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## Symposium

## A Lipid Gate for the Peripheral Control of Pain

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Cells in injured and inflamed tissues produce a number of proalgesic lipid-derived mediators, which excite nociceptive neurons by activating selective G-protein-coupled receptors or ligand-gated ion channels. Recent work has shown that these proalgesic factors are counteracted by a distinct group of lipid molecules that lower nociceptor excitability and attenuate nociception in peripheral tissues. Analgesic lipid mediators include endogenous agonists of cannabinoid receptors (endocannabinoids), lipid-amide agonists of peroxisome proliferator-activated receptor- $\alpha$ , and products of oxidative metabolism of polyunsaturated fatty acids via cytochrome P<sub>450</sub> and other enzyme pathways. Evidence indicates that these lipid messengers are produced and act at different stages of inflammation and the response to tissue injury, and may be part of a peripheral gating mechanism that regulates the access of nociceptive information to the spinal cord and the brain. Growing knowledge about this peripheral control system may be used to discover safer medicines for pain.

## Introduction

Harmful stimuli are detected by a class of specialized sensory neurons, called nociceptors, which are housed in the trigeminal and DRG and project their axons to the periphery of the body. These neurons are divided into two subclasses that are both structurally and functionally distinct. Medium-sized “A $\delta$ ” nociceptors convey the localized sharp pain sensation that acts as a warning sign of injury, whereas small-sized “C” nociceptors mediate the more diffused and delayed pain that promotes defensive behaviors and supports tissue repair. In addition to mechanical and thermal insults (two common causes of body damage), nociceptors also respond to a variety of chemical irritants (e.g., defensive compounds produced by plants and insects) as well as to endogenous chemicals, such as protons, nucleotides, peptides, and lipid-derived mediators (for review, see Piomelli and Sasso, 2014).

Cells in injured and inflamed tissues generate a variety of proalgesic (pain-inducing or pain-enhancing) lipid mediators, which include membrane-derived phospholipids (e.g., lysophosphatidic acid and lysophosphatidylinositol), and oxidative

metabolites of polyunsaturated fatty acids (PUFAs) (e.g., prostaglandin E<sub>2</sub>, hydroxylated derivatives of linoleic acid) (Piomelli and Sasso, 2014) (Fig. 1). These molecules increase the excitability of nociceptive neurons by engaging selective G-protein-coupled receptors or ligand-gated ion channels. The specific roles played by each of these substances, if any, are often unknown, but their importance in inducing and maintaining pain has been recognized since the 1970s (Ferreira, 1972). Only recently has it become clear, however, that the proalgesic influence of these mediators is countered by the actions of a distinct set of bioactive lipids that modulate nociception by lowering sensory neuron excitability. These analgesic lipids include endocannabinoids, such as anandamide and 2-arachidonoyl-*sn*-glycerol (2-AG), lipid-amide agonists of peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ), such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), and various products of oxidative PUFA metabolism (Fig. 1). Here we review evidence indicating that these lipid-derived mediators modulate pain initiation by regulating the transmission of nociceptive signals from injury sites in peripheral tissues to the CNS.

## Endogenous cannabinoid agonists

Anandamide and 2-AG are part of a signaling complex that also comprises G-protein-coupled cannabinoid receptors that mediate their effects (CB<sub>1</sub> and CB<sub>2</sub>) as well as proteins responsible for their production, transmembrane transport, and breakdown. These agents are produced and degraded through distinct enzyme-mediated routes (for review, see Guindon and Hohmann, 2009). Anandamide is formed by cleavage of a membrane phospholipid in which the amine group of phosphatidylethanolamine is covalently linked to arachidonic acid. Newly released anandamide acts near its sites of production, as an autocrine or paracrine messenger, and is rapidly eliminated through a process consisting of carrier-mediated transport into cells (the molecular mechanism of which remains unclear) followed by hydrolysis catalyzed

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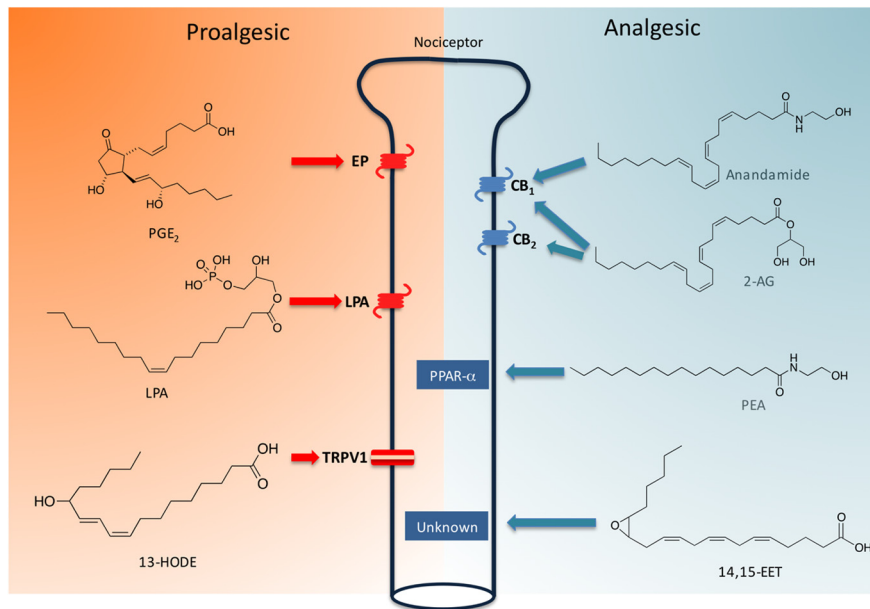
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Conflicts of interest: D.P. and B.D.H. are inventors in issued patents and patent applications filed by the University of California and the Fondazione Istituto Italiano di Tecnologia, which disclose pharmaceutical agents targeting molecular pathways described in the present article. B.D.H. is a cofounder of Eicosis LLC, which develops soluble epoxide hydrolase inhibitors. D.P. is a cofounder of Antena Therapeutics Inc, which develops endocannabinoid-based medications, and Thesano Pharmaceuticals Inc, which develops dermatological therapies unrelated to the topic of the present article. The remaining authors declare no competing financial interests.

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**Figure 1.** Peripheral gating of nociception by lipid-derived mediators. Lipid messengers generated by neural and non-neural cells during injury or inflammation regulate the excitability of peripheral nociceptors. Proalgesic lipids, which heighten nociceptor excitability, include prostanoids, such as prostaglandin  $E_2$  (which binds to G-protein-coupled EP-type receptors), phospholipids, such as lysophosphatidic acid (LPA) (which binds to LPA receptors), and oxidized PUFA derivatives, such as 13-hydroxyoctadecenoic acid (13-HODE), which activates TRPV-1. Persistent TRPV-1 activation can lead to desensitization and consequent reduction in nociceptive signaling. Analgesic lipids, which dampen nociceptor excitability, include endocannabinoids, such as anandamide and 2-AG (which bind to  $CB_1$  and  $CB_2$  cannabinoid receptors), endogenous ligands for PPAR- $\alpha$ , such as PEA, and oxidized PUFA derivatives, such as 14,15-EET. Anandamide can also activate TRPV-1, but at concentrations that are unlikely to be reached under most physiological conditions.

by the intracellular serine amidase, fatty acid amide hydrolase (FAAH) (Ueda et al., 2013). Anandamide can be also transformed by cyclooxygenase-2 into proalgesic metabolites called prostamides (Gatta et al., 2012). There are no known inhibitors of anandamide formation. By contrast, anandamide deactivation can be interrupted using agents that block membrane transport (e.g., AM404 or ARN272) (Fu et al., 2012), FAAH-mediated degradation (e.g., URB597) (Kathuria et al., 2003) (Fig. 2), or substrate-selective cyclooxygenase metabolism (e.g., LM-4131) (Hermanson et al., 2013).

2-AG is produced in a series of reactions that starts with the conversion of phosphatidylinositol-4,5-bisphosphate into 1,2-diacylglycerol (1,2-DAG), which is catalyzed by phospholipase C- $\beta$ . 1,2-DAG is then cleaved by diacylglycerol lipase- $\alpha$  (DGL- $\alpha$ ) to form 2-AG and free fatty acid (Stella et al., 1997). Monoacylglycerol lipase (MGL) is the main serine esterase involved in 2-AG deactivation (Dinh et al., 2002; Hohmann et al., 2005), with  $\alpha$ - $\beta$  hydrolase domain 6 (ABHD-6) participating in some cases (Marrs et al., 2010). Like anandamide, 2-AG may be also metabolized by cyclooxygenase-2 to produce oxidized proalgesic derivatives (for review, see Guindon and Hohmann, 2008). 2-AG formation can be inhibited using relatively nonselective probes that block either PLC (e.g., U73122) or DGL (e.g., tetrahydrolipstatin) (Gregg et al., 2012). DGL- $\alpha$ -preferring inhibitors have been recently disclosed but remain to be fully characterized (Appiah et al., 2014). Agents interfering with 2-AG deactivation include compounds that target MGL (e.g., URB602 and JZL-184) (Hohmann et al., 2005; Long et al., 2009) and ABHD-6 (e.g., WWL-70) (Marrs et al., 2010) (Fig. 2).

## Endocannabinoid control of peripheral pain

Although highly expressed in neurons of the brain and spinal cord (Herkenham, 1991),  $CB_1$  cannabinoid receptors are also present in neural and non-neural cells throughout the body (for review, see Guindon and Hohmann, 2009). They are synthesized in cell bodies of DRG neurons and are transported to peripheral nerve terminals, where they are localized appropriately to control pain initiation in response to agonist stimulation (Hohmann and Herkenham, 1999). Indeed, cell-specific deletion of  $CB_1$  in mouse nociceptive neurons impairs the antinociceptive effects of local or systemic (but not intrathecal) administration of cannabinoid agents (Agarwal et al., 2007).  $CB_2$  receptors, on the other hand, are primarily found in immune cells, such as B-lymphocytes and macrophages, but are also present in skin keratinocytes and other cell types (Dhopeswarkar and Mackie, 2014). Their expression in nociceptors is very low under baseline conditions but can be enhanced by injury or inflammation (Wotherspoon et al., 2005; Svízenská et al., 2013). Antihypersensitivity mechanisms mediated by peripheral  $CB_1$  receptors were first documented using local injections of anandamide (Calignano et al., 1998; Richardson et al., 1998).

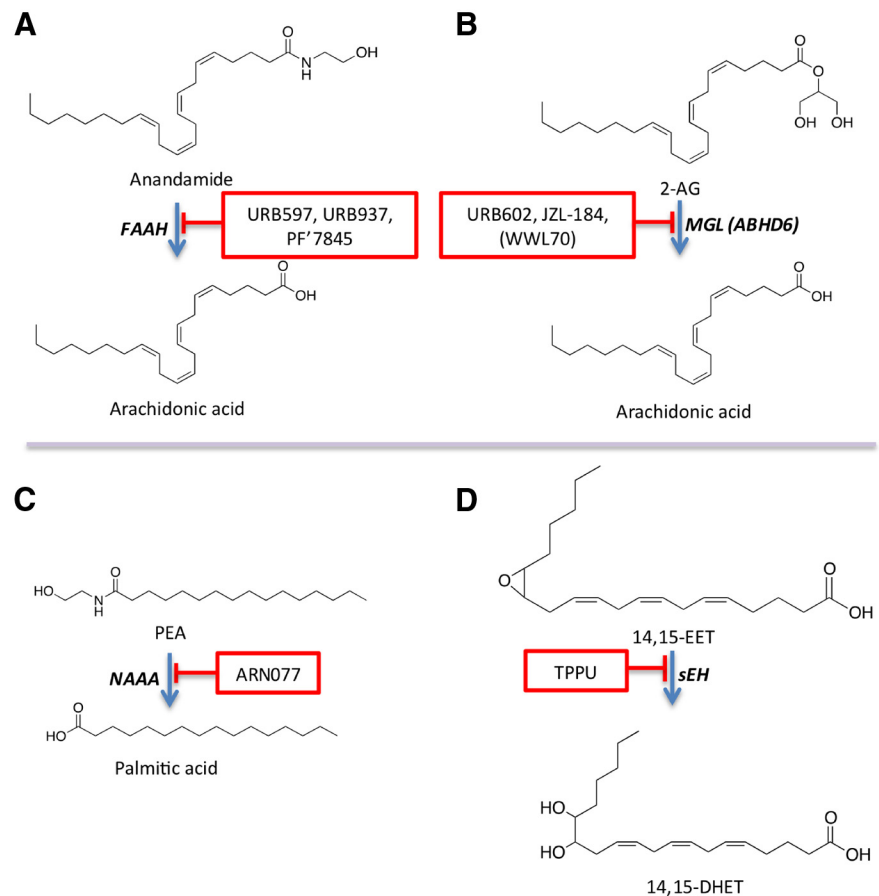
In subsequent studies, the antinociceptive actions of this compound were confirmed to be  $CB_1$ -dependent, whereas the antinociceptive effects of 2-AG were shown to require activation of both  $CB_1$  and  $CB_2$  receptors (Guindon and Hohmann, 2009). When administered at the site of injury, cannabinoid agonists suppress the activity of nociceptive neurons in the spinal cord, suggesting that cannabinoid receptor occupancy outside the CNS is sufficient to control nociception (Nackley et al., 2003a, b, 2004; Sagar et al., 2005). In addition to cannabinoid receptors, anandamide can activate various ligand-gated ion channels, including transient receptor potential vanilloid-1 (TRPV-1) (Akopian et al., 2009). This activation requires, however, relatively high concentrations of anandamide, which are unlikely to occur *in vivo*, and its physiological significance remains unclear. For example, submicromolar concentrations of anandamide suppress the activity of somatosensory neurons (Khasabova et al., 2008), whereas the same neurons are excited by anandamide at concentrations  $\geq 10 \mu\text{M}$  through a TRPV-1-dependent mechanism (Price et al., 2004). Notably, a variety of lipid mediators have been shown to engage TRPV-1 more potently than does anandamide, including the nonendocannabinoid FAAH substrate OEA (LoVerme et al., 2006).

Several laboratories have attempted to harness the therapeutic potential of peripheral endocannabinoid signaling using inhibitors of anandamide and 2-AG deactivation, with a goal of alleviating pain states without causing unwanted CNS-based side effects. Pharmacological blockade of either MGL or FAAH at injury sites produces marked antinociception: FAAH and MGL inhibitors, administered locally into the paw, reduce behavioral hypersensitivity provoked by intraplantar injections of capsaicin

in a modality-specific fashion and with nonoverlapping patterns of pharmacological selectivity (Spradley et al., 2010). Local MGL inhibition with the compound JZL-184 suppresses capsaicin-evoked nocifensive behavior and heat hypersensitivity through both CB<sub>1</sub>- and CB<sub>2</sub>-selective mechanisms, without altering capsaicin-evoked mechanical allodynia. By contrast, local injection of the FAAH inhibitor URB597 selectively blocks capsaicin-evoked mechanical allodynia, through CB<sub>1</sub> activation, without changing nocifensive behavior or heat hypersensitivity (Spradley et al., 2010). In a model of inflammatory nociception, MGL inhibitors act peripherally to heighten the effects of endogenously produced 2-AG via both CB<sub>1</sub> and CB<sub>2</sub> receptors (Guindon et al., 2011). By contrast, in the same model, the antinociceptive effects of FAAH inhibitors are exclusively mediated by CB<sub>1</sub>, whereas their anti-inflammatory actions require CB<sub>2</sub> and PPAR- $\alpha$  (presumably via PEA/OEA augmentation, see Endogenous PPAR- $\alpha$  agonists) (Guindon and Hohmann, 2009; Clapper et al., 2010). The development of a brain-impermeant FAAH inhibitor, URB937, allowed researchers to demonstrate unambiguously that anandamide controls pain initiation through a peripheral CB<sub>1</sub>-dependent mechanism (Clapper et al., 2010). URB937, which is actively extruded from the CNS by the action of an ATP-binding cassette transporter (Moreno-Sanz et al., 2011), suppresses formalin-evoked pain behaviors and neuronal activation in the spinal cord and produces CB<sub>1</sub>-mediated antinociception in models of nerve injury and inflammation (Clapper et al., 2010; Sasso et al., 2012). Collectively, these observations indicate that anandamide and 2-AG play nonredundant roles in the control of peripheral nociception.

In a model of peripheral neuropathy caused by the chemotherapeutic agent, cisplatin, the globally active FAAH inhibitor, URB597, prevents the development of allodynia, normalizes cisplatin-induced decrease in conduction velocity of A $\alpha$ /A $\beta$ -fibers and reduces the increase in immunoreactivity for TRPV-1 and the injury marker ATF3 in DRG neurons (Khasabova et al., 2012). Its brain-impermeant counterpart, URB937, and the MGL inhibitor JZL-184 are also effective in suppressing mechanical and cold allodynia but act through distinct mechanisms: CB<sub>1</sub>, CB<sub>2</sub>, and TRPV-1 antagonists block the antiallodynic effects of URB937 (and URB597), whereas those of JZL-184 are prevented by CB<sub>1</sub> and CB<sub>2</sub> antagonists only (Guindon et al., 2013). The finding that TRPV-1 antagonists block the antinociceptive actions of URB937 underscores the complexity of the molecular response to FAAH inhibition, which cannot be simplistically equated to enhanced anandamide activity at cannabinoid receptors.

Cisplatin stimulates the mobilization of anandamide and 2-AG in lumbar spinal cord tissue but lowers 2-AG content in DRG (Guindon et al., 2013). In lumbar spinal cord, cisplatin heightens FAAH transcription, suggesting that compensatory



**Figure 2.** Targeting analgesic lipid-derived mediators for pain control. Protecting analgesic lipid messengers from enzyme-mediated degradation enhances the intrinsic actions of these agents in animal models and offers multiple opportunities to develop medications that control pain without exerting unwanted centrally mediated side effects. **A**, Anandamide is hydrolyzed by FAAH, which is inhibited by globally active compounds, such as URB597 and PF'7845, and by peripherally restricted compounds, such as URB937. **B**, 2-AG is hydrolyzed by MGL and, to a minor extent, by ABHD-6. MGL is inhibited by URB602 and JZL-184, whereas ABHD-6 is inhibited by WWL-70. **C**, PEA and OEA are hydrolyzed by NAAA. ARN077 inhibits NAAA with high potency and selectivity but is metabolically unstable and cannot be used systemically. **D**, Epoxides of polyunsaturated fatty acids, such as 14,15-EET, are hydrolyzed by sEH, which is inhibited by compounds such as 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU).

changes in FAAH activity may result from the ability of this cytotoxic drug to elevate anandamide levels (Guindon et al., 2013). Moreover, traumatic nerve injury increases anandamide and 2-AG content, as well as CB<sub>1</sub> expression, in DRG (Mitrirattanakul et al., 2006). It appears, therefore, that antinociceptive endocannabinoid signaling is upregulated in both CNS and peripheral nervous system during painful states.

The data summarized above suggest that activation of CB<sub>1</sub>, CB<sub>2</sub>, and possibly other receptors downstream of inhibition of endocannabinoid degradation alleviates pain via a peripheral mechanism. Are both CB<sub>1</sub> and CB<sub>2</sub> required for this response or is CB<sub>2</sub> activation alone sufficient to control pain? To address this question, the antinociceptive efficacy of the CB<sub>2</sub>-preferring agonist AM1710 was evaluated in a model of chemotherapy-induced pain. Chronic dosing with AM1710 caused a marked suppression of pain responses (Deng et al., 2014). Importantly, these effects were absent in mutant mice lacking CB<sub>2</sub> receptors and occurred in the absence of tolerance, CB<sub>1</sub>-dependent withdrawal or cardinal signs of CB<sub>1</sub> activation (Deng et al., 2014). Additionally, treatment with AM1710 decreased transcription of mRNAs encoding for proinflammatory cytokines (e.g., tumor necrosis factor- $\alpha$ ) and chemokines (e.g., monocyte chemoattractant protein-1) in lumbar spinal cord (Deng et al., 2014). Thus, similarly to periph-

eral blockade of endocannabinoid degradation, CB<sub>2</sub> receptors activation exhibits a favorable therapeutic ratio marked by sustained efficacy in the absence of tolerance, physical withdrawal, CB<sub>1</sub>-mediated side effects, and drug abuse liability.

### Endogenous PPAR- $\alpha$ agonists

PPAR- $\alpha$  is a lipid-activated nuclear receptor that serves key functions in the control of energy metabolism (Gervois and Mansouri, 2012). In addition to liver and muscle, where PPAR- $\alpha$  is highly expressed, the receptor is also found in other cell types, including DRG neurons (LoVerme et al., 2006) and macrophages (Gervois and Mansouri, 2012). Consistent with this localization, synthetic PPAR- $\alpha$  agonists (e.g., GW7647 and Wy-14643) suppress pain-related behaviors produced in rats and mice by injection of carrageenan, formalin, or magnesium sulfate (Taylor et al., 2002; LoVerme et al., 2006), prevent formalin-induced firing of rat spinal cord neurons (LoVerme et al., 2006), and show remarkable anti-inflammatory properties in animal models (Taylor et al., 2002; Kostadinova et al., 2005; LoVerme et al., 2005). Moreover, PPAR- $\alpha$  agonists reduce thermal and mechanical hyperalgesia evoked in mice by nerve injury or inflammation (LoVerme et al., 2006). PPAR- $\alpha$ -null animals are insensitive to the antinociceptive effects of PPAR- $\alpha$  agonists but are hypersensitive to various proalgesic and proinflammatory stimuli (Devchand et al., 1996; Ruiz-Medina et al., 2012), which is suggestive of a role for PPAR- $\alpha$  in the tonic control of nociception and inflammation.

A variety of naturally occurring fatty-acid derivatives are agonists for PPAR- $\alpha$ . These include low-potency ligands, such as free fatty acids, and high-potency ligands, such as OEA and PEA (Fig. 1). The median effective concentration (EC<sub>50</sub>) values for PPAR- $\alpha$  activation are 0.12  $\mu$ M for OEA and 3  $\mu$ M for PEA (Fu et al., 2003; LoVerme et al., 2005). Evidence indicates that OEA and PEA exert a tonic inhibitory control over the induction of nociceptive responses. First, DRG neurons produce substantial amounts of these lipid amides, even in the absence of external stimuli. Because of this constitutive production, OEA and PEA reach single-digit micromolar concentrations in nonstimulated cells, which should be sufficient to engage a substantial fraction of local PPAR- $\alpha$  (Piomelli and Sasso, 2014). Second, proinflammatory stimuli suppress the formation of OEA and PEA. For example, macrophages exposed to bacterial endotoxin respond with a persistent decrease in OEA and PEA content (Solorzano et al., 2009), which results from a downregulation in the transcription of *N*-acylphosphatidylethanolamine-selective phospholipase D (Zhu et al., 2011), the enzyme responsible for the biosynthesis of these lipid mediators (Rahman et al., 2014). Consistent with a role for OEA and PEA in inflammatory pathology, synovial fluid from subjects with rheumatoid arthritis and osteoarthritis contains lower concentrations of these lipid amides than does synovial fluid from healthy controls (Richardson et al., 2008).

The idea that lipid-amide agonists of PPAR- $\alpha$  are homeostatic regulators of nociception is supported by experiments using pharmacological agents that block *N*-acylethanolamine acid amidase (NAAA) (Solorzano et al., 2009; Khasabova et al., 2013; Sasso et al., 2013), a cysteine amidase that catalyzes the hydrolysis of PEA and OEA in macrophages and, possibly, other cells (Ueda et al., 2013) (Fig. 2). For example, topical applications of the potent and selective, but metabolically unstable, NAAA inhibitor ARN077 restore baseline OEA and PEA levels in inflamed skin tissue and attenuate nociceptive responses elicited in mice and rats by carrageenan injection, sciatic nerve ligation, or ultraviolet B-radiation (Sasso et al., 2013). These effects are absent in PPAR-

$\alpha$ -null mice and are prevented, in rats, by the PPAR- $\alpha$  antagonist GW6471 (Sasso et al., 2013). A possible interpretation of the findings outlined above, and those discussed in the next section, is that PEA and OEA contribute to the maintenance of host-defense homeostasis by preventing the launch of inappropriate nociceptive and inflammatory responses. A full test of this hypothesis will require, however, the development of new experimental tools, including systemically active NAAA inhibitors.

### The role of lipid amides in tumor-evoked hyperalgesia

The tumor microenvironment contains a variety of lipid substances (among other factors) that heighten the sensitivity of primary sensory neurons (Mantyh et al., 2002), thereby promoting hyperalgesia and spontaneous pain. Lipid mediators that activate PPAR- $\alpha$  (e.g., OEA and PEA) or CB<sub>1</sub> receptors (e.g., anandamide) reduce the release of these substances by suppressing the immune system (O'Sullivan and Kendall, 2010) as well as the proliferation of tumor cells (Guindon and Hohmann, 2011; Pisanti et al., 2013). In addition to these indirect mechanisms, lipid amides also stimulate PPAR- $\alpha$  or CB<sub>1</sub> in somatosensory neurons to reduce nociception directly. Estimates that >75% of cancer patients experience moderate to severe pain that is poorly managed by opioid treatment (Mercadante, 1999) generates interest in the analgesic properties of these lipid mediators.

The roles of endogenous PPAR- $\alpha$  and cannabinoid ligands in tumor-evoked hyperalgesia have been studied both *in vitro* and *in vivo*. In an early animal model, osteolytic sarcoma cells were injected into the intramedullary space of the femur in a syngeneic mouse (Schwei et al., 1999). However, this protocol did not readily lend itself to electrophysiological recordings and was modified by injecting tumor cells into the calcaneus bone (Cain et al., 2001; Wacnik et al., 2001). In this latter model, osteolytic damage occurs within 6 d and is accompanied by mechanical hyperalgesia, spontaneous nocifensive behavior, and spontaneous C-fiber activity (Cain et al., 2001; Wacnik et al., 2001; Khasabova et al., 2013). An intimate relationship between sensory neurons and tumor cells develops: innervation of tumors with fibers immunoreactive for calcitonin gene-related peptide (a proalgesic and proinflammatory peptide) parallels the development of mechanical hyperalgesia (Wacnik et al., 2005).

An *in vitro* coculture model was developed to study the effects of chemical mediators released from cancer cells on DRG neurons: in this model, sarcoma cells plated on a cover glass condition the medium bathing a second cover glass on which mouse DRG neurons are plated (Khasabova et al., 2007). Parallel changes in lipid-amide signaling in small-diameter DRG neurons from tumor-bearing mice or naive mice maintained in medium conditioned by sarcoma cells (Khasabova et al., 2008, 2012, 2013) validate the reliability of this *in vitro* system to study tumor-evoked changes in neurons that are most likely to give rise to nociceptors (Hiura and Sakamoto, 1987; Pearce and Duchon, 1994). Moreover, the data generated in this model support the conclusion that factors released by cancer cells produce long-term alterations in sensory neurons, which contribute to tumor-evoked pain.

Reductions in the levels of PEA and anandamide in DRG that innervate the tumor as well as in DRG from naive mice cultured with sarcoma cells have been observed. One factor that contributes to lowering lipid amide levels is increased enzyme-mediated hydrolysis. The activities of both FAAH (Khasabova et al., 2008) and NAAA (Khasabova et al., 2012) are higher in DRG from tumor-bearing mice and DRG cocultured with sarcoma cells than in control DRG. The increase in enzyme activity is accompanied by accrued FAAH transcription (Khasabova et al., 2008).

Diminished levels of bioactive lipid amides are also likely to contribute to tumor-related nociception because local injections of URB597 or ARN077, two selective inhibitors of FAAH and NAAA activities, respectively (Fig. 2) (Kathuria et al., 2003; Sasso et al., 2013), each reduce hyperalgesia in tumor-bearing paws (Khasabova et al., 2008, 2012). A CB<sub>1</sub> antagonist prevented the effects of URB597. In addition, local administration of PPAR- $\alpha$  or CB<sub>1</sub> antagonists in naive mice evokes hyperalgesia (Khasabova et al., 2008, 2012), underscoring the importance of basal activation of these receptors by endogenous ligands in setting the threshold for nociception in naive subjects.

When DRG cells are cocultured with sarcoma cells, the physiological changes in small-diameter DRG neurons parallel those seen *in vivo*. Small DRG neurons from tumor-bearing or naive mice maintained in coculture with tumor cells exhibit larger Ca<sup>2+</sup> transients following depolarization with potassium chloride, compared with control neurons (Khasabova et al., 2007, 2012). In the coculture condition, CB<sub>1</sub> agonists reduce the amplitude of the depolarization-evoked Ca<sup>2+</sup> transient, an effect mimicked by FAAH inhibition. CB<sub>1</sub> blockade prevents the actions of both CB<sub>1</sub> agonists and FAAH inhibitors.

The effects of the NAAA inhibitor ARN077 on DRG neurons parallel those of URB597 but are mechanistically different in that they require PPAR- $\alpha$  rather than CB<sub>1</sub> receptors (Khasabova et al., 2012). This is confirmed by the ability of the endogenous PPAR- $\alpha$  agonist, PEA, to mimic the actions of ARN077 (Khasabova et al., 2012). Interestingly, PPAR- $\alpha$  activation has no effect on neurons maintained *in vitro* in the absence of sarcoma cells, but PPAR- $\alpha$  blockage increases the amplitude of depolarization-evoked Ca<sup>2+</sup> transient (Khasabova et al., 2012). This result supports the possibility, mentioned above, that PPAR- $\alpha$  may be saturated by endogenous ligands under basal conditions (Piomelli and Sasso, 2014).

### Epoxy fatty acids in inflammatory and neuropathic pain

Epoxides of arachidonic acid and other PUFAs (collectively called epoxy fatty acids [EpFAs]) (Fig. 1) are powerful modulators of nociception (Wagner et al., 2011; Inceoglu et al., 2012). They are generated by the cytochrome P<sub>450</sub> pathway (A–C, E) (Morisseau and Hammock, 2013), which is divided into two branches: one leads to products of  $\omega$  and  $\omega$ -1 hydroxylation, which are generally proalgesic and proinflammatory, and another generates the EpFAs. The latter class includes epoxyeicosatrienoic acids (EETs) produced from the  $\omega$ -6 PUFA, arachidonic acid, which are predominantly analgesic and anti-inflammatory and are rapidly converted to corresponding dihydroxyeicosatrienoic acids (Wagner et al., 2011). The EpFAs also include derivatives of  $\omega$ -3 PUFAs, such as eicosapentaenoic acid and docosahexaenoic acid (Morisseau et al., 2010). Evidence suggests that  $\omega$ -3 EpFAs are important contributors to the positive biological outcomes of diets high in fish-derived  $\omega$ -3 PUFAs (Wagner et al., 2011; Morisseau and Hammock, 2013; Zhang et al., 2014). Even though epoxides are strained and sometimes reactive, three-membered cyclic ethers, most EpFAs are quite stable chemically. They are rapidly degraded, however, by a  $\alpha/\beta$ -hydrolase fold enzyme termed soluble epoxide hydrolase (sEH or EH2) (Morisseau and Hammock, 2013) (Fig. 2). Because of the low K<sub>m</sub> and high k<sub>cat</sub> of sEH for most EpFAs (Morisseau et al., 2010), these mediators are controlled as much by their degradation as by their biosynthesis. The fact that EpFA activity is regulated by biochemical mechanisms similar to those discussed above for other analgesic lipid mediators emphasizes the general roles played by such mechanisms in the control of analgesic lipid signaling.

Research on the cytochrome P<sub>450</sub> pathway of PUFA metabolism lagged behind other branches for multiple reasons. A major one is that it was difficult to show biological activities of EpFAs because of their rapid cleavage by sEH. Once this problem was overcome by the invention of potent sEH inhibitors (Shen and Hammock, 2012), other factors, including low titers of bioactive metabolites, difficulties in analysis, and lack of high-quality standards, continued to slow down progress. Nevertheless, experiments using sEH inhibitors have clearly demonstrated that EpFAs are involved in a variety of biological processes (Panigrahy et al., 2013; Zhang et al., 2013; Ulu et al., 2014). Some of the most dramatic effects of these agents (and thus, by inference, the EpFAs they protect) have been observed in animal models of inflammation. Rodent sepsis models were used to show that sEH blockade increases EET levels and concomitantly attenuates the burst in proalgesic prostaglandins triggered by bacterial endotoxin (Schmelzer et al., 2005).

Based on observations made in the sepsis model, sEH inhibitors were used to modulate levels of EET and other EpFAs in a variety of experimental contexts. The compounds prevent and reverse cardiac and pulmonary dysfunction (i.e., atrial fibrillation, fibrosis and cardiac hypertrophy, reduced onset of atherosclerosis, pulmonary fibrosis, chronic obstructive pulmonary disease, pulmonary hypertension) as well as a variety of pathological inflammatory states (e.g., gastrointestinal inflammation, vascular inflammation, stroke, ischemia-reperfusion injury, renal inflammation, and fibrosis), mitochondrial dysfunction, and chemical-induced nephrotoxicity (Morisseau and Hammock, 2013). Probably related to reduced sensitivity to reactive oxygen species and endoplasmic stress responses, sEH inhibitors increase pancreatic islet size, improve glucose homeostasis, and lower insulin resistance (Xu et al., 2006; Shen and Hammock, 2012; Morisseau and Hammock, 2013).

Consistent with their anti-inflammatory effects, sEH inhibitors attenuate nociceptive responses produced by a variety of inflammatory agents in rodents (Schmelzer et al., 2006). Interestingly, the inhibitors reduce pain responses produced by administration of PGE<sub>2</sub>, suggesting that they may work downstream of cyclooxygenase and its proalgesic metabolites (Inceoglu et al., 2011). Equally intriguing is the ability of sEH blockage to reduce pain behaviors in models of diabetes and nerve damage (Inceoglu et al., 2012). Whether monitoring mechanical allodynia or using a conditioned placement preference test, sEH inhibitors outperform gabapentin yet are devoid of the cognitive and motoric side effects commonly associated with use of this centrally active analgesic (Inceoglu et al., 2012). Interestingly, cyclooxygenase inhibitors, which have no effect on neuropathic pain responses when administered alone, synergistically reduce such responses when they are combined with sEH blockers (Guedes et al., 2013). The mechanism of action of sEH inhibitors in analgesia is still unknown, although neurosteroids appear to be involved (Inceoglu et al., 2013).

In conclusion, putting the available data together, we can conjecture that three distinct classes of bioactive lipid mediators (endocannabinoids, endogenous PPAR- $\alpha$  activators, and oxidative products of PUFA metabolism) regulate the transmission of nociceptive information from peripheral sites of injury and inflammation to the CNS. The data also raise several interesting questions. The first pertains to the existence of mechanisms, both local and systemic, which might regulate the correct deployment of analgesic lipid signals after tissue damage. Particularly important in this context may be the role of the autonomic nervous system, which is known to control endocannabinoid and OEA

signaling in the gut (DiPatrizio et al., 2011) and the adipose organ (LoVerme et al., 2006). Another relevant question concerns the stages of injury or inflammation at which analgesic lipids might intervene. As mentioned above, there is evidence that the endogenous PPAR- $\alpha$  agonists, PEA and OEA, help set the threshold for nociception in intact tissues and that proinflammatory stimuli may act, at least in part, by disabling this homeostatic control system. By contrast, endocannabinoids, such as anandamide, may be released on demand during injury to offset the effects of local proalgesic signals, whereas products of oxidative PUFA metabolism, including the EpFAs and others, such as lipoxins and resolvins (Serhan et al., 2008), may help restore normal nociceptive responses during resolution and tissue healing (Piomelli and Sasso, 2014). Disruptions in the temporal unfolding of this program may contribute to the development of pathological pain conditions and might be targeted to discover better medicines for pain.

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