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Peripheral gating of pain signals by endogenous analgesic lipids

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Abstract

Primary sensory afferents and their neighboring host-defense cells are a rich source of lipid-derived mediators that contribute to the sensation of pain caused by tissue damage and inflammation. But an increasing number of lipid molecules have been shown to act in an opposite way, to suppress the inflammatory process, restore homeostasis in damaged tissues and attenuate pain sensitivity by regulating neural pathways that transmit nociceptive signals from the periphery of the body to the central nervous system.

The ‘gate control theory’ of pain postulates that neural circuits in the central nervous system (CNS) dynamically regulate nociceptive signals arising in the periphery of the body. Since its formulation in 1965[1], this theory has provided a framework in which to interpret the actions of central analgesic circuits, such as those recruited during acute stress or placebo responses. Emerging evidence indicates, however, that nociceptive signals may be subject to a dynamic filtering process even before they reach the spinal cord. Primary sensory neurons and the host-defense cells surrounding them release a variety of analgesic factors that control the traffic of nociceptive information to the CNS (Figure 1). Lipid-derived mediators are crucial players, albeit not the only ones[2], in this peripheral gating mechanism. Here, we review the properties of endogenous bioactive lipids that modulate pain initiation by interacting with receptor systems on primary sensory afferents and neighboring cells that are not neurons – including macrophages, mast cells and keratinocytes. We also outline stages of tissue injury and inflammation at which these analgesic lipids may intervene, and speculate on how their actions might be exploited to discover better medicines for pain.

Proalgesic lipids

The cell bodies of nociceptors – the primary sensory neurons specialized in detecting harmful stimuli – are housed in anatomical structures located outside the CNS, the trigeminal and dorsal root ganglia (DRG), and generate axonal stalks that split into two sets of fibers running in opposite directions. One reaches the skin and most internal organs, with the exception of the brain, where it senses noxious stimuli and converts them into electrical

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signals. The other set of fibers extends toward the dorsal horn of the spinal cord to form synapses with local neurons, which process the information arriving from the periphery and transmit it to supraspinal sites. Nociceptors are divided into two broad classes with distinct structure and function. 'Aδ' nociceptors, which have medium-sized cell bodies and fast-conducting axons, mediate the localized sharp pain sensation that serves as a warning signal of injury. 'C' nociceptors, on the other hand, have small-diameter cell bodies and slow-conducting axons, and convey the poorly localized delayed pain that supports tissue repair by inducing defensive behaviors. Many (but not all) nociceptors are considered to be polymodal because they share the ability to recognize as harmful external inputs of widely different modalities – thermal, chemical and mechanical. At the root of this perceptual flexibility is the existence of multiple nociceptor subclasses, each expressing a distinctive repertoire of membrane ion channels, receptors and intracellular signaling proteins. For example, the presence of cation channels of the transient receptor potential (TRP) family renders nociceptors differentially sensitive to heat (TRPV1), cold (TRPM8) and chemical irritants (TRPA1). After tissue damage, this heterogeneous palette of transduction molecules undergoes profound changes, giving rise to a state of hyper-excitability, called peripheral sensitization, in which non-noxious events are perceived as noxious (allodynia) and mildly noxious events are perceived as highly noxious (hyperalgesia). Sensitization is often accompanied by neurogenic inflammation – a local vasodilatory response caused by release of substance P and calcitonin gene-related peptide (CGRP) from primary afferent terminals – and by activation of a group of primary sensory neurons, called silent nociceptors, which becomes responsive to harmful stimuli only after tissue damage has occurred[3].

In addition to external stimuli, nociceptors are exquisitely sensitive to a number of endogenous proalgesic (pain-inducing or pain-enhancing) factors that either are quickly released after an injury or produced more slowly during inflammation, peripheral neuropathy and tumor growth (Figure 1). Intracellular nucleotides such as ATP and ADP, which spill out of damaged cells, and peptides such as bradykinin, which is liberated from a plasma globulin during blood clotting, may supply a first wave of proalgesic chemicals by virtue of their ability to activate excitatory receptors on primary afferents. A second, slower wave of sensitizing and proinflammatory agents includes substance P and CGRP as well as a diverse array of bioactive lipid-derived mediators generated by primary afferents and close-by host-defense cells. The key roles that two such mediators, prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2), play in nociceptor sensitization were first identified in the 1970s[4] and are now firmly established[5]. PGE_2 and PGI_2 are produced at sites of inflammation and activate specific G protein-coupled receptors on sensory neurons to increase membrane excitability and enhance secretion of substance P and CGRP[6]. The rate-limiting step in this signaling cascade is provided by two functionally similar, but molecularly distinct enzymes – cyclooxygenase (Cox)-1 and Cox-2 – which convert the membrane-derived polyunsaturated fatty acid (PUFA), arachidonic acid, into PGH_2, a common precursor for all prostanoids[7]. By interrupting Cox-1 and Cox-2 activities, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen suppress primary afferent sensitization and strongly reduce inflammatory pain[4]. In addition to Cox-1 and Cox-2 products, many other lipid molecules formed through enzymatic or non-enzymatic PUFA oxidation are able to excite nociceptors and cause hyperalgesic states. Recently described examples are the

hydroxylated derivatives of linoleic acid[8], hepoxilin A_3[9], PGE_2-glycerol ester[10], and prostamide $F_2\alpha$[11]. Other lipid-derived proalgesic agents include lysophosphatidic acid[6] and lysophosphatidyl inositol[12]. While the specific functions served by each of these substances are in most cases unclear, the overall significance of lipid-mediated signaling in the induction and maintenance of persistent pain is widely recognized[3]. Research in the last ten years has unexpectedly revealed, however, the existence of bioactive lipids that contrast the effects of proalgesic agents and modulate pain initiation at sites of injury and inflammation. These analgesic lipid mediators – which include endogenous cannabinoids, lipid-amide agonists of peroxisome proliferator-activated receptor-α (PPAR-α), and various products of oxidative PUFA metabolism (Table 1) – play non-redundant roles in the dynamic modulation of peripheral responses to noxious stimuli.

Endogenous cannabinoids: antinociceptive response to injury

Neural and non-neural cells in damaged and inflamed tissues produce a family of arachidonic acid derivatives, called endocannabinoids, which suppress sensitization and neurogenic inflammation by activating CB_1 and CB_2 cannabinoid receptors – the same $G_{i/o}$ protein-coupled receptors that are targeted by Δ^9-tetrahydrocannabinol in marijuana[13].

Cannabinoid receptors

CB_1 receptors are highly expressed in the CNS, where they are concentrated in axon terminals of inhibitory interneurons and excitatory projection neurons[14]. Thanks to this presynaptic localization and to their ability to modulate calcium and potassium channel activities, CB_1 receptors have important functions in the regulation of neurotransmitter release in the brain and spinal cord[14]. Substantial levels of CB_1 expression are also found in nociceptive and non-nociceptive sensory neurons of the DRG[15,16,17,18] and trigeminal ganglion[19], as well as in host-defense cells that make contact with those neurons (e.g., macrophages, mast cells and epidermal keratinocytes)[20,21] (Figure 2). Experimental interventions that evoke persistent hyperalgesia in animals are associated with profound changes in CB_1 expression in spinal and supraspinal structures of the CNS[22]. Similar alterations have been shown to occur in the DRG. For example, prolonged peripheral inflammation elicited by injection of complete Freund's adjuvant (CFA) in the rat hind paw, is accompanied by elevation of CB_1 receptor density in dermal nerve endings and cell bodies of nociceptive neurons that project to that paw[23]. Similarly, growth of painful histolytic tumors in the mouse heel bone is associated with increased CB_1 expression in nociceptors that innervate the bone and its surrounding tissues[24].

Studies in primary cultures of DRG neurons have shown that cannabinoid receptor agonists – dual CB_1/CB_2 ligands such as CP-55940 and Win-55212-2 or CB_1-selective ligands such as arachidonyl-2-chloroethylamide (ACEA) – inhibit voltage-gated calcium channels[25] and acid-sensing ion channels (ASICs)[26], reduce calcium transients evoked by capsaicin activation of TRPV1[24,27], and block nerve growth factor-induced TRPV1 sensitization[28]. Selective CB_1 receptor antagonists (e.g., rimonabant and AM251) prevent these effects[27,28,29]. Consistent with those results, *in vivo* pharmacological studies have demonstrated that cannabinoid agonists suppress nociceptive behaviors and CGRP release in rodents through a peripheral CB_1-mediated mechanism[30,31,32,33]. The peripheral nature of

these actions is strikingly illustrated by results obtained in mutant mice that specifically lack CB_1 in nociceptive neurons[17,18]. Compared to their wild-type littermates, these mice show decreased sensitivity to the analgesic effects exerted by local or systemic (but not intrathecal) administration of cannabinoid agents. Importantly, these mice also display an exaggerated hyperalgesic response to CFA injection, suggesting that CB_1 receptors on primary afferents modulate the initiation of inflammatory pain.

The second cannabinoid receptor subtype, CB_2, has limited structural similarity with CB_1 (the two proteins are only 40% identical) and a distinctive distribution in mammalian tissues[13]. Substantial CB_2 receptor levels are found in cells of hematopoietic origin, including those interacting with primary afferents during tissue injury (e.g., macrophages and mast cells)[20,34] (Figure 2). CB_2 receptors are sparsely expressed in the brain, spinal cord and DRG of healthy rats and mice, but their numbers increase markedly after peripheral nerve damage[35,36]. For example, ligation of the L5–L6 spinal nerve and transection or compression of the sciatic nerve all heighten CB_2 expression in sensory neurons of rodent DRG[37,38]. Moreover, CB_2 receptors have been identified in TRPV1-positive nociceptive neurons of human DRG, where their expression is also stimulated by nerve damage[39]. In line with these morphological findings, CB_2 activation by selective agonists (e.g. AM1241 and JWH-133) exerts profound anti-nociceptive effects in animal models of acute, inflammatory and neuropathic pain[36,40,41]. That such effects may be primarily due to a peripheral mechanism is not only suggested by the localization of CB_2 receptors, outlined above, but also by experiments showing that administration of CB_2 agonists into the rat paw suppresses hyperalgesia evoked by injection of capsaicin and reduces the response of spinal neurons to mechanical stimulation[42,43]. Both neural and non-neural cell populations are likely to contribute to these effects, as indicated by the ability of CB_2 agonists to attenuate calcium transients elicited by capsaicin in rat and human DRG neurons[27,39], enhance release of β-endorphin from keratinocytes[44], and reduce the secretion of immune mediators from macrophages and mast cells[45] (Figure 2).

In addition to CB_1 and CB_2, endocannabinoid lipids are also known to interact with non-cannabinoid sites, such as TRPV1 channels and G protein-coupled GPR55 receptors. These interactions have attracted a great deal of attention and are discussed in several recent reviews[46,47], but their relevance to the peripheral control of nociception is still unclear.

Endogenous cannabinoids

The two best-known members of the endocannabinoid family of lipid mediators, anandamide and 2-arachidonoyl-*sn*-glycerol (2-AG), are produced through distinct biochemical pathways, each starting with the enzyme-mediated hydrolysis of a specific phospholipid precursor in cell membranes[48]. Anandamide is formed by cleavage of a relatively uncommon and still poorly understood lipid molecule, in which phosphatidylethanolamine (PE) is covalently linked to arachidonic acid *via* an amide bond (Figure 3). In neurons, this reaction is stimulated by calcium ions and is catalyzed by a structurally unique phospholipase D (PLD) that exclusively recognizes *N*-acyl-substituted PE species[49]. In macrophages, on the other hand, the same reaction may occur in two separate steps: a molecularly uncharacterized phospholipase C (PLC) converts *N*-

arachidonoyl-PE into phospho-anandamide, which then loses its phosphate group by the action of a phosphatase[29]. Newly formed anandamide acts near its sites of production, as an autocrine or paracrine messenger (Figure 2), and is rapidly eliminated through a process consisting of carrier-mediated transport into cells (the molecular mechanism of which has so far resisted elucidation)[50] followed by intracellular breakdown to arachidonic acid and ethanolamine, catalyzed by the serine hydrolase fatty acid amide hydrolase (FAAH)[48,49]. Alternatively, anandamide may be directly transformed by Cox-2 (but not Cox-1) into proalgesic prostamides[11] (Figure 3). A novel class of Cox-2 inhibitors that specifically stop this reaction, without affecting the oxygenation of arachidonic acid to prostaglandins, has been recently disclosed[51].

Inflammation and nerve injury cause marked changes in anandamide mobilization. For example, in mice and rats, intraplantar injection of CFA[17] or ligation of the sciatic nerve increases anandamide levels in the affected paw[40], whereas an opposite effect is produced by tumor implantation in the heel bone[52] or topical skin application of a pro-inflammatory phorbol ester[53]. Importantly, marked changes in anandamide levels have also been documented in biopsies of human subjects with acute pancreatitis[54]. The molecular mechanisms underlying such alterations are still incompletely understood. Heightened anandamide mobilization may be due to enhanced calcium entry in nociceptors and/or to stimulation of Toll-like receptor-4 (TLR4) in macrophages[29] (Figure 2) [TLR4 is a member of the pattern recognition family of receptors, which recognize exogenous pathogen-associated molecules, such as lipopolysaccharide (LPS), as well as endogenous damage-associated molecules, such as uric acid]. Conversely, lowered anandamide availability may result from injury-induced changes in FAAH expression. In the DRG of healthy rats, FAAH is localized to small-diameter C nociceptors, but peripheral nerve damage (sciatic nerve axotomy or lumbar spinal nerve transection) induces its ectopic expression in large-diameter sensory neurons[55]. Moreover, growth of painful bone tumors is associated with enhanced FAAH expression in the paw bearing the tumor and the DRG innervating it[24]. This effect – along with a parallel, but independent increase in carrier-mediated anandamide uptake by neurons and/or other cells of the DRG[56] – may be responsible for the observed deficit in anandamide mobilization[52].

Irrespective of the regulatory mechanisms involved, anandamide released at sites of injury may modulate nociceptive signals by activating local CB_1 receptors. Experiments with pharmacological agents that block intracellular FAAH activity support this idea. For example, intraplantar delivery of the brain-penetrant FAAH inhibitor URB597, at doses that presumably do not interfere with FAAH activity in the CNS, reduces mechanically evoked responses of rat spinal neurons through a mechanism that requires CB_1 activation[35]. Furthermore, systemic administration of the peripherally restricted FAAH inhibitor, URB937, which selectively interrupts anandamide degradation outside the brain and spinal cord[57], causes striking anti-hyperalgesic effects in rodent models of visceral and inflammatory pain[57,58]. Such effects are exclusively dependent on peripheral FAAH blockade and are prevented by the CB_1 antagonists, rimonabant and AM251, but not by the CB_2 antagonist AM630[57,58]. Interestingly, FAAH inhibitors act in a synergistic manner with NSAIDs to attenuate pain-related behaviors elicited, in mice, by carrageenan or sciatic nerve constriction[58,59]. This super-additive interaction underscores the deep functional links

existing between endocannabinoids and prostanoids, as well as the interest in developing dual inhibitors that target both FAAH and Cox activities.

Unlike anandamide, 2-AG derives from the hydrolysis of a well-known inositol-containing phospholipid, phosphatidylinositol-4,5-bisphosphate (PIP_2), the centerpiece of a lipid pathway that generates multiple intracellular and transcellular messengers[48] (Figure 3). The first reaction in this pathway is catalyzed by the β-isoform of PLC (PLC-β), which is activated by G_q-coupled receptors and converts PIP_2 into the second messenger 1,2-diacylglycerol (DAG). DAG regulates protein kinase C and other cellular effectors, but also serves as substrate for the hydrolytic activity of diacylglycerol lipase-α (DGL-α), which cleaves DAG into 2-AG[48]. The serine hydrolase, monoacylglycerol lipase (MGL), is the main enzyme involved in terminating 2-AG-mediated endocannabinoid signaling, which it accomplishes by hydrolyzing this glycerol ester and releasing free arachidonic acid. Like anandamide, 2-AG may be metabolized by Cox-2 to produce proalgesic derivatives such as PGE_2 glycerol ester[10].

In the brain and spinal cord, 2-AG mobilization is controlled by receptors linked to transducing G_q proteins[46,60] and is crucially involved in the descending modulation of pain during acute stress[61]. Similarly, in cultures of rat DRG neurons, the proalgesic peptide bradykinin causes a rapid release of 2-AG, presumably through activation of B_2 bradykinin receptors[62], while platelet-activating factor (PAF) produces a similar effect in Raw264.7 macrophages by engaging PAF receptors[63]. In addition to these G_q-mediated responses, generation of 2-AG may be initiated, in rat platelets, by TLR4 activation[64]. Mechanisms such as those described above probably underlie the localized increase in 2-AG levels observed in CFA-inflamed mouse paws[17]. The relevance of localized 2-AG release to nociceptive signaling is confirmed by the finding that intraplantar injections of MGL inhibitors (URB602 or JZL-184) counteract the pain-related behaviors evoked by formalin administration in rats[65] or tumor growth in mice[52]. The dependence of these effects on CB_2 receptor activity[52,66] distinguishes MGL inhibitors from FAAH inhibitors, and indicates that 2-AG and anandamide may influence pain initiation through complementary, rather than overlapping mechanisms (Figure 3).

In summary, many lines of evidence suggest that endocannabinoid lipids are rapidly recruited during tissue injury to provide a first line of control over arising nociceptive signals. The therapeutic opportunity offered by this peripheral gating mechanism – the possibility of achieving effective analgesia without causing untoward central effects – has fueled intense efforts to discover new agents that influence endocannabinoid signaling outside the CNS (Table 2). Such efforts are not without challenges, however. Among the problems to be faced are the selection of specific endocannabinoid mechanisms that must be targeted to achieve adequate analgesic efficacy (e.g., peripheral CB_1 *vs* CB_2 agonism, global *vs* peripheral FAAH inhibition), and the identification of clinical pain conditions that would most likely respond to pharmacological interference with those mechanisms.

Endogenous PPAR-α agonists: homeostatic control of nociception

PPAR-α is a ligand-operated transcription factor that plays essential roles in energy metabolism and inflammation[67]. An unusually spacious ligand-binding pocket allows this receptor to recognize, albeit with different affinities, a variety of endogenous fatty-acid derivatives. These include low-potency agonists (e.g., free fatty acids), which act at mid-micromolar to high-micromolar concentrations[68], and high-potency agonists [e.g., 8(S)-hydroxyeicosatetraenoic acid and amides of ethanolamine with saturated and monounsaturated fatty acids (FAE)], which act at submicromolar to single-digit micromolar concentrations[69,70,71]. The FAEs, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Figure 4), engage PPAR-α with relatively high potency (median effective concentration, EC_{50}: 0.12 μM for OEA and 3 μM for PEA)[69,70] and have emerged as potential homeostatic regulators of nociception.

Peroxisome proliferator-activated receptor-α

Like other members of the nuclear receptor superfamily, PPAR-α is activated through ligand binding, which causes the formation of a multi-protein complex that comprises an obligatory partner, the retinoic acid receptor (RXR), along with a variable set of protein co-activators[67]. In its active form, PPAR-α binds to responsive elements on DNA, enhancing the transcription of various anti-inflammatory proteins, such as inhibitor of κB-α (IκB-α), while concomitantly interrupting the activity of pro-inflammatory transcription factors through direct protein-protein interactions (a process called 'transrepression')[67]. Transcription regulators modulated by PPAR-α include nuclear factor κB (NF-κB), signal transducers and activators of transcription (STATs), activator protein 1 (AP1), and nuclear factor of activated T cells (NFAT)[67]. In addition to inducing changes in gene expression, which unfold over a period of hours or even days, PPAR-α activation may also produce rapid non-genomic effects, as briefly discussed below.

PPAR-α is expressed in many organs and tissues[67] – including DRG neurons[72], macrophages[67] and other host-defense cells[67,73]. Consistent with this localization, local or systemic administration of synthetic PPAR-α agonists (e.g., GW7647 and Wy-14643) exerts profound anti-inflammatory effects in mice[70,74,75], suppresses pain-related behaviors elicited in rats and mice by injection of carrageenan, formalin or magnesium sulfate[72,74], and prevents formalin-induced firing of rat spinal cord neurons[72]. The endogenous PPAR-α agonist, PEA, has similar anti-hyperalgesic properties, which are synergistic with those of CB_1 receptor agonists[30,72,76]. Additionally, PPAR-α activation strongly reduces thermal and mechanical hyperalgesia evoked in mice by nerve injury (constriction of the sciatic nerve) or inflammation (CFA injection)[72]. These anti-hyperalgesic effects are absent in PPAR-α–deficient mice[72], which also show enhanced responses to several pro-inflammatory and proalgesic stimuli[77,78], an important indication that PPAR-α participates in the tonic control of inflammation and nociception.

The analgesic actions of PPAR-α ligands appear within minutes of drug administration, suggesting that they may be mediated, at least initially, by a transcription-independent mechanism. In support of this idea, studies with selective inhibitors have identified two subtypes of calcium-activated potassium channels that may be rapidly regulated by PPAR-

α: large-conductance BK_{ca} ($K_{ca}1.1$) and intermediate-conductance IK_{ca} channels ($K_{ca}3.1$)[72] (Figure 5). BK_{ca} and IK_{ca} are known to modulate the excitability of primary sensory neurons[79], but the molecular steps connecting PPAR-α activation to the gating of these channels remain to be defined. Anti-hyperalgesic activity may also result from the interaction of PPAR-α with transcription factors of the NFκB complex, which suppress the expression of genes targeted by immune-derived proalgesic mediators, including tumor necrosis factor-α (TNF-α) and interleukin 1β (IL1β) (Figure 5). Lastly, it is worth noting that PPAR-α agonists can also attenuate hyperalgesia after infusion into the cerebral ventricles[80]. This finding underscores the growing awareness about the role of the PPAR family of receptors (including PPAR-γ and PPAR-α) in the central modulation of pain signals[81].

Endogenous agonists of PPAR-α

OEA and PEA are chemically related to anandamide and, in neurons, they are produced by the activity of the same PLD that catalyzes anandamide release[82] (Figure 4). Nevertheless, the mechanisms governing the formation of these FAEs differ from those involved in the production of anandamide in two important aspects. First, many cell types in the body – including DRG neurons and skin cells – generate substantial amounts of OEA and PEA even in the absence of external stimuli, which are required instead to trigger on-demand anandamide release. Because of this tonic production, the levels of OEA and PEA in healthy tissues are much higher than those of anandamide. For example, the baseline concentration of PEA in mouse ear skin is approximately 3.5 μM, more than 175 times greater than that of anandamide[53]. Thus, based on the known affinities of PPAR-α for OEA and PEA (EC_{50}: 0.12–3 μM), it is reasonable to assume that a substantial fraction of PPAR-α is constitutively bound to FAEs in non-stimulated cells. Second, many pro-inflammatory stimuli dampen the production of OEA and PEA even as they trigger anandamide formation in the same cell. For example, macrophages exposed to the bacterial toxin LPS respond with a rapid burst of anandamide release[29] followed by a slower persistent decrease in FAE content[83,84]. Experiments with Raw264.7 macrophages suggest that the PLC/phosphatase pathway mediates the initial spike in anandamide production[29] (Figure 3), whereas the delayed decline in OEA and PEA results from a down-regulation in NAPE-PLD transcription due to reduced acetylation of histone proteins bound to the NAPE-PLD promoter[84]. The ability of tissue damage to suppress FAE mobilization has been documented using multiple animal models of inflammation and peripheral neuropathy[53,56,70,83]. Adding clinical relevance to these observations, one study found that the concentrations of OEA and PEA in synovial fluid are lower in subjects with rheumatoid arthritis and osteoarthritis than in healthy controls[85].

The results outlined above suggest that endogenous FAE signaling at PPAR-α exerts a tonic inhibitory control over the induction of inflammatory and nociceptive responses. This idea was tested using pharmacological agents that selectively block the cysteine hydrolase, *N*-acylethanolamine acid amidase (NAAA)[53,56,83], which catalyzes the deactivating hydrolysis of OEA and PEA in macrophages[49] (Figure 4). Topical administration of the NAAA inhibitor, N-[(3S)-2-oxo-3-oxetanyl]-3-phenylpropanamide, in mice reinstates normal PEA levels in activated leukocytes and blunts inflammatory responses induced by LPS or

carrageenan[83]. Exogenous PEA mimics these effects while PPAR-α deletion abolishes them. Importantly, under the same conditions, the FAAH inhibitor URB597 fails to normalize PEA levels, highlighting the critical role of NAAA in PEA and OEA degradation by innate-immune cells. A second NAAA inhibitor, the compound ARN077, attenuates heat hyperalgesia and mechanical allodynia elicited in mice and rats by carrageenan injection, sciatic nerve ligation or ultraviolet B-radiation[53]. These anti-nociceptive effects are absent in PPAR-α-deficient mice and are prevented, in rats, by the PPAR-α antagonist GW6471. Similarly, ARN077 reduces mechanical hyperalgesia in fibrosarcoma-bearing mice and corrects, through a mechanism mediated by PPAR-α abnormal calcium signaling in DRG neurons co-cultured with fibrosarcoma cells[86]. Confirming its proposed mechanism of action, ARN077 restores baseline FAE levels in diseased tissues of neuropathic and tumor-bearing mice[53,86].

To sum up, a plausible interpretation of the available data is that endogenous FAEs acting at PPAR-α help maintain host-defense homeostasis by preventing the launch of inappropriate inflammatory and nociceptive reactions (Figure 5). Thus, the function of these lipid mediators may be compared to that of an electronic high-pass filter, which attenuates signals that fail to reach a set threshold. Two strategies may be used to exploit this mechanism for therapeutic purposes (Table 2). The first is to directly activate PPAR-α using synthetic or endogenous ligands[67,76]. Though theoretically possible, this approach is riddled with practical problems due to the safety risks posed by excessive direct-agonist activation of PPAR-α[87], and the inherent complexity of adapting natural lipid molecules to therapeutic usage (e.g., quick metabolism, high plasma protein binding, low solubility). An alternative strategy is to create small drug-like molecules that enhance intrinsic PPAR-α signaling by interrupting the NAAA-mediated degradation of OEA and PEA [53,83,86]. Since the main effect of NAAA inhibitors is to normalize FAE signaling in injured or inflamed tissues, rather than indiscriminately activating PPAR-α throughout the body, this approach may offer a degree of cellular selectivity that might translate into greater efficacy and safety.

Bioactive products of PUFA oxygenation: return to nociceptive homeostasis

The lipid profile of an exudate – the fluid that filters from the blood into a site of inflammation – undergoes dramatic changes in the course of a self-limited inflammatory reaction. As this reaction phases out into resolution, the initial complement of potent proalgesic and proinflammatory lipid mediators – prostanoids such as PGE_2 and PGI_2, and leukotrienes such as LTB_4 – is replaced by a different set of lipid molecules that promote the return of the inflamed tissue to homeostasis by stopping neutrophil infiltration, accelerating the clearance of cellular debris, enhancing the antimicrobial activity of epithelial cells, and stimulating wound healing[88]. As part of their effort to restore homeostasis, these bioactive lipids may also normalize nociceptive signaling by binding to selective receptors present on the surface of neurons and immune cells.

Epoxygenated fatty acids

The cytochrome P_{450} family of enzymes catalyzes the oxygenation of various long-chain PUFAs – including arachidonic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – each giving rise to a distinct group of bioactive epoxide-containing fatty acids (EpFAs), which act near their site of synthesis to regulate vascular tone, kidney function and inflammation[89] (Figure 6). The EpFAs are quickly converted by the enzyme soluble epoxide hydrolase (sEH) into 1,2-dihydroxy-fatty acids, which are either less active or have different activities than the parent molecules. The discovery of potent and selective inhibitors of intracellular sEH activity has allowed researchers to explore the functions of endogenously produced EpFAs in animal models of inflammatory and neuropathic pain. These investigations have shown that systemic administration of sEH inhibitors or local injection of synthetically prepared EpFAs attenuate pain-related responses evoked by LPS or carrageenan in rats[89]. A more recent study has examined the impact of three structurally different sEH inhibitors in rats rendered hypersensitive to pain stimuli by treatment with streptozotocin (STZ), a toxin that causes type-1 diabetes by selectively killing insulin-secreting β-cells in the pancreas[90]. The inhibitors reduce mechanical allodynia in STZ-treated rats, without influencing normal nociceptive responding in healthy animals, and concomitantly increase the levels of non-metabolized EpFAs in plasma and spinal cord. Even though the receptor(s) responsible for these effects are still unknown, the marked anti-hyperalgesic properties of sEH inhibitors support a role for endogenous EpFAs in the modulation of nociceptive signaling, and identify sEH as a potential target for analgesic drugs[89] (Table 2).

Lipoxins, resolvins and allied compounds

The lipoxins are generated through the sequential enzyme-mediated oxygenation of membrane-derived arachidonic acid[88] (Figure 6). Their biosynthetic routes are multiple and complex, and have been reviewed in detail elsewhere[91]. One important point to mention here is that several key pathways of lipoxin biosynthesis involve the cooperation among different cell types that physically contact one another during an inflammatory response. For example, neutrophils produce lipoxin A_4 (LXA_4), one of the best-known members of this class, utilizing as substrate a short-lived metabolite of arachidonic acid released from nearby macrophages[88]. This unusual mode of production, called transcellular biosynthesis, reflects the physiological need to match the complement of bioactive lipid mediators present at an inflammatory site with the varying cellular profiles progressively recruited to that site. The anti-inflammatory and pro-resolution effects of LXA_4 are mediated by G protein-coupled ALX/FPR2 receptors (also called formyl peptide receptor-like 1, FPRL-1)[88], but the attenuated nociception that accompanies such effects might also involve CB_1 cannabinoid receptors. Pharmacological studies suggest that LXA_4 acts as a positive allosteric CB_1 modulator, potentiating CB_1-mediated responses evoked by anandamide *in vitro* and *in vivo* and increasing the ability of this compound to displace the binding of a radiolabeled CB_1 ligand to mouse brain membranes[92].

Resolvins and protectins are produced, like the lipoxins, through the multi-step oxygenation of membrane-derived PUFAs; however, they do not utilize as precursor arachidonic acid, an omega-6 PUFA, but rather the omega-3 PUFAs, EPA and DHA (Figure 6). EPA gives rise

to E-series resolvins such as resolvin E_1 (RvE_1), while DHA is converted into D-series resolvins, such as resolvin D_1 (RvD_1), as well as protectin D_1[91]. Additionally, in human macrophages and platelets, DHA may be transformed by 12-lipoxygenase to form maresin 1 (Figure 6)[91]. The pro-resolving and immune-modulating properties displayed by these bioactive lipids are impressive[88], but are not sufficient to explain the array of anti-nociceptive effects exerted by some of them. For example, intraplantar administration of RvE_1 or RvD_1 in mice suppresses pain-related responses evoked by a broad variety of proalgesic stimuli – including formalin, capsaicin, CFA, TNF-α and PGE_2[93,94]. RvE_1 and RvD_1 are also highly effective at reducing nociceptive behaviors caused by skin incision or muscle retraction in mice and rats, two models of human post-operative pain[94]. The receptor mechanisms that mediate these analgesic actions are only partially understood. There is evidence that the Gi-coupled receptor ChemR23, which also binds the adipokine chemerin, is involved in the effects of RvE_1. This idea is consistent with the presence of ChemR23 in DRG neurons and the ability of chemerin to mimic the anti-nociceptive actions of RvE_1[93]. RvD_1 activates the LXA_4 receptor, ALX/FPR2, but the functional significance of this interaction is still unclear[95].

With their high pharmacological potencies and short life spans, lipoxins, resolvins and allied molecules fit the profile of ‘soft drugs’ – agents that undergo quick and predictable deactivating metabolism after having accomplished their therapeutic goal. Not surprisingly, therefore, pharmaceutical development in this area is focused on the use of resolvin analogs for the topical treatment of localized inflammatory conditions, such as dry eye (http://WWW.RESOLVYX.COM/products/index.asp). Nevertheless, there may be several pain-related applications for resolvin-based soft drugs, including the intra-articular treatment of pain arising from knee surgery or osteoarthritis.

Conclusions

The evidence presented in this article allows us to conjecture that bioactive lipid mediators regulate the access of nociceptive information to the CNS at three distinct stages. In healthy tissues, the tonic release of endogenous PPAR-α agonists, such as PEA and OEA, may help set the threshold for nociception by regulating the baseline transcriptional activity of the NFκB complex and the opening of membrane ion channels in primary sensory afferents and nearby host-defense cells. After an injury has occurred, the temporary interruption of OEA and PEA biosynthesis caused by cell damage and infection may disable the inhibitory influence exerted by these lipid messengers, allowing inflammation to unfold and nociceptive thresholds to decrease. At the same time, the localized on-demand formation of the endocannabinoids, anandamide and 2-AG, may mitigate the effects of exogenous and endogenous proalgesic agents by attenuating nociceptor excitability and contrasting local pro-inflammatory signals. Lastly, as the response to tissue damage moves toward its resolution phase, a wave of analgesic products of oxidative PUFA metabolism, such as lipoxins and resolvins, may help normalize nociceptive responses in the healing tissue. It is likely, but remains to be proven, that the correct deployment of this signaling program is subject to local and systemic regulatory mechanisms. At the local level, lipid mediators released in the early phases of injury may govern the generation of substances acting at later stages: this has been demonstrated for proresolving lipoxins, for example, the production of

which is partly controlled by proalgesic Cox-2-derived prostaglandins[88]. At the system level, neural and hormonal signals may help integrate peripheral and central gating mechanisms during conditions of altered nociception, such as acute stress or body-wide inflammation. The autonomic nervous system, which modulates the biosynthesis of endogenous PPAR-α agonists in some peripheral tissues (e.g., white adipose)[96], might play an important role in this context[97]. In addition to addressing such issues, future studies will also need to determine whether errors in the correct unfolding of the intrinsic analgesic mechanism outlined here might contribute to the development and maintenance of pathological pain states. Aided by continuing advances in mass spectrometry-based lipidomics, such studies may lead to the discovery of novel analgesic lipids and the development of innovative medicines that effectively control pain without interfering with central neurotransmission.

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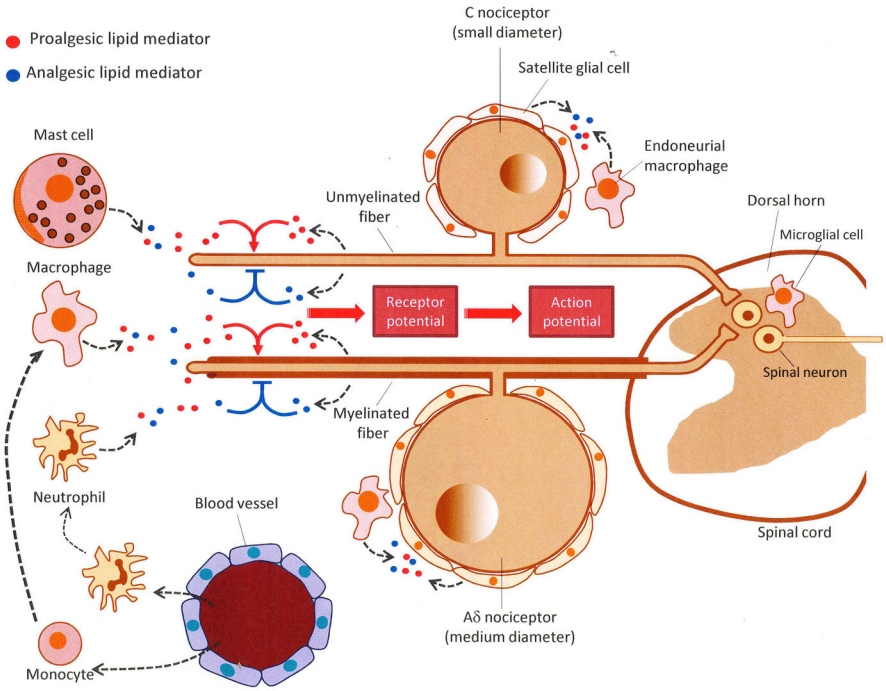

Figure 1. Peripheral gating of nociceptive signals by endogenous lipid mediators

From the dorsal root ganglia (DRG), where the cell bodies of C and Aδ sensory neurons are located, nociceptive fibers travel both peripherally (to innervate skin, bone, viscera and other internal organs) and centrally (to form synapses with neurons in the dorsal horn of the spinal cord). Peripheral nociceptor terminals respond to acute injury directly, through cell-surface receptors specialized in detecting harmful physical and chemical stimuli, as well as indirectly, through receptors that sense proalgesic (pain-inducing) factors released by cell damage, inflammation, neuropathy or tumor growth (e.g., nucleotides, peptides and various lipid mediators). These sensitizing effects may be modulated by analgesic lipid-derived mediators generated at different stages of the tissue response to damage and under the control of neural and hormonal signals (e.g., autonomic nervous system, circulating corticosteroids). Some of these compounds may be released from the sensory nerve endings themselves, while others may derive from local host-defense cells, such as resident mast cells and macrophages, epidermal keratinocytes and blood-borne leukocytes recruited to the injury site. Nociceptive nerve endings integrate the inputs provided by proalgesic (excitatory) and analgesic (inhibitory) signals, converting them into graded receptor potentials and, eventually, all-or-none action potentials. Alterations in the production of analgesic lipid mediators may occur not only at the nerve endings of primary afferents, but also within the DRG and dorsal horn of the spinal cord, with neurons and other cells (e.g., satellite cells, endoneurial macrophages, microglia) likely contributing to this response.

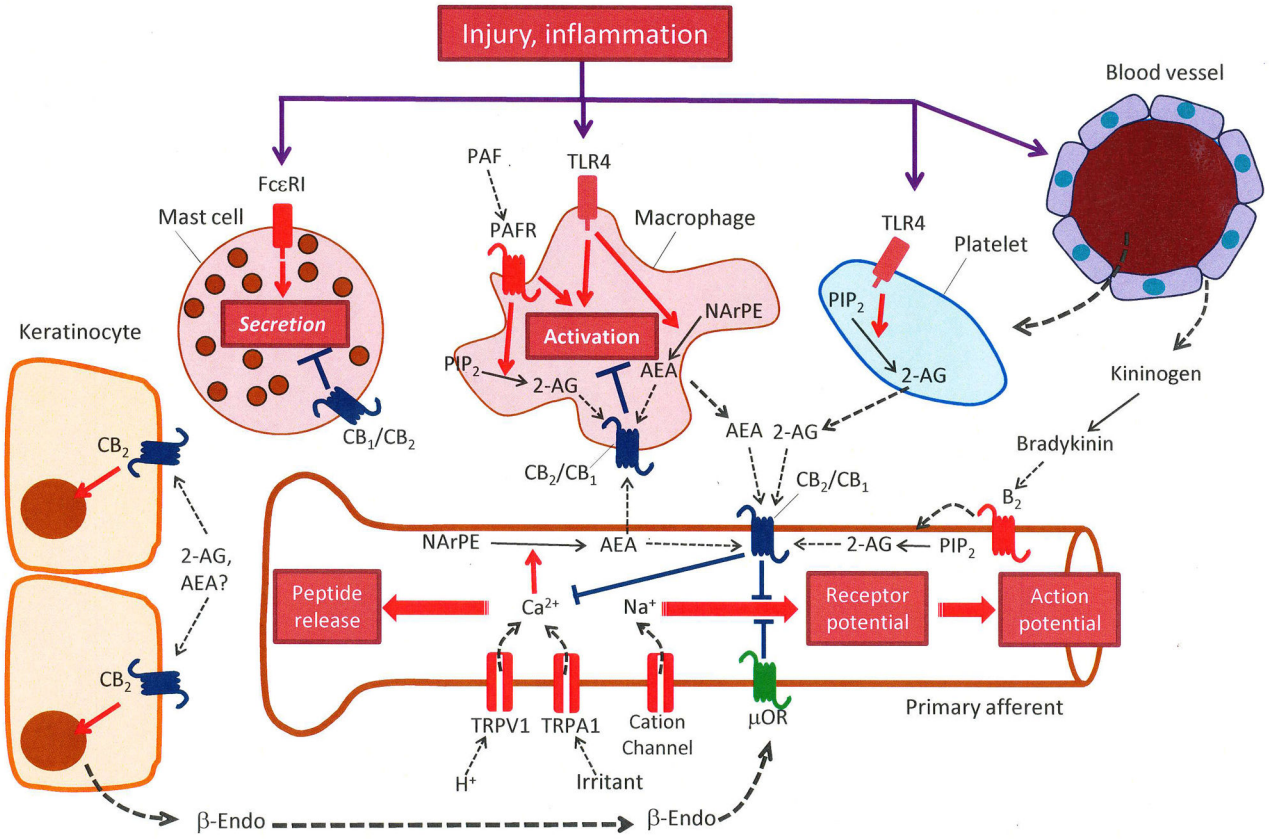

Figure 2. Endocannabinoid control of peripheral nociception

Acute tissue injury and inflammation stimulate the localized release of anandamide (AEA) and 2-arachidonoyl-*sn*-glycerol (2-AG) from primary sensory afferents and neighboring non-neural cells – including macrophages, mast cells and blood-derived platelets. AEA release from its membrane precursor, *N*-arachidonoyl-phosphatidylethanolamine (NArPE), may be initiated by calcium entry in nociceptors and/or stimulation of Toll-like receptor-4 (TLR4) in macrophages. 2-AG mobilization from phosphatidylinositol-4,5-bisphosphate (PIP_2) may be stimulated by activation of B_2 bradykinin receptors in nociceptors, platelet activating factor (PAF) receptors in macrophages, and/or TLR4 in platelets. Newly formed AEA and 2-AG activate CB_1 and/or CB_2 cannabinoid receptors modulating membrane excitability and calcium signals in primary afferent terminals, stimulating the secretion of β-endorphin (β-endo) from keratinocytes (which binds to μ-opioid receptors [μOR] on nociceptors), and inhibiting macrophage activation and mast-cell degranulation. FcεRI is the high affinity immunoglobulin E receptor.

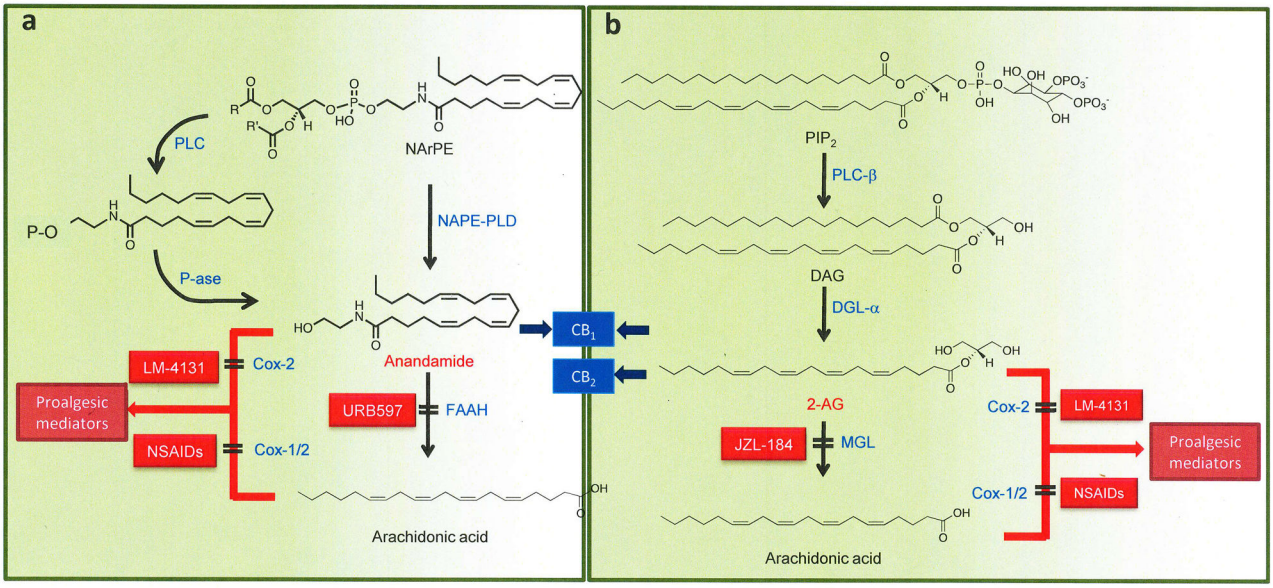

Figure 3. Formation and deactivation of endocannabinoid lipids

(A) Anandamide is generated by hydrolysis of the membrane lipid, *N*-arachidonoyl-phosphatidylethanolamine (NArPE), which is catalyzed in neurons by a phospholipase D that selectively recognizes *N*-acylated species of PE (NAPE-PLD). The concentrations of NArPE and anandamide are very low in resting neurons, but quickly increase in response to neural activity and/or neurotransmitter receptor occupation. In macrophages, activation of Toll-like receptor 4 (TLR4) by the bacterial endotoxin lipopolysaccharide stimulates an as-yet-uncharacterized phospholipase C (PLC), which converts NArPE into phospho-anandamide. This intermediate is dephosphorylated by a phosphatase (P-ase) to form anandamide. Endogenously produced anandamide modulates nociception by binding to CB_1 cannabinoid receptors. In both neurons and macrophages, anandamide is converted into arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH), which is selectively blocked by inhibitors such as the O-arylcarbamate derivative, URB597. Anandamide can also be transformed by cyclooxygenase-2 (Cox-2) into proalgesic prostamides, a reaction that is prevented by substrate-selective inhibitors such as LM-4131. Non-steroidal anti-inflammatory drugs (NSAIDs) stop the conversion of arachidonic acid into prostaglandins by inhibiting both Cox-1 and Cox-2 activities.

(B) 2-arachidonyl-*sn*-glycerol (2-AG). G_q-coupled receptors and Toll-like receptor-4 (TLR4) stimulate phospholipase C-β (PLC-β), which converts phosphatidylinositol-4,5-bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG). DAG is hydrolyzed by diacylglycerol lipase-α (DGL-α) to yield 2-AG, which inhibits nociceptive responses by activating CB_1 and CB_2 cannabinoid receptors. Monoacylglycerol lipase (MGL) terminates 2-AG signaling by hydrolyzing this glycerol ester into arachidonic acid and glycerol. 2-AG and arachidonic acid may be transformed by cyclooxygenase (Cox-1) and/or Cox-2 into various families of proalgesic lipid mediators, which include PGE_2 glycerol ester (derived from 2-AG) and PGE_2 (derived from arachidonic acid).

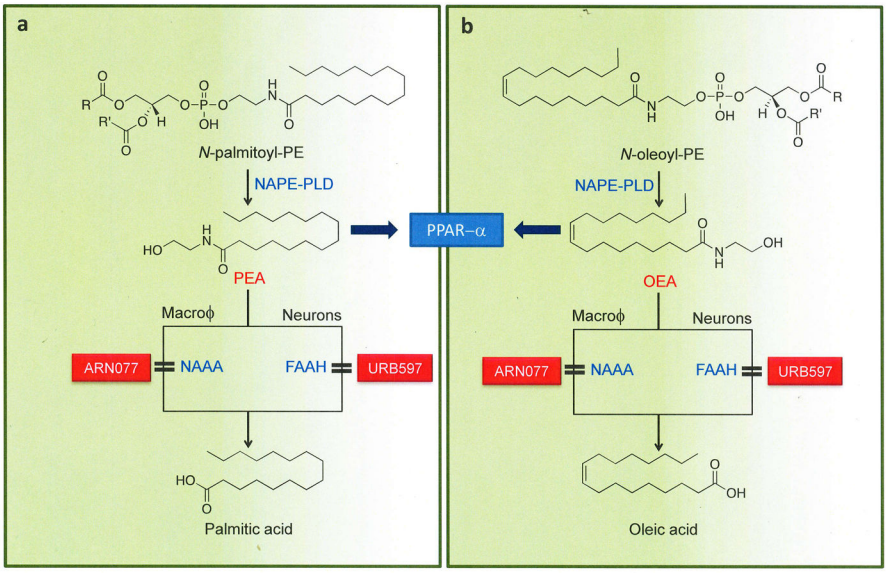

Figure 4. Formation and deactivation of OEA and PEA in macrophages

The fatty acid ethanolamides (FAEs), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), are generated by cleavage of two distinct membrane phospholipids, *N*-palmitoyl-phosphatidylethanolamine (PE) and *N*-oleoyl-PE. This reaction is catalyzed by the same N-acyl PE-specific phospholipase D (NAPE-PLD) that mediates anandamide release in neurons. Under basal conditions, macrophages produce substantial amounts of *N*-palmitoyl-PE, *N*-oleoyl-PE and the corresponding FAEs, which inhibit inflammatory responses and increases nociceptive thresholds by activating PPAR-α. In macrophages and, possibly, primary sensory neurons of the DRG, FAEs are converted into fatty acid and ethanolamine by the cysteine hydrolase, *N*-acylethanolamine acid amidase (NAAA), which is selectively inhibited by the threonine β-lactone ARN077. In neurons and other cells, FAE hydrolysis is primarily mediated by fatty acid amide hydrolase (FAAH), which is blocked by various classes of inhibitors, including the O-aryl carbamate URB597.

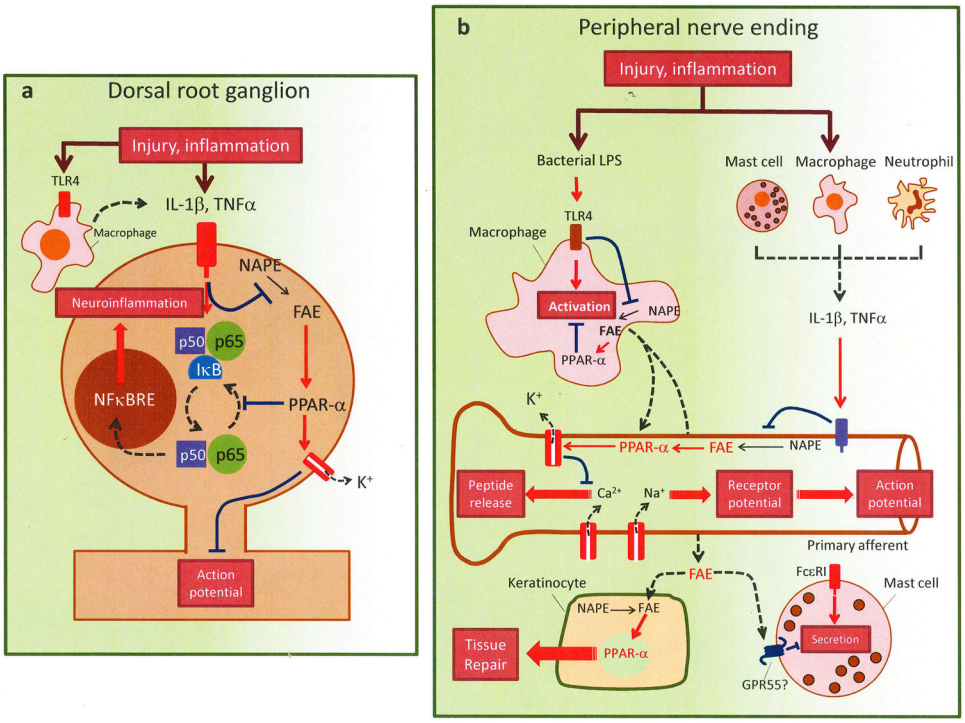

Figure 5. Control of peripheral nociception by endogenous PPAR-α agonists

In the absence of stimulation, macrophages and dorsal root ganglia (DRG) neurons produce substantial amounts of oleoylethanolamide and palmitoylethanolamide. These fatty acid ethanolamides (FAEs) exert a tonic inhibitory control over macrophage activity and nociceptor excitability by recruiting PPAR-α-dependent mechanisms, which may include opening of BK_{ca} and IK_{ca} potassium channels and regulation of the nuclear factor-κB (NFκB) transcription complex (composed of p50, p65 and inhibitor of κB-α [IκB-α]). After tissue damage or during inflammation, microbial toxins, such as lipopolysaccharide (LPS), and/or immune-derived cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 1β (IL1β), suppress FAE biosynthesis and stop FAE signaling at PPAR-α, temporarily disabling its inhibitory function. These molecular events may take place both in (A) neurons and other cells of the DRG, and (B) peripheral nerve endings of primary sensory neurons. In the periphery, FAEs may also influence tissue repair, by engaging PPAR-α in keratinocytes, and mast cell degranulation, possibly by interacting with G protein-coupled GPR55 receptors. Abbreviation: NFκBRE: NFκB responsive elements.

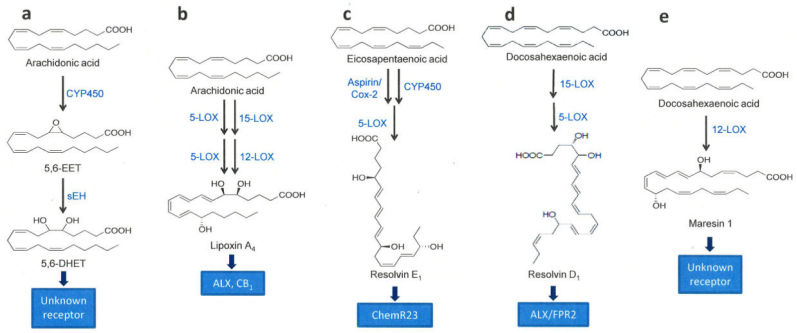

Figure 6. Biosynthetic pathways for analgesic PUFA-derived mediators

This simplified scheme illustrates the biosynthetic pathways for representative polyunsaturated fatty acid (PUFA)-derived mediators involved in the peripheral control of nociception. (A) Biosynthesis and degradation of 5,6-epoxyeicosatrienoic acid (5,6-EET), a member of the epoxygenated fatty acid (EpFA) family of lipids. Cytochrome P_{450} converts arachidonic acid into 5,6-EET, which is subsequently hydrolyzed by soluble epoxide hydrolase (sEH) into 5,6-dihydroxyeicosatrienoic acid. (B–D) Biosynthetic pathways for (B) lipoxin A_4, (C) resolvin E_1, and (D) maresin 1. Abbreviations: LOX, lipoxygenase, Cox-2, cyclooxygenase-2.

Table 1

Analgesic lipid-derived mediators produced at sites of acute tissue injury, inflammation and neuropathy.

Lipid Mediator	Metabolic Precursor	Biosynthetic Enzyme	Molecular Target	Select Ref.
Anandamide	N-arachidonoyl-PE	NAPE-PLD	CB_1	30
2-Arachidonoyl-*sn*-glycerol	PIP_2	PLC-β and DGL-α	CB_1 and CB_2	5
Palmitoyl ethanolamide	N-palmitoyl-PE	NAPE-PLD	PPAR-α	30, 72
Oleoyl ethanolamide	N-oleoyl-PE	NAPE-PLD	PPAR-α	69, 72
Resolvin D_1	DHA	15-lipoxygenase and 5-lipoxygenase	GPR32	93
Resolvin E_1	EPA	Cytochrome P_{450} and 5-lipoxygenase	ChemR23	93
Lipoxin A_4	Arachidonic acid	15-lipoxygenase and 5-lipoxygenase	ALX, CB_1	88, 92
Epoxyeicosa-trienoic acids	Arachidonic acid	Cytochrome P_{450}	Unknown	89

Abbreviations: ALX, lipoxin A_4 receptor; CB_1 and CB_2, type-1 and -2 cannabinoid receptors; ChemR23, Chemerin receptor-23; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GPR32, G protein-coupled receptor-32; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-selective phospholipase D; PE, phosphatidylethanolamine; PIP_2, phosphatidylinositol-4,5-bisphosphate; PPAR-α, peroxisome proliferator-activated receptor type-α; TRPV1, transient receptor potential vanilloid-1 channel.

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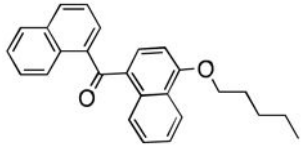
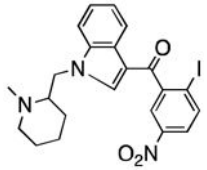
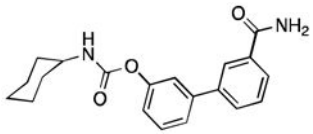
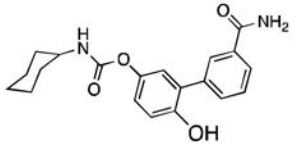
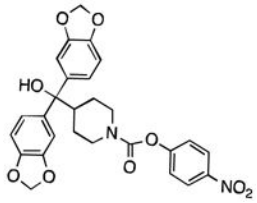
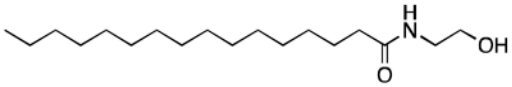
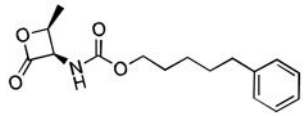
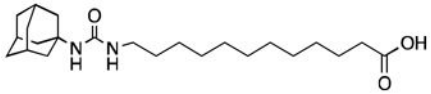
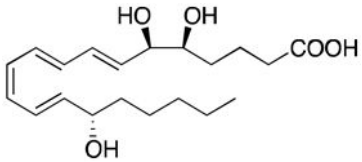
Table 2

Peripheral lipid-mediated signaling as a source of new analgesic medicines

The current therapy of chronic pain relies heavily on opiates (e.g., morphine and oxycodone) and anticonvulsants (e.g., gabapentin and pregabalin), two classes of drugs that primarily act on neural circuits of the brain and spinal cord. These agents help many, but their use is associated with central side effects and abuse potential. The existence of peripheral lipid-mediated mechanisms that regulate the flow of nociceptive signals to the central nervous system (CNS) offers the opportunity to develop medications that control pain without producing undesired central effects. Activation of CB_1 cannabinoid receptors in the brain is psychotropic, but peripherally restricted CB_1 agonists or globally active CB_2 agonists (e.g., AM1241) reduce nociceptive responses in rodent models without eliciting overt signs of cannabinoid intoxication. Marked anti-nociceptive actions, mediated by CB_1 and/or CB_2 receptors, are also observed with inhibitors of the endocannabinoid-hydrolyzing enzymes, fatty acid amide hydrolase (FAAH, e.g. URB597 or URB937) and monoacylglycerol lipase (MGL, e.g. JZL184). PPAR-α-mediated antinociception can be achieved either using direct PPAR-α agonists, such as the endogenous ligand palmitoylethanolamide (PEA), or inhibitors of the PEA-hydrolyzing enzyme *N*-acylethanolamine acid amidase (NAAA, e.g. ARN077). Similar approaches may be used to exploit the analgesic properties of endogenous bioactive lipids generated by oxygenation of polyunsaturated fatty acids (PUFAs): inhibition of soluble epoxide hydrolase (sEH), which converts epoxygenated PUFAs into inactive dihydroxy-acids, and administration of resolvins both produce marked antinociception in rodent models.

Molecular Target	Representative Probe	Chemical Structure	Select Ref.
Peripheral CB_1 receptor	Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone		32
CB_2 receptor	AM1241		41
FAAH	URB597		98

Molecular Target	Representative Probe	Chemical Structure	Select Ref.
Peripheral FAAH	URB937		57
MGL	JZL184		99
PPAR-α	Palmitoylethanolamide		76
NAAA	ARN077		100
Soluble EH	*t*-AUCB		89
CB_1 receptor (Allosteric Modulation)	Lipoxin A_4		92

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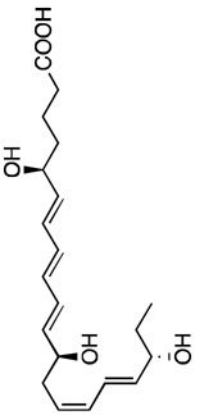
Molecular Target	Representative Probe	Chemical Structure	Select Ref.
ChemR32	Resolvin E_1		88