manner to attenuate pain responses in rodent models. We used the peripherally restricted FAAH inhibitor URB937, which has no access to the brain and spinal cord yet causes pronounced antihyperalgesic effects that are due to its ability to elevate anandamide levels outside the brain and spinal cord [28]. sEH blockade was achieved employing the potent inhibitor 1-trifluoromethoxyphenyl-3-(1-propionylpiperidine-4-yl)urea (TPPU) [29]. As experimental models, we utilized a mouse model of acute inflammation (carrageenan) and a rat model of neuropathy (insulin-dependent diabetes evoked by streptozocin).

2. Materials and methods

2.1. Animals

We used male CD1 mice (25–30 g; Charles River, Calco, Italy) and Sprague–Dawley male rats (250–300 g; Charles River, Wilmington, MA, USA). All procedures performed in Italy were in accordance with the Ethical Guidelines of the International Association for the Study of Pain, Italian regulations on the protection of animals used for experimental and other scientific purposes (D.M. 116192), and European Economic Community regulations (O.J. of E.C. L 358/1 12/18/1986). Procedures and animal care performed at the University of California, Davis adhered to the guidelines of the National Institutes of Health guide for the care and use of Laboratory animals and were performed in accordance with the protocols approved by the Animal Use and Care Committee (IACUC) of the University of California, Davis. Great care was taken to minimize suffering of the animals and to reduce the number of animals used. Mice were housed in groups of 5 in ventilated cages containing autoclaved cellulose paper as nesting material and rats were housed 2 per cage on corncob bedding all with free access to food and water. They were maintained under a 12 h light/dark cycle (lights on at 08:00 a.m.), at controlled temperature ($21 \pm 1\,^{\circ}C$) and relative humidity ($55 \pm 10\%$). The animals were randomly divided in groups of 6. Behavioral testing was performed between 9:00 a.m. and 5:00 p.m. Scientists running the experiments were not aware of the treatment protocol at the time of the test (blind procedure).

2.2. Chemicals

λ-Carrageenan, PEG 400 and TWEEN 80, were purchased from Sigma–Aldrich (Milan, Italy). The sEH inhibitors TPPU, 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea, *t*-TUCB, *trans*-4-[4-(3-trifluoromethoxyphenyl-l-ureido)-cyclohexyloxy]-benzoic acid and the FAAH inhibitor URB937,3-(3-carbamoylphenyl)-4-hydroxy-phenyl] N-cyclohexylcarbamate, were synthesized and characterized in house as previously described [30–32]. URB937 was prepared immediately before use in 80% sterile saline solution/10% PEG 400/10% Tween 80 and orally administered to mice in a volume of 2.5 mL kg^{-1}. TPPU, *t*-TUCB and URB937 administered to rats were prepared immediately before use in PEG 400 and intraperitoneally administered in a volume of 1 mL kg^{-1}.

2.3. Carrageenan-induced inflammation

We induced paw edema by injecting λ-carrageenan (1% w/v in sterile water, 50 μL) into the left hind paw of lightly restrained adult male CD1 mice. Edema was measured with a plethysmometer (Ugo Basile, Comerio, Italy). Fresh inhibitor solutions were prepared immediately before use in 80% sterile saline solution/10% PEG-400/10% Tween 80 and orally administered in a volume of 2.5 mL kg^{-1}. All experiments were performed in a quiet room, and experimenters were blinded to the treatment protocol at the time of the test. Mechanical hyperalgesia was evaluated by measuring the latency (in seconds) to withdraw the paw from a constant mechanical pressure exerted onto the dorsal surface [31]. A 15-g calibrated glass cylinder (diameter = 10 mm) drawn to conical point (diameter = 3 mm) was used to exert the mechanical force. The weight was suspended vertically between two rings attached to a stand and was free to move vertically. A cut-off time of 180 s was used. Heat hyperalgesia was assessed by the method of Hargreaves et al. [33], measuring the latency to withdraw the hind paw from a focused beam of radiant heat (thermal intensity: infrared 3.0) applied to the plantar surface in a plantar test apparatus (Ugo Basile). The cutoff time was set at 30 s.

2.4. Diabetic neuropathic pain model

Diabetic neuropathic pain was modeled using streptozocin, an antibiotic that selectively destroys pancreatic beta cells. The decrease in mechanical withdrawal thresholds (MWTs) measured with the von Frey assay (IITC, Woodland Hills, CA) develops within 5–7 days after streptozocin administration and persists for the lifetime of the animal. Baseline naive MWTs were established prior to induction of diabetes. For this, rats were acclimated to the laboratory and apparatus for 1 h and MWT were probed at least three times at 1-min intervals; the scores averaged per animal. After naïve MWTs were assessed, streptozocin (55 mg kg^{-1}) in saline was injected *via* the tail vein as previously reported [34]. After 1 week, the MWTs of diabetic rats were measured and allodynia (a painful response to innocuous stimulation) was confirmed. Rats that scored 65% or lower of the original naïve baseline were considered allodynic and included in the experimental groups. For the bioassay, rats were assessed for their diabetic neuropathic MWTs and then injected intraperitoneally (i.p.) with TPPU or URB937 and MWT were measured 30, 60, 90, 120, 150, and 180 min after the injection. The procedure was followed for all doses (0.03, 0.1, 0.3, 1, 3 mg kg^{-1}) of TPPU, 3 mg kg^{-1} *t*-TUCB and (0.03, 0.1, 0.3, 1, 3 mg kg^{-1}) URB937. The diabetic neuropathic pain baselines and subsequent MWTs are reported as grams of force needed to elicit hind paw withdrawal.

2.5. Isobolographic analyses

The interaction between TPPU and URB937 was characterized by isobolographic analysis assuming that the combinations were