Trends in Molecular Medicine

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Review

Endocannabinoid and Prostanoid Crosstalk in Pain

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Interfering with endocannabinoid (eCB) metabolism to increase their levels is a proven anti-nociception strategy. However, because the eCB and prostanoid systems are intertwined, interfering with eCB metabolism will affect the prostanoid system and inversely. Key to this connection is the production of the cyclooxygenase (COX) substrate arachidonic acid upon eCB hydrolysis as well as the ability of COX to metabolize the eCBs anandamide (AEA) and 2-arachidonoylglycerol (2-AG) into prostaglandin-ethanolamides (PG-EA) and prostaglandin-glycerol esters (PG-G), respectively. Recent studies shed light on the role of PG-Gs and PG-EAs in nociception and inflammation. Here, we discuss the role of these complex systems in nociception and new opportunities to alleviate pain by interacting with them.

Highlights

The eCB and PG systems have been studied separately for a long time. Increasing evidence has revealed a strong connection between the two systems, supporting a change in paradigm whereby both systems are studied together.

eCBs and PGs are key players in nociception, acting via peripheral, spinal, and supraspinal mechanisms. As we suggest here, targeting multiple enzymes and receptors will represent new opportunities to treat pain, using the cooperative effects of modulating both the eCB and prostanoid systems.

Increasing evidence supports the bioactivity of PG-EA and PG-Gs, including in inflammatory and painful situations.

Bioactive Lipids: Important Players in Nociception

Pain is a common symptom that accompanies several pathologies and affects patients' quality of life. Different mechanisms lead to pain (Box 1) and constitute the basis for drug development. Nevertheless, current medications, such as opioids or anti-inflammatory drugs, are reaching limitations in the management of chronic and neuropathic pain. The identification of new therapeutic strategies requires a better understanding of the role of endogenous mediators during pain processes.

Among endogenous mediators, **bioactive lipids** (see Glossary) have been described as crucial mediators during **hyperalgesia**, from the initiation to the maintenance of peripheral and central **sensitization**. In this context, the **endocannabinoid (eCB)** and **prostanoid** systems are of particular interest. The two main eCBs, *N*-arachidonoylethanolamine (AEA, anandamide) and 2-arachidonoylglycerol (2-AG), are involved in numerous pathophysiological processes (reviewed in [1]), including inflammation and **nociception**. As we discuss here, several studies have shown that eCBs and related lipids [such as ***N*-acylethanolamines** (NAEs) and **monoacylglycerols**] reduce pain in acute and chronic models of inflammatory and neuropathic pain. Multiple studies evidenced alterations in these bioactive lipid levels during inflammation and pain in both animal models and patients. Similarly, prostanoids [e.g., prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2)] are well-known lipid mediators for which a clear role in inflammatory pain has been described.

More recently, an interconnection between these two bioactive lipid systems (i.e., eCBs and prostanoids) [2–6] has emerged. Indeed, AEA and 2-AG can be metabolized by the enzymes producing the prostanoids [namely cyclooxygenase (COX)-2) and the prostaglandin (PG) synthases] (Figure 1). This metabolic pathway produces prostaglandin glycerol esters (PG-Gs) from 2-AG and prostaglandin ethanolamides (PG-EAs; also known as prostamides) from AEA. These eCB metabolites are increasingly described as lipid mediators in their own right, involved notably in inflammation and pain [2,6–10]. Here, we review the complex interplay between the eCB and prostanoid systems and their implications in the (patho)physiology of pain.

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Box 1. Pain Classification and Processing

Pain Classification

The International Association for the Study of Pain (IASP) defines pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage' [115]. Pain can be classified into three types that differ by the site, stimulus type, and clinical setting in which they are encountered: (i) nociceptive pain arises from the activation of nociceptors (a high-threshold sensory receptor of the peripheral nervous system capable of transducing and encoding noxious stimuli). This transient pain type is vital and represents an alarm system informing of the presence of (potential) harmful stimulus. Nociceptors are divided into three main classes: thermal, mechanical, and chemical; (ii) inflammatory pain is triggered by receptors expressed by nociceptive neurons that detect inflammatory mediators released during an inflammatory event, such as tissue injury; and (iii) neuropathic pain arises from lesions or diseases affecting the CNS and peripheral nervous system. It can be encountered in the context of trauma (nerve lesions), metabolic disturbances (diabetic neuropathy), or infection (postherpetic neuralgia), or be drug induced (chemotherapy).

Pain Processing

The perception of noxious stimuli activates primary sensory neurons. Cation channels gated by pressure, temperature, and chemical ligands giving rise to action potentials mediate this activation. The frequency and intensity of these action potentials reflect the intensity and duration of the noxious stimuli. The DRG contain the cell bodies of primary sensory neurons responsible for the transduction, modulation, and transmission of signals from the periphery to the dorsal horn of the spinal cord. Within the dorsal horn, the primary afferent signals activate secondary neurons. Once relayed and modulated by neurons of the dorsal horn, noxious signals are transduced to the thalamus and the cortex by the spinothalamic tract to supraspinal structures, such as the parabrachial nucleus, periaqueductal gray, thalamus, amygdala, and the somatosensory and prefrontal cortices (see Figure 2 in main text). The descending pain pathway from the hypothalamus, periaqueductal gray, and rostroventral medulla reaches the dorsal horn of the spinal cord through the dorsolateral funiculus. These supraspinal projections interact with afferent fibers and interneurons and actively modulate noxious signals from the periphery.

Satellite glial cells (in the DRG), microglia and astrocytes (in the CNS), Schwann cells (in the periphery), and immune cells all have a pivotal role in pain perception and are directly implicated in peripheral and central sensitization processes. eCBs have been shown to modulate several of these processes, as illustrated in Figure 2, main text.

Metabolism of Endocannabinoids and Prostanoids: COX-2 as a 'Metabolic Hub'

The eCB 2-AG is synthetized from diacylglycerols upon the action of diacylglycerol lipases (DAGL) α and β. The main catabolic pathway of 2-AG is its hydrolysis into arachidonic acid (AA) and glycerol. Three enzymes are responsible for 2-AG hydrolysis: monoacylglycerol lipase (MAGL), also known as monoglyceride lipase (MGL), and α/β hydrolase domain 6 and 12 (ABHD6 and ABHD12) [11–13]. Although MAGL largely controls 2-AG levels, the contribution of the different enzymes is likely tissue and condition dependent [11].

By contrast, AEA and related NAEs are synthetized from *N*-acylphosphatidylethanolamines (NAPEs) via several pathways involving at least two enzymes. The canonical pathway for NAE synthesis involves *N*-acyltransferase activity followed by the action of a NAPE-preferring phospholipase D (NAPE-PLD) [14]. NAEs are hydrolyzed by two enzymes, fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), into the corresponding fatty acid and ethanolamine [14].

Thus, hydrolysis of both 2-AG and AEA provides AA that can be metabolized by COX-1 and COX-2 into PGs. In this context, 2-AG was described as a major source of AA in the brain, liver, and lungs, while in the gut and the spleen, cytosolic phospholipase A_2 ($cPLA_2$)-mediated AA production appears to be the predominant pathway [15]. PG synthesis relies on sequential enzymatic reactions by COXs and specific PG synthases and occurs on demand, depending on enzyme expression levels and activity. Metabolism of AA by COXs is considered the pivotal step in PG production [16]. It is generally admitted that COX-1 is a constitutively expressed enzyme, whereas COX-2 is induced during inflammation. Major exceptions to this paradigm are the kidney, gastrointestinal tract, thymus, and central nervous system (CNS), where COX-2 is constitutively expressed [17].

Glossary

Bioactive lipids: a lipid is considered bioactive when variation in its levels leads to functional consequences through the activation of receptors as opposed to simply a source of energy or a structural cell component.
Dorsal horn of the spinal cord: the first relay of primary afferent fibers from muscles, skin, and viscera. It is a bilateral structure situated on the dorsolateral side of the spinal cord and is part of the gray matter. This structure is also actively involved in the processing of nociceptive signals because it is the region where the descending pain pathway modulates inputs from the periphery.
Dorsal root ganglia (DRG): anatomical structures regrouping the cell bodies of pseudounipolar neurons that relay peripheral sensory signals to the spinal cord. They are located in the intervertebral foramina.
Endocannabinoids (eCBs): lipid mediators that bind to and activate the cannabinoid receptors (CB_1 and CB_2). Thus, a *bona fide* eCB should be endogenously produced and bind at least one of the two cannabinoid receptors.
Hyperalgesia: increased pain arising from stimuli that normally evoke pain.
Monoacylglycerols: esters of a fatty acid with glycerol. 2-Arachidonoylglycerol is an eCB monoacylglycerol. 2-Palmitoylglycerol and 2-oleoylglycerol (a ligand for the GPR119 receptor) are examples of monoacylglycerols not binding the cannabinoid receptors. Monoacylglycerols are produced from diacylglycerols via the action of DAGL α or β.
***N*-acylethanolamines (NAEs):** amides of a fatty acid and ethanolamine. AEA is an eCB, while PEA and *N*-oleoylethanolamine (OEA) are two examples of NAEs that do not bind the cannabinoid receptors. The major pathway leading to NAEs involves an *N*-acyltransferase (NAT) producing *N*-acylphosphatidylethanolamines followed by the release of NAEs by NAPE-PLD.
Nociception: sensory process of encoding a 'potential threat' signal (heat, cold, trauma, etc.) by the nervous system.
Prostanoids: a family of lipid mediators generated by the action of COX on AA.
Sensitization: characterized by increased sensitivity of neurons to signals and results in the formation of

Besides hydrolysis, another major fate of 2-AG and AEA is their metabolism by COX-2, resulting in the production of PG-Gs and PG-EAs, respectively. Indeed, due to their arachidonoyl moiety, these eCBs are COX-2 substrates [18]. Although COX-1 was initially reported as unable to metabolize eCBs, some reports have also implicated COX-1 in PG-G and PG-EA production [8,19]. Further hydrolysis of PG-Gs and PG-EAs may release PGs in some settings [6,20]. However, while PG-G hydrolysis can be catalyzed by several enzymes (i.e., MAGL, ABHD6, LyPLA$_2$, and CES1-2 [21–23]), no specific enzyme has been described for PG-EA hydrolysis. Accordingly, PG-EAs are reported to be metabolically more stable than PG-Gs [24].

Therefore, COX-2 is to be considered as a metabolic hub that can metabolize AA and eCBs and generate PGs or PG-Gs and PG-EAs (Figure 1) with complex and intertwined kinetics. Indeed, depending on substrate concentration and colocalization of other enzymes, substrate competition occurs at every step of prostanoid synthesis [25,26]. Hence, measurement of both enzyme expression and lipid levels is needed to decipher the involvement of PGs, PG-EAs, and PG-Gs in pathophysiological processes, including nociception. However, PG-G and PG-EA quantification *in vivo* remains a challenge, with few studies reporting their levels in pathophysiological conditions [4,5,7–9]. Indeed, while PG-Gs and PG-EAs were quantified *in vitro* and in intact cells [2,4,19,27], their quantification *in vivo* has remained elusive and has sometimes necessitated rather drastic conditions. For instance, increased PG-G levels were reported in the brains of mice overexpressing COX-2 and receiving a MAGL inhibitor [28], while PG-EA levels were quantified *in vivo* in control and FAAH$^{-/-}$ mice receiving an intravenous injection of AEA [29]. Nevertheless, a few studies measured these lipids in pathophysiological conditions. PGE$_2$-G levels were quantified in **dorsal root ganglia** (DRG) of mice by using ten DRGs per mouse [5] and in the rat hind paw [7], while the PGD$_2$-G metabolite 15d-PGJ$_2$-G was quantified in the colon of mice with colitis [9]. PGF$_{2\alpha}$-EA levels were increased in the spinal cord of rats receiving carrageenan to induce knee inflammation (Box 2), although the levels of PG-Gs and other PG-EAs were below detection limits [8].

This overview supports the key role of the enzymes of the prostanoid system in eCB metabolism and actions and emphasizes the need to quantify these bioactive lipids when characterizing the effects of inhibitors of the enzymes described earlier.

Endocannabinoids and Prostanoids in Pain Modulation

Endocannabinoids

The different components of the eCB system (receptors and enzymes) are localized throughout the nociceptive and modulatory pathways and their expression is altered in several pain states (Figure 2). The link between pain and the eCB system is further supported by the increased eCB levels found in several models [30–33]. The implication and modulation of the eCB system in pain as well as the molecular mechanisms mediating the effects of eCBs have been extensively studied (reviewed in [34]) and are briefly summarized here.

In DRG, eCBs are increased after spinal nerve ligation (SNL, Box 2)-induced neuropathic pain [35]. 2-AG levels were also elevated in the spinal cord of rats that underwent plantar incision, a model of postoperative pain [33]. In the chronic constriction injury (CCI, Box 2) model, eCB levels were increased in the spinal cord, periaqueductal gray (PAG) and rostroventral medulla (RVM) of rats 3 and 7 days after surgery [32]. This increase was thought to be a homeostatic response aimed at decreasing pain. Supporting this hypothesis, decreased AEA levels were reported to contribute to pain maintenance in a mouse model of bone cancer [36]. Moreover, in a model of knee arthritic pain, 2-AG levels were decreased in mouse midbrain and restored by hyperalgesia reversal [37].

action potentials in response to low-intensity inputs that do not usually trigger them. Sensitization can be peripheral (peripheral nervous system) and/or central (CNS) and occurs during inflammatory or neuropathic pain processes.

Substrate-selective inhibitors: chemical compounds able to block the activity of an enzyme towards one of its substrates but not the others. In the context of this review, substrate-selective inhibitors of COX-2 inhibits its action on 2-AG and AEA (thus preventing the production of PGH$_2$-G and PGH$_2$-EA, respectively) but not on AA (thus allowing the synthesis of the prostaglandin precursor PGH$_2$).

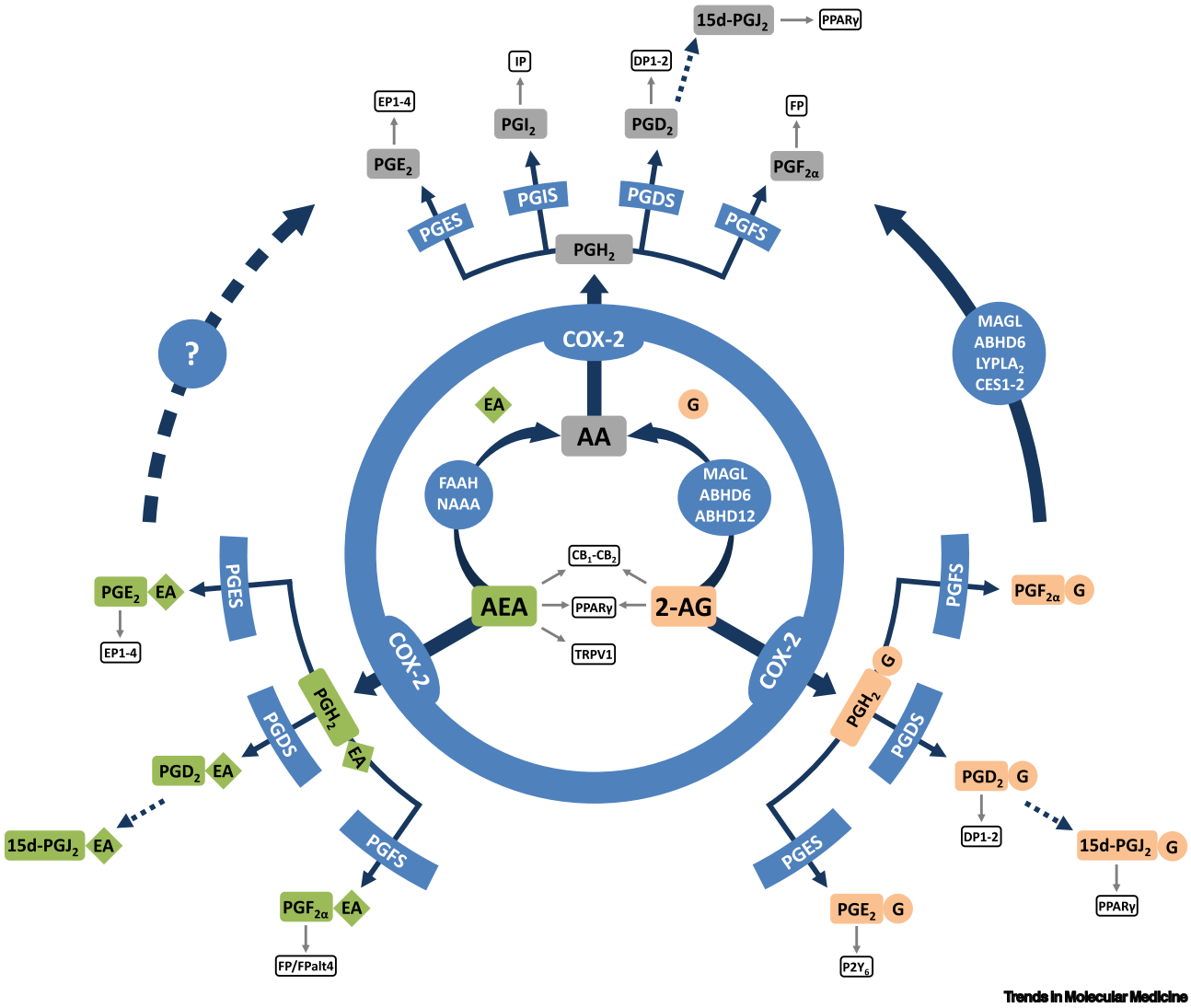

Figure 1. Simplified Endocannabinoid (eCB) Metabolism. In the center, the eCBs anandamide (AEA, green) and 2-arachidonylglycerol (2-AG, orange) bind to cannabinoid receptors 1 and 2 (CB_1 and CB_2) and peroxisome proliferator-activated receptor (PPAR)γ. Furthermore, AEA (an endovanilloid) binds to transient receptor potential cation channel subfamily V member 1 (TRPV1). Hydrolysis of eCBs releases arachidonic acid (AA, gray) and this is controlled by specific enzymes: fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) hydrolyze AEA, monoacylglycerol lipase (MAGL), and α/β hydrolase domain 6 (ABHD6) and 12 (ABHD12) hydrolyze 2-AG. Via a multiple-step process, AA is oxygenated into prostaglandins (PG) (upper part, gray). First, PGH_2 is produced by cyclooxygenase (COX) action on AA. Then, specific PG synthases further catabolize PGH_2 to PGE_2, PGD_2, $PGF_{2\alpha}$, and PGI_2. PGs act on specific receptors (EP1-4, DP1-2, FP, and IP, respectively). Oxygenation of AEA and 2-AG by COX-2 results in PG-EAs (lower left, green) and PG-Gs, respectively (lower right, orange). Pharmacological studies of PG-EAs revealed that PGE_2-EA binds to all EP receptors and $PGF_{2\alpha}$-EA activates a heterodimer of FP and its splice variant (FP/FPalt4). PGE_2-G activates the $P2Y_6$ receptor, while PGD_2-G activates both DP receptors but is more potent at activating the DP1 receptor. The metabolite of PGD_2-G, 15d-PGJ_2-G, activates PPARγ. Hydrolysis of PG-Gs releases the corresponding PGs and can be mediated by MAGL, ABHD6, lysophospholipase A_2 ($LYPLA_2$), and carboxylesterase (CES)1-2 activity (upper right). To date, no enzymatic activity has been shown towards PG-EAs. White text on a light-blue background indicates enzymes, while black text on a white background indicates receptors.

Accordingly, eCB administration was shown to mediate analgesia but the effectors involved depend on the mechanism of pain initiation and can differ over time. In models of inflammatory pain (e.g., formalin or carrageenan administration), local or systemic eCB administration decreased hyperalgesia during the inflammatory phase [38–40]. The effects of 2-AG were CB_2 dependent in one study [38] and CB_1 dependent in another [40], while the effects of AEA were CB_1 dependent [39].

Box 2. Classical Animal Models in Pain

Animal models of pain can be classically divided between the types of pain elicited. Inflammatory pain models usually require intraplantar injection of irritants (e.g., carrageenan, formalin, complete Freund's adjuvant, LPS, etc.) in the skin, muscle, or paw of the animal. In these models, the subsequent local inflammation results in the release of mediators, such as inflammatory cytokines, chemokines, neurotrophic factors, and lipid mediators, that stimulate nerve endings and result in the generation of action potential. Depending on the irritant and its dose, each model is defined by a specific time-course in which both inflammation and subsequent pain subside at different rates. One important subtype of inflammatory pain is osteoarthritis pain, which relies on the injection of sodium monoiodoacetate, carrageenan, or kaolin into the knee and is characterized by its chronicity.

Neuropathic pain is induced by damages to the somatosensory system (peripheral and/or CNS) and the animal models can be subdivided into two classes. The first relies on a direct nerve injury through several surgical procedures. The spared nerve injury (SNI) comprises the axotomy or ligation of branches of the sciatic nerve. Partial sciatic nerve ligation (pSNL) involves the ligation of one-third to a half of the sciatic nerve width. Another surgical approach comprises the ligation of two spinal nerves (usually L5 and L6 in rodents) and is known as spinal nerve ligation (SNL). These three neuropathic pain models rely on either transection or very tight ligation of nerves. Chronic constriction injury (CCI) is another model where the sciatic nerve is constricted by loose ligation. Other anatomical regions are also studied in the context of neuropathic pain. As an example, the inferior orbital nerve injury (ONI) targets the trigeminal nerve through ligation of the infraorbital nerve.

The second class of neuropathic pain models relies on the use of compounds that are deleterious to the somatosensory system. Anticancer drugs (e.g., cisplatin, oxaliplatin, or paclitaxel) and antiretroviral drugs (e.g., 2,3-dideoxycytidine or didanosine) are the most commonly used. Of note, neuropathic pain can also arise as a collateral symptom of metabolic disturbances (e.g., diabetes) or follow ischemia, and some animal models are based on these specific triggers. Finally, some genetic models of pain have also been devised, such as mice genetically predisposed to spontaneous osteoarthritis.

These different models of pain are diverse in their etiology and subsequent neurophysiological processes and have contributed greatly to a better understanding of pain processes.

Moreover, eCB administration also decreases hyperalgesia in neuropathic pain models induced by antiretroviral or chemotherapy drugs, partial sciatic nerve ligation (pSNL) (Box 2) and CCI [41–45]. Again, depending on the study, the receptors implicated in the effects of eCBs were different. In one study, the effects of 2-AG were dependent on both cannabinoid receptors, while the effects of AEA were CB_1 dependent [41]. In another study, the effects of AEA were CB_1 and CB_2 dependent, while the effects of 2-AG were CB_1 and GPR55 dependent [44] (even though 2-AG does not bind GRP55 [46]).

Cannabinoid receptors have a distinct expression profile and implication in nociception. CB_1 is localized on presynaptic neurons and to a lower extent on astrocytes, microglia, and oligodendrocytes in the CNS. By contrast, CB_2 is more extensively expressed on immune cells, such as microglia and macrophages, compared with other cell types, resulting in immunomodulatory effects [47]. eCBs are involved in multiple brain structures that process nociceptive signaling from the periphery and are also responsible for inhibitory descending signals (Box 1 and Figure 2). Generally, CB_1 is shown to mediate most of the effects of eCBs in the brain, although CB_2 was also implicated in some settings [48]. Moreover, the effects of eCBs on nociception appear to be dependent on both peripheral and central cannabinoid receptors. Therefore, eCBs are able to modulate nociception in the periphery, spinal cord, or supraspinal structures through several mechanisms where neurons, microglia, and macrophages interact [49]. In fact, activation of cannabinoid receptors in the periphery is sufficient to exert analgesic effects [30,50–53]. This is important from a translational perspective because cannabinoid receptor activation in the CNS comes with behavioral side effects. However, the role of the CB_1 receptor in pain is not as straightforward. Indeed, activation of CB_1 receptors specifically on inhibitory dorsal horn interneurons was shown to increase the excitability of nociceptors, thus leading to hyperalgesia [54]. These elements support eCBs and their receptors as key players involved in the control of inflammatory and neuropathic pain.

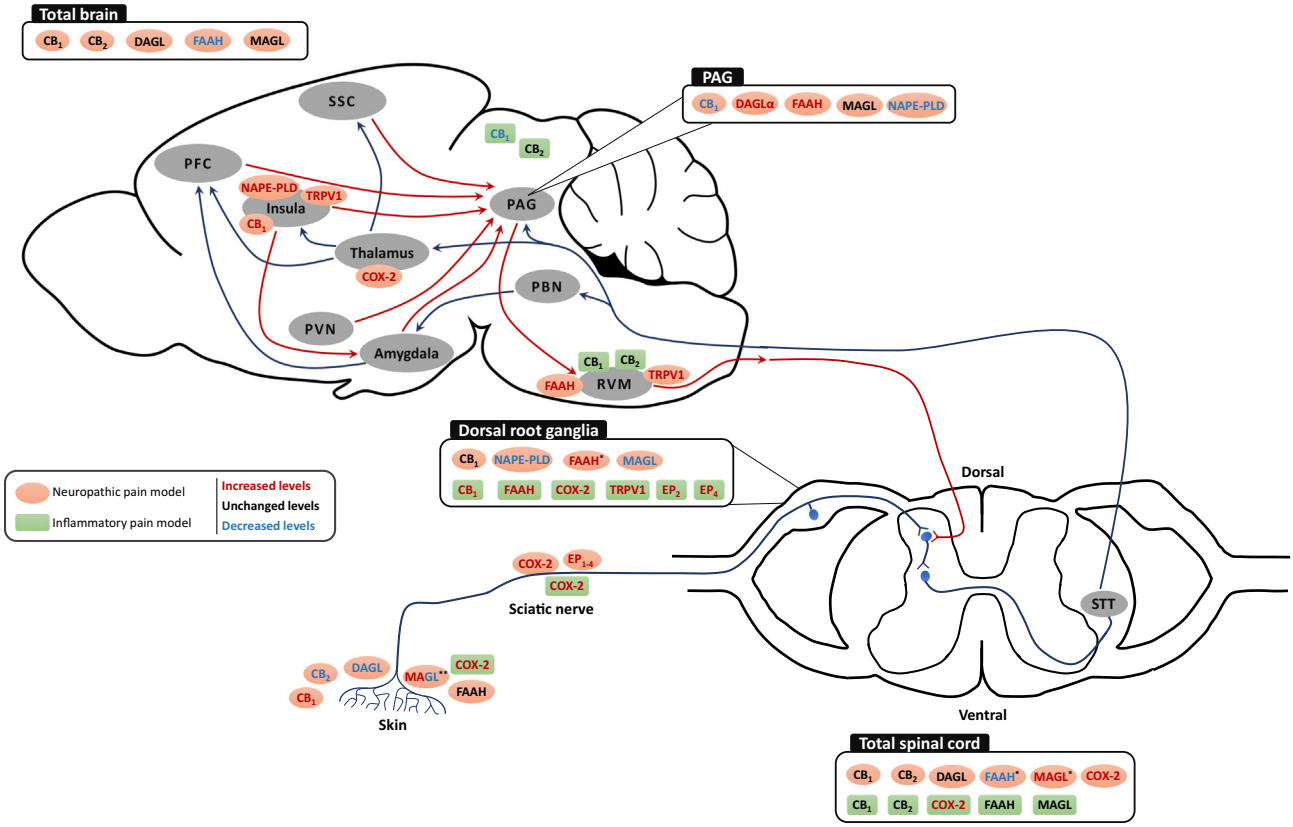

Figure 2. Alterations of the Cyclooxygenase (COX)-2 Metabolic Hub in Ascending and Descending Pain Pathways. Nociceptive stimuli are relayed from the skin (and internal organs) to the dorsal horn of the spinal cord by sensory fibers. The somata of these sensory neurons are located within the dorsal root ganglia (DRG). The primary afferent signals activate secondary neurons that are connected with interneurons and the descending pain pathway. The signals are then transduced to supraspinal regions through the spinothalamic tract (STT), processed and relayed in the brain by the parabrachial nucleus (PBN), amygdala, and prefrontal cortex (PFC) as well as periaqueductal gray (PAG), thalamus, and regions of the cortex, such as PFC, somatosensory cortex (SSC), and insula. The descending modulating pain pathway originates from the paraventricular nucleus (PVN), PFC, SSC, and amygdala, and converges on the PAG. PAG projections then reach on the rostral ventromedial medulla (RVM) where the descending pain pathways are further relayed to the dorsal horn of the spinal cord, where supraspinal signals are integrated and modulate primary afferent signals. The endocannabinoid (eCB) and prostanoid systems are present at each step of nociceptive stimuli processing and are impacted in several models of inflammatory (orange) and neuropathic (green) pain. Increased levels are in red, unchanged levels are in black, and decreased levels are in blue. * Indicates variations obtained in three surgically induced models of neuropathic pain [spared nerve injury (SNI) , spinal nerve ligation (SNL), and chronic constriction injury (CCI)]. However, the opposite variations were observed in a chemically induced model of neuropathic pain (cisplatin). ** Indicates that MAGL was either increased or decreased in two distinct models of neuropathic pain. See Table S1 in the supplemental information online for references.

Prostaglandins

Among the different PGs, PGE_2, PGD_2, and prostacyclin (PGI_2) are the most studied in nociception. PGE_2 is frequently associated with hyperalgesia induction and involved in proinflammatory processes. Functional coupling of mPGES-1 and COX-2 might explain the pivotal role of PGE_2 in the initiation and maintenance of inflammation and nociception [55]. PGE_2 acts through four G-protein-coupled receptors (GPCRs), namely the EP1–4 receptors. Regarding nociception, EP1 receptor activation can directly modulate ion channel activation (i.e., TRPV1 and NaV1.8) on peripheral nerve endings [56,57]. Thus, peripheral application of PGE_2 is used to trigger mechanical hyperalgesia in animal models of inflammatory pain [58,59]. EP4 receptor activation

contributes to prolonged peripheral sensitization [60]. In the dorsal horn, PGE_2 facilitates transmission of pronociceptive mediators by EP2 receptor activation at the presynaptic and postsynaptic levels [61,62]. Therefore, PGE_2 controls nociceptive input perception and duration at the central and peripheral levels.

PGI_2 has a crucial role in peripheral and central sensitization. PGI_2 activates the IP receptor (also a GPCR), resulting in vasodilatation and writhing response following its intraplantar injection to mice [63]. Antagonist and IP receptor deletion studies decrease hyperalgesia in arthritis models [64]. In neuropathic pain, microglial tumor necrosis factor (TNF)-α production stimulates PGI_2 production by endothelial cells. PGI_2 then activates the IP receptor expressed on DRGs and **dorsal horn of the spinal cord** neurons, further enhancing neuronal excitability [65].

Contrary to PGE_2 and PGI_2 that demonstrate clear proinflammatory and pro-algesic properties, PGD_2 can increase or decrease nociception through peripheral and central mechanisms. Two GPCRs have been described to bind PGD_2, DP1, and DP2. Reports of DP1 activation in the periphery are contradictory [66]. For instance, PGD_2 could be pronociceptive by decreasing the action potential threshold in cultured DRG neurons through DP1 activation, although the magnitude of the effect was dependent on both DP1 and DP2 receptor activation [66]. However, in another setting, DP1 appeared to decrease nociception because $DP1^{-/-}$ mice exhibited an increased flinching response to formalin [67]. Moreover, depending on the other PGs involved, the overall outcome of PGD_2 on nociception is different, resulting in seemingly paradoxical effects [66,67]. Indeed, depending on the dose administered, PGD_2 potentiated or inhibited the action of PGE_2 on nociceptive pathways, both through a DP1-dependent mechanism [68]. While these studies showed a role for DP1 in the effects of PGD_2 on nociception, DP1 receptors were not implicated in neuropathic nociception in a rat model of SNI [69]. Instead, PGD_2 produced by microglia following SNI triggered hyperalgesia through DP2 activation in dorsal horn neurons [69]. Therefore, PGD_2 appears to be necessary for pain signal transduction but, depending on the expression of DP receptors and the model, is not sufficient per se to induce hyperalgesia, and could even exert analgesic effects.

COX-2 Metabolites of Endocannabinoids

The exact functions of PG-Gs and PG-EAs are still being unraveled. As mentioned earlier, the low levels of these compounds complicate their quantification in pathophysiological settings. However, advances have been made in recent years to understand their role via exogenous administration, notably in inflammatory settings (Box 3).

PG-Gs can be hydrolyzed by several enzymes into their corresponding PGs. Additionally, some PG-Gs can activate the same receptors as the corresponding PGs. Therefore, it is difficult to distinguish whether the effects of PG-Gs are specific or merely due to their hydrolysis into PGs. Indeed, in some settings, PG-G effects were solely due to their hydrolysis into PGs [6,20], while in other cases, the effects of PG-Gs were not recapitulated by the corresponding PGs [2,9,70].

Among PG-Gs, intraplantar administration of PGE_2-G induces hyperalgesia in mice [5,7]. This effect was mediated by nuclear factor-kappa B (NF-κB) activation [7]. NF-κB activation has been associated with neuropathic and inflammatory pain and is involved in both peripheral and central nociceptive structures [71,72]. The hyperalgesic effect of PGE_2-G was partially mediated by EP1–4 receptors [7]. However, PGE_2-G showed low affinity for these receptors (Box 3 and Table 1). This suggests that some effects of PGE_2-G are due to its hydrolysis into PGE_2 because the latter was shown to recapitulate those effects in several studies [6,7,20]. While this may be the case in some settings, PGE_2-G also exerts effects independently of the activation of EP receptors. Indeed, PGE_2-G was also shown to activate the $P2Y_6$ receptor with high affinity [10] (Box 3 and

Box 3. Prostaglandin-Glycerol Esters, Prostaglandin-Ethanolamides, and Inflammation

PG-Gs and PG-EAs have gained interest in recent years as bioactive lipids in their own right. These lipid mediators have been mostly studied in inflammatory settings; however, their exact functions and the receptors mediating their effects (see Table 1 in main text) are still being unraveled. Among PG-Gs, PGE_2-G is described as being proinflammatory by increasing cytokine expression in macrophages and exerting neurotoxic effects [2,3]. The mechanism of action of PGE_2-G involves NF-κB activation and $P2Y_6$ activation, as well as EP receptor activation via its hydrolysis into PGE_2 [6,7,10]. Similar to PGE_2-G, $PGF_{2\alpha}$-G also increases cytokine expression by LPS-activated macrophages [2]. However, not all PG-Gs are proinflammatory. PGD_2-G has anti-inflammatory effects *in vitro* and *in vivo* [2,9,70]. In a colitis model, PGD_2-G counteracted colon inflammation induced by dextran sulfate sodium (DSS) in mice. This effect was partially mediated by DP1 and PPARγ activation [9]. Furthermore, inflammation induced by intraplantar carrageenan administration was decreased following local PGD_2-G administration [70].

The most studied PG-EA is $PGF_{2\alpha}$-EA, which has been identified as a crucial mediator controlling intraocular pressure. $PGF_{2\alpha}$-EA is known to bind the FP/FP-alt4 receptor, which is a heterodimer of the FP receptor and one of its splicing variants [116]. $PGF_{2\alpha}$-EA has a role in inflammatory pain and its levels were increased in the spinal cord in a murine model of knee inflammation. Moreover, $PGF_{2\alpha}$-EA shows pro-algesic properties when applied via intrathecal administration [8]. *In vitro*, PGE_2-EA showed neuroprotective effects by preventing apoptosis of neurons [117]. Furthermore, PGE_2-EA decreases TNF-α synthesis by human mononuclear cells [118]. Although PGE_2-EA has not been extensively studied, its pharmacological profile has been characterized. PGE_2-EA is able to bind EP receptors and its effect on mononuclear cells is dependent on EP2 receptor activation [118,119].

Further studies *in vivo* are needed to determine the exact role of the PG-Gs and PG-EAs in inflammatory conditions. These studies will also help us understand the role of these mediators in inflammation-driven pain.

Table 1). This receptor was implicated in hyperalgesia development in neuropathic pain in rats [73]. Moreover, peripheral activation of $P2Y_6$ is pro-algesic in formalin-induced hyperalgesia in rats [74]. $P2Y_6$ was also implicated in the hyperalgesic effect of PGE_2-G in sickle cell disease-induced hyperalgesia in mice [5]. Taken together, these results point towards a deleterious role for PGE_2-G in pain.

However, this is not the case for all PG-Gs. In fact, PGD_2-G has beneficial effects in inflammation and pain [2,9,70]. It was shown to be a DP1 agonist with a similar affinity to PGD_2 and could activate DP2 with 30-times lower affinity than PGD_2 [9]. Consistent with its anti-inflammatory

Table 1. Receptors Mediating the Effects of Prostaglandin-Ethanolamides and Prostaglandin-Glycerol Esters

Compound	Receptor	Activity, EC_{50} (nM)	Binding, Ki (nM)	Refs
PGE_2-G	$P2Y_6$	0.002		[10]
	EP1		979	[120]
	EP2		>19 800	[120]
	EP3		378	[120]
	EP4		737	[120]
	DP, TP, FP, IP		>10 000	[120]
PGD_2-G	DP1	39		[9]
	DP2	412		[9]
PGE_2-EA	EP1	848		[24,119]
	EP2	>10 000	468	[24,119]
	EP3	123	200	[24,119]
	EP4	>10 000	513	[24,119]
	DP, TP, FP, IP	Not active		[24]
	TRPV1		> 10 000	[121]
PGD_2-EA	DP, EP1-4, FP, IP, TP	Not active		[24]
$PGF_{2\alpha}$-EA	DP, EP1-4, FP, IP, TP	Not active		[24]

effects, PGD_2-G decreased inflammatory edema formation and hyperalgesia in a mouse model of carrageenan-induced inflammatory pain [70]. These effects were not mediated by metabolite formation or DP1 activation, suggesting the involvement of an as yet unknown target [70]. The exact role of these PG-Gs needs further characterization in these settings because their formation might likely occur in crucial pain-processing structures, such as the spinal cord, where 2-AG levels and COX-2 expression are increased in various pain models.

Concerning PG-EAs, little is known about their effects in pain. $PGF_{2\alpha}$-EA levels were increased in the spinal cord in a murine arthritis model [8]. Intrathecal administration of $PGF_{2\alpha}$-EA increased the excitability of dorsal horn nociceptive neurons and exerted pro-algesic effects [8]. Accordingly, administration of a $PGF_{2\alpha}$-EA receptor (a heterodimer of the FP receptor and one of its splice variants) antagonist reduced carrageenan-induced hyperalgesia [8]. To date, the role of other PG-EAs in nociception has not been studied *in vivo*.

In conclusion, considering the interplay between the eCB and prostanoid systems (Figure 1), the consequences at the molecular level of elevated eCB levels in pain are not clear because this can result in either analgesic or pro-algesic effects. Indeed, eCBs can act in a beneficial way through cannabinoid receptor activation or exert detrimental effects through AA production and subsequently of pro-algesic PGs, such as PGE_2. Formation of PG-Gs and PG-EAs through COX-2 metabolism of eCBs could also lead to anti- or pro-algesic effects depending on the metabolite produced.

Interacting with the Endocannabinoid and Prostanoid Systems to Alleviate Pain

Endocannabinoid Biosynthesis

NAEs (including AEA) are synthetized from NAPEs by NAPE-PLD, although multiple pathways can lead to NAE formation [75]. Little is known about the role of NAPE-PLD in pain. The expression of NAPE-PLD was not affected in paw skin, spinal cord, and brain in a mouse model of antiretroviral-induced neuropathic pain [44], whereas NAPE-PLD protein levels were decreased in DRG neurons following nerve injury or inflammation-induced pain in mice [76,77]. While this discordance in NAPE-PLD expression profile could be attributed to the different models, further work is needed to evaluate the role of this enzyme in nociception. The recent development of NAPE-PLD inhibitors could help shed light on the matter [78].

DAGL-α is the major DAGL involved in the release of 2-AG in neurons, while DAGL-β is expressed on microglia and macrophages [79]. Interestingly, only DAGL-β activity is necessary for the development of lipopolysaccharide (LPS)-induced hyperalgesia in mice [80,81]. The mechanism implicated is a reduction of 2-AG levels and subsequent AA and PG biosynthesis [81]. Therefore, prevention of 2-AG synthesis by DAGL-β could be an interesting approach to treat pain without cannabimimetic side effects or tolerance [81]. However, to date, there are no selective DAGL-β inhibitors available (e.g., ABHD6 is reported as an off-target) [82,83].

Of note, interfering with eCB biosynthesis will affect not only AEA or 2-AG levels, but also a large cohort of lipid mediators produced via the same pathways (i.e., NAEs in the case of AEA and monoacylglycerols in the case of 2-AG). These lipid mediators activate distinct receptors and can result in different outcomes compared with eCBs. Moreover, decreasing 2-AG synthesis will result in some tissues in reduced arachidonic acid levels.

Endocannabinoid Catabolism

FAAH Inhibition

Inhibition of FAAH leads to increased NAE (and, therefore, AEA) levels. FAAH expression is increased during neuropathic pain in the PAG and the RVM, hence the possibility that blocking

FAAH activity and restoring AEA levels centrally could counteract neuropathic pain [84,85]. However, FAAH expression appears to be model dependent because antiretroviral administration decreased FAAH mRNA in the total brain [44]; therefore, FAAH inhibitors might lack efficacy depending on the origin of neuropathic pain. Various chemical classes of FAAH inhibitor have been developed and are active in different preclinical inflammatory and neuropathic pain models [86–88]. Depending on the studies, the effects of FAAH inhibition were dependent on one or both cannabinoid receptors and sometimes on peroxisome proliferator-activated receptor (PPAR)α activation [50,51,89,90]. This is consistent with not only the different receptors activated by AEA, but also the multiple NAEs increased upon FAAH inhibition, and typically *N*-palmitoylethanolamine (PEA).

Acute FAAH inhibition using URB597 increased NAE levels in the spinal cord, but not in the inflamed paw, and decreased hyperalgesia in a rat model of carrageenan-induced inflammatory pain. Conversely, repeated administrations of the FAAH inhibitor had no effect [89]. This was attributed to a tolerance or adaptation of the eCB system to sustained FAAH inhibition. However, in chemotherapy-induced neuropathic pain in mice, both acute and chronic regimens of URB597 were efficient in decreasing hyperalgesia [48,50]. Moreover, a peripherally restricted FAAH inhibitor, URB937, also decreased hyperalgesia in inflammatory and neuropathic pain models and highlighted the therapeutic analgesic potential of increasing AEA levels in the periphery [48,50,53]. So far, four inhibitors have been used in clinical trials with various indications, including two trials in pain (NCT00981357 and NCT01748695) that have not shown any benefits. As suggested by some authors, the affective component in humans might affect outcomes of FAAH inhibition in clinical trials [91,92]. While this remains to be confirmed, it is too early to draw conclusions on the lack of effectiveness of FAAH inhibitors in pain.

NAAA Inhibition

Similar to FAAH inhibition, NAAA inhibition affects NAE levels *in vitro* and *in vivo* [14]. However, NAAA has been less studied than FAAH in the control of AEA levels. Blockade of NAAA leads to a decrease of hyperalgesia in models of inflammatory and neuropathic pain, although the lipid mediators and receptors implicated were not always investigated [93–95]. Indeed, while it was shown that NAAA also controls AEA levels [14], most studies using NAAA inhibitors focus on PEA levels and PPARα-mediated effects.

MAGL Inhibition

MAGL expression in pain varies depending on the models studied. No variations were observed in the CNS in antiretroviral-induced neuropathic pain [44], while MAGL was increased in the spinal cord of mice with inferior orbital nerve-induced neuropathy [96]. These results are in line with a decrease in 2-AG levels in the spinal cord in nociceptive models [97,98]. Accordingly, MAGL inhibition increased 2-AG levels in the spinal cord and counteracted hyperalgesia in neuropathic pain (e.g., CCI or chemotherapy induced) and formalin-induced inflammatory pain in mice [99,100]. The analgesic effects of MAGL inhibition by JZL184 were mediated by both CB_1 and CB_2 in inflammatory pain in mice [101]. However, in the same study, the anti-inflammatory effect of JZL184 was independent of cannabinoid receptors [101]. MAGL inhibition increased 2-AG in the brain and may reduce AA and subsequent PG formation [15,102]. Therefore, this antiedematous effect may be due to a reduction in PG production or even to increased PG-G production, although this remains to be investigated. The effect of MAGL inhibition on PG levels appears to be restricted to particular tissues (e.g., brain, liver, and lungs) [15,102]. Therefore, these results point to MAGL as a promising target with low probability of nonsteroidal anti-inflammatory drug (NSAID)-like adverse effects, because it does not decrease PG production in the gut [102]. However, elevated central 2-AG levels might induce cannabimimetic side effects and desensitization of CB receptors.

ABHD6 Inhibition

Similar to MAGL, ABHD6 hydrolyzes 2-AG; however, its inhibition results in smaller increases in 2-AG levels [2] and, therefore, was suggested as a potential way of increasing 2-AG levels without the potential adverse effects of MAGL inhibition. However, ABHD6 inhibition has not been extensively studied in the context of pain. In CCI-induced neuropathic pain in mice, the ABHD6 inhibitor WWL70 decreased hyperalgesia independently of cannabinoid receptor activation [103]. This could point to ABHD6 as a favorable target to counteract neuropathic pain. However, further work is needed to characterize the mechanisms involved and to assess the involvement of ABHD6 in inflammatory pain.

COX-2 Inhibition

COX-2 inhibition with NSAIDs is used in humans to tackle the most common forms of hyperalgesia but reaches limitation in specific forms of pain (i.e., neuropathic and chronic inflammatory pain). NSAIDs inhibit pro-algesic prostanoid (e.g., PGE_2 and PGI_2) production. However, as discussed in this review, the reality is more complex because COX-2 inhibition will also decrease PG-G and PG-EA production. Moreover, some of the analgesic effects of COX-2 inhibition on inflammatory pain were shown to be CB_1 dependent and due to increased eCB levels [104,105].

The complexity of the COX-2 functional heterodimer allows small molecules, such as the *R*-enantiomers of NSAIDs (*R*-flurbiprofen, *R*-naproxen, and *R*-ibuprofen), to act as **substrate-selective inhibitors** based on allostery and competition. These compounds selectively inhibit 2-AG metabolism by COX-2 without affecting AA metabolism [106]. Therefore, while once considered inactive counterparts of *S*-profens, *R*-profens could have a place in pain therapy by increasing eCB levels. As a case in point, *R*-flurbiprofen was reported in 1995 to be an effective treatment for pain in humans [107]. Moreover, *R*-flurbiprofen decreased neuropathic pain, in a cannabinoid receptor-dependent manner, in nerve injury models by restoring the eCB tone in the peripheral nervous system [76]. The analgesic effects of *R*-profens could also be mediated by a reduction in detrimental PGE_2-G production in the periphery. Indeed, blockade of PGE_2-G synthesis by systemic or local *R*-flurbiprofen administration rescued sickle cell disease-induced hyperalgesia in mice [5]. Substrate-selective inhibition remains attractive in pain to avoid adverse effects due to inhibition of AA metabolism, such as the gastrointestinal adverse effects seen with NSAIDs. However, the exact involvement of decreased PG-G and PG-EA levels remains to be studied in nociception because some PG-Gs could be beneficial.

At the Crossroad of Two Systems

Several targets appear promising in the complex interplay between eCBs and PGs. Besides classical 'single-target' drugs, multiple targeting strategies have been tested with small molecules. Dual FAAH/MAGL inhibition has been investigated in inflammatory and neuropathic pain models using JZL195 and was shown to be greater than that of separate FAAH and MAGL inhibition [108,109]. This multiple targeting of the eCB system might be a strategy to increase the efficacy of modulation of the eCB system in human pain.

Another strategy targeting both FAAH and the $PGF_{2\alpha}$-EA receptor is effective in reducing formalin-induced inflammatory pain in mice [110]. Indeed, FAAH inhibition increases AEA levels and, therefore, pro-algesic $PGF_{2\alpha}$-EA formation by COX-2.

NSAIDs exert their analgesic effect on pain via COX-2 inhibition and decreased PG levels. In fact, ibuprofen and flurbiprofen derivatives can decrease FAAH activity and, therefore, act as dual FAAH/COX-2 inhibitors [111]. Specific FAAH/COX-2 inhibitors have been synthetized [112,113]. One of these (ARN2508) reduced inflammation in a model of inflammatory bowel

Clinician's Corner

We might be on the verge of future changes in pain management via bioactive lipid modulation. Although with mitigated success, some molecules are already used in clinical trials to inhibit eCB hydrolysis. For instance, FAAH inhibitors showed efficiency in preclinical models of pain, but were not successful in clinical trials. This raises the question as to which is the right combination of model and enzyme and underlines the complexity of this system.

Further studies, including in patients, are needed to understand fully the dynamic changes that occur during pain states in these complex and interconnected signaling systems. This will entail quantification of the lipid mediators (Figure 1) during clinical trials involving FAAH, MAGL, or other enzyme inhibitors.

From a cellular perspective, changes in effector expression and enzyme activity create a dynamic environment that controls both eCB and prostanoids levels, depending on the model studied. This is of course reminiscent of the clinic, where one pain state is different from another and further supports the notion that a 'one drug fits all' approach will not work with this system.

Pain as a condition and pain treatments come with a high risk of addiction. However, thus far, studies on the eCB and prostanoid systems have not evidenced such risk.

disease (IBD) and peripheral inflammation in mice [112] without gastric damage, as a result of FAAH inhibition, providing an interesting pharmacological profile for this molecule [113]. However, no report of their effect on nociception emerged, although promising results may arise due to the two proven anti-nociceptive strategies of FAAH and COX-2 inhibition.

Pharmacological inhibition of eCB hydrolysis can decrease prostanoid synthesis in specific tissues, partly contributing to the beneficial effect observed in nociception. The link between 2-AG hydrolysis and PG production is well described, whereas the link between AEA, AA, and PGs is less studied. Accordingly, $FAAH^{-/-}$ mice showed elevated AEA levels and no variation in PG levels in the brain, whereas $MAGL^{-/-}$ mice had decreased PG amounts in all brain regions analyzed [114]. Therefore, the link between AEA concentration and PG levels is not as direct as observed for 2-AG hydrolysis. This could be explained in part by the higher tissue levels of 2-AG compared with AEA, resulting in higher levels of AA being released upon its hydrolysis.

As mentioned earlier, MAGL and ABHD6 are among the hydrolases that can break down PG-Gs into PGs. Therefore, inhibiting MAGL or ABHD6 could also increase PG-G levels and effects through direct hydrolysis blockade and increased 2-AG availability for COX-2. Indeed, some effects of inhibiting 2-AG hydrolysis were cannabinoid receptor independent [2,3,44,103]. Therefore, inhibition of 2-AG hydrolysis has a double positive mechanism of action in pain by increasing 2-AG levels and decreasing PG production. However, the positive effect observed by inhibition of 2-AG hydrolysis might be dampened by pro-algesic PGE_2-G production or increased by analgesic PGD_2-G production. Further work is needed to determine the exact settings during nociceptive processes that can result in deleterious PGE_2-G synthesis or beneficial PGD_2-G synthesis [2,3,7,70].

By contrast, the effects of 2-AG might also involve other receptors, such as GPR55 [44]. However, the exact mechanism is still unclear because 2-AG is not a GPR55 ligand and GPR55 was shown to modulate CB_2 activation [46]. Therefore, further work on the mechanism of cross-antagonism between cannabinoid receptors and GPR55 could unravel new ways to modulate nociception.

Concluding Remarks

From the discussion provided here, it is clear that the interplay between eCBs and prostanoids is crucial and should be taken into consideration when interfering with these systems. Given that multiple enzymes are involved in this crosstalk, inhibiting their activities results in multiple changes in lipid levels. Adding to the complexity, these intricate systems are dynamic through time and space. As summarized here, hyperalgesia affects enzyme and receptor expression as well as lipid levels in both the periphery and CNS.

Modulation of the eCB system in these structures is a proven strategy to counteract neuropathic and inflammatory pain. However, eCB modulation, especially regarding 2-AG levels, is not devoid of adverse effects, due to central CB_1 activation. Therefore, a future challenge might involve fine-tuning eCB levels as well as metabolite (PG, PG-G, and PG-EA) production. Indeed, as mentioned, these metabolites are bioactive and can modulate pain. However, while PGs are well characterized, more studies are needed to decipher the exact role of PG-Gs and PG-EAs in nociception (see Outstanding Questions). Nevertheless, based on current knowledge, the combination of 2-AG hydrolysis inhibitors and blockade of PGE synthase activity would benefit from the positive effects of 2-AG while avoiding the detrimental effect of PGE_2-G formation. Given the competition between the PG synthases, inhibiting PGE synthase in this setting could also favor PGD_2-G production. Similarly, inhibiting FAAH while preventing the formation or effects of

Outstanding Questions

The pharmacological characterization of PG-Gs and PG-EAs is still in its infancy (Box 3). Some of these lipid mediators still lack identified receptor(s), while for most of the others mainly functional assays were used. Thus, a thorough and robust pharmacological evaluation is needed to move the field forward. This would also involve comparing PG-Gs and PG-EAs to the corresponding PGs when a receptor is shared, because biased agonism could help distinguish the effects of those ligands.

Stable analogs of PG-Gs would be interesting, especially for *in vivo* studies. However, this is easier said than done because changes in the structure could lead to different pharmacology (e.g., affinity and biased agonism). Thus, this strategy will have to wait for the identification of the receptors binding the PG-Gs to compare the pharmacology of the endogenous mediator and that of its stable analog.

To what extent do MAGL and FAAH inhibition result in changes in PG-G and PG-EA levels? This remains to be fully characterized and calls for highly sensitive bioanalytical methods due to the relatively low abundance of these lipids and to limited amounts of tissue available when considering specific regions of the pain-processing pathways. This is even more important as FAAH and MAGL inhibitors move to clinical trials. Indeed, changes in these lipid mediator levels might explain some of the 'fortuitous' effects recorded (i.e., not related to the primary substrates, such as AEA, PEA or 2-AG).

The extent to which PG-Gs and PG-EAs contribute to the effects observed upon COX or PG synthase inhibition remains an open question in most models. Besides being a fundamental research question, this is also relevant for the clinical practice, where NSAIDs are largely used.

Substrate-selective COX inhibitors are crucial pharmacological tools in the context of inflammation and pain. Thus, improving their potency, selectivity, and pharmacokinetic profile will be beneficial to the field.

$PGF_{2\alpha}$-EA might help push forward FAAH inhibition as a therapeutic strategy in pain. Thus, multiple targeting strategies or, when achievable, substrate-selective inhibition might be the way to go to fully exploit the potential of these bioactive lipids.

Supplemental Information

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