

COX-2-derived endocannabinoid metabolites as novel inflammatory mediators

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Cyclooxygenase-2 (COX-2) is an enzyme that plays a key role in inflammatory processes. Classically, this enzyme is upregulated in inflammatory situations and is responsible for the generation of prostaglandins (PGs) from arachidonic acid (AA). One lesser-known property of COX-2 is its ability to metabolize the endocannabinoids, *anandamide* (AEA) and *2-arachidonoylglycerol* (2-AG). Endocannabinoid metabolism by COX-2 is not merely a means to terminate their actions. On the contrary, it generates PG analogs, namely PG-glycerol esters (PG-G) for 2-AG and PG-ethanolamides (PG-EA or prostamides) for AEA. Although the formation of these COX-2-derived metabolites of the endocannabinoids has been known for a while, their biological effects remain to be fully elucidated. Recently, several studies have focused on the role of these PG-G or PG-EA *in vivo*. In this review we take a closer look at the literature concerning these novel bioactive lipids and their role in inflammation.

COX-2, AA, and PGs in inflammation

Inflammation is a physiological response to infection and injury, aimed at eliminating the threat and re-establishing homeostasis. However, failure of resolution and chronic inflammation lead to tissue damage and are implicated in the pathogenesis of many diseases such as inflammatory bowel diseases, arthritis, multiple sclerosis, and atherosclerosis [1].

COX-2 is an enzyme that plays a key role in inflammatory processes. Classically, this enzyme is upregulated in inflammatory situations and is responsible for the generation of PGs from AA. Generally, these prostanoids contribute to the development of inflammation and exert proinflammatory effects, and thus COX inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), constitute potent anti-inflammatory compounds. There are two isoforms of COX, the constitutively expressed COX-1

and the inducible COX-2. However, in the brain and spinal cord, COX-2 is constitutively expressed in neurons and radial glia (but not in astrocytes, oligodendrocytes, or microglia) and plays a role in the early hyperalgesic response to tissue injury [2]. COX enzymes are functional heterodimers (Box 1) that catalyze two distinct and consecutive reactions. The cyclooxygenase reaction is a bisoxygenation leading to the formation of PGG₂ from AA, followed by the peroxidase reaction leading to PGH₂ [3]. PGH₂ is then quickly converted by specific synthases – PGD, PGE, PGF, PGI, and thromboxane synthases – to give the specific PGs and thromboxane A₂ that constitute the biologically active products. Four principal PGs are generated *in vivo*, PGD₂, PGE₂, PGI₂ (or prostacyclin), and PGF_{2α} (Figure 1A). The ‘2’ refers to the number of carbon-carbon double bonds in the product, and therefore to AA-derived prostanoids, which are the most abundant. Other fatty acids can also be metabolized by COX-2, such as adrenic acid (7,10,13,16-docosatetraenoic acid) and eicosapentaenoic acid, whereas docosahexaenoic acid is a very poor substrate of COX-2. Prostanoids derived from eicosapentaenoic acid (EPA) are the ‘3-series’ compounds. Prostanoids exert their effects by activating one or more specific G protein-coupled receptors (GPCRs), as well as the nuclear peroxisome proliferator activated receptors (PPARs) [3].

The clinical efficacy of NSAIDs in painful and inflammatory settings reinforced the role of COXs as implicated in the generation of inflammatory processes. However, numerous studies also point to a role for COX-2 in the resolution of inflammation [4], and the increased risk of gastrointestinal and cardiovascular events with COX inhibitors highlights the fact that PGs are also implicated in physiological effects [5,6].

We outline here the role played by COX-2 in bringing together the endocannabinoid and eicosanoid systems and take a look at the recently described biological activities of the COX-2-derived metabolites of the endocannabinoids. This interaction between the endocannabinoid and eicosanoid systems is complex, and the full picture concerning the physiological and pathological consequences of COX-2 oxidation of endocannabinoids remains to be elucidated. New pharmacological tools are emerging to modulate the levels or actions of these novel lipid mediators and will be discussed in the last section.

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Box 1. COXs, the homodimers that function as heterodimers

COX enzymes are sequence homodimers, composed of tightly associated monomers with identical primary structures. However, COXs function as conformational heterodimers with an allosteric and a catalytic monomer [60]. Ligand-dependent crosstalk between monomers influences the catalytic activity of COXs. Occupation of the active site of the allosteric monomer induces modifications at the interface between the monomers, which leads to alteration of the active site of the catalytic monomer. Although several mechanisms have been proposed, the specific modifications that are induced remain to be elucidated [3]. The modulation of COX activity by non-substrate fatty acids is an example of allosteric modulation of COX activity by endogenous molecules [60]. Palmitic acid, for instance, potentiates AA oxygenation by COX-2, but reduces COX-1 activity [3].

This functional heterodimer has consequences regarding COX inhibition. COX inhibitors were classified into two groups depending on the type of inhibition: the rapid reversible inhibitors, such as ibuprofen and mefenamate, and the slow irreversible inhibitors such as celecoxib, naproxen, flurbiprofen, and aspirin [3].

However, the story has proved more complex because some inhibitors function by binding to the allosteric monomer, others to the catalytic monomer, or even by binding to both monomers. For instance, when considering the slow irreversible inhibitors, naproxen and flurbiprofen only bind to the allosteric monomer whereas celecoxib and aspirin bind to the catalytic monomer [60]. Additionally, aspirin modifies the catalytic monomer by acetylation [61,62]. On the other hand, rapid reversible inhibitors such as ibuprofen and mefenamate bind to both monomers to inhibit AA metabolism by COX-2 [27,49].

Moreover, there is a difference in the mode of action of inhibitors depending on the substrate. For instance, when endocannabinoids are considered as substrates, binding of the rapid reversible inhibitors to the allosteric monomer is enough to prevent endocannabinoid metabolism by COX-2 [27,49]. Thus, although these rapid reversible inhibitors are competitive regarding AA metabolism, they are non-competitive versus the endocannabinoids.

Finally, the *R* enantiomers of profens, which were considered to be inactive towards COX-2 because only the *S* enantiomers are able to inhibit AA metabolism, were recently shown to inhibit COX-2 with endocannabinoids as substrates [27]. The notion of substrate-selective inhibitors of COX-2 was therefore introduced.

COX-2 at the interface of the eicosanoid and endocannabinoid systems

COX-1 has a strong requirement for a free carboxyl group in the substrate, which does not apply to COX-2. Therefore COX-2 can metabolize neutral derivatives of AA such as esters and amides [7]. The first demonstration of this substrate-based functional difference between COX-1 and COX-2 was the discovery that COX-2 could oxygenate the endocannabinoid *N*-arachidonylethanolamine (AEA), although less efficiently than AA [7,8]. Subsequently it was shown that COX-2 can also oxidize 2-arachidonoylglycerol (2-AG) with a similar efficiency as for AA [7,9]. Site-directed mutagenesis studies have attempted to dissect the structural requirements for COX-2-mediated oxygenation of the endocannabinoids with somewhat discrepant results. Tyr385, the COX-2 active-site residue, is required for oxygenation of AA and the endocannabinoids [10,11], whereas Arg120 and Leu531 facilitate oxygenation of both substrates [10–12]. Tyr355 seems, however, to be more important for oxygenation of AA than for endocannabinoids [10–12]. Arg513, one of three amino acid residues that differ between the side pockets of COX-2 and COX-1 (His513 in the case of COX-1) was hypothesized to be

crucial for endocannabinoid oxygenation by COX-2. The studies by Kozak *et al.* confirmed this hypothesis by measuring reduced oxygenation rates for endocannabinoids, but not AA, following Arg513 mutation to His [10,11]. However, another study found no difference in the oxygenation rates of AA and endocannabinoids with the Arg513His mutant [12].

The endocannabinoids AEA and 2-AG are endogenous bioactive lipids which exert numerous biological effects. They modulate food intake and energy balance, and exert anxiolytic effects as well as analgesic and anti-inflammatory effects [13–15]. Classically cannabinoids exert their effects by binding to and activating two GPCRs, the cannabinoid receptors CB₁ and CB₂. AEA and 2-AG can also activate the PPAR receptors, and AEA is a ligand for the transient receptor potential cation channel subfamily V member 1 (TRPV1). The biological actions of these endocannabinoids are terminated by their hydrolysis by specific lipases, fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine hydrolyzing acid amidase (NAAA) for AEA and monoacylglycerol lipase (MAGL), and α/β hydrolase domain 6 (ABHD6) for 2-AG [16]. These enzymes hydrolyze AEA and 2-AG into AA and ethanolamine or glycerol, respectively (Figure 2). In addition to COX-2 and the lipases mentioned here, endocannabinoids can also be metabolized by other enzymes such as lipoxygenases and cytochrome P450 (Box 2).

The dual metabolism of endocannabinoids (hydrolysis into AA by specific lipases and their COX-2-mediated oxygenation) puts forth a bilateral interaction between the endocannabinoid and eicosanoid systems.

On the one hand, endocannabinoid hydrolysis could affect AA levels and therefore prostanoid synthesis. Accordingly, although endocannabinoids exert anti-inflammatory effects in their own right, 2-AG has been shown to be a source of AA in the brain [17–19], and some of the effects of inhibiting endocannabinoid hydrolysis and increasing their levels were imputed to the subsequent decrease in AA and thus blocking of the prostanoid pathway [17].

On the other hand, some of the effects of COX-2 inhibition could be more complex than the inhibition of PG synthesis by a reduction in AA availability, and could involve inhibiting endocannabinoid metabolism and activation of the cannabinoid receptors. Several observations support this hypothesis: (i) COX-2 is constitutively expressed in the brain and was shown to affect endocannabinoid metabolism [20] and control endocannabinoid-mediated effects on GABAergic transmission [21,22]; (ii) antinociceptive effects of NSAIDs and COX-2-selective inhibitors in rodent pain models can be blocked by a CB₁-selective antagonist in some settings [23–25]; (iii) COX inhibitors can increase endocannabinoid levels in murine models of inflammation [25]; and (iv) substrate-selective COX-2 inhibitors exert anti-allodynic effects through activation of cannabinoid receptors [26] and increase endocannabinoid levels *in vitro* [27,28] and *in vivo* [29].

This led to the supposition that exploiting the endocannabinoid component of COX-2 inhibition might be a method to enhance the effects of NSAIDs and reduce their toxicity. Indeed, if endocannabinoids exert analgesic and

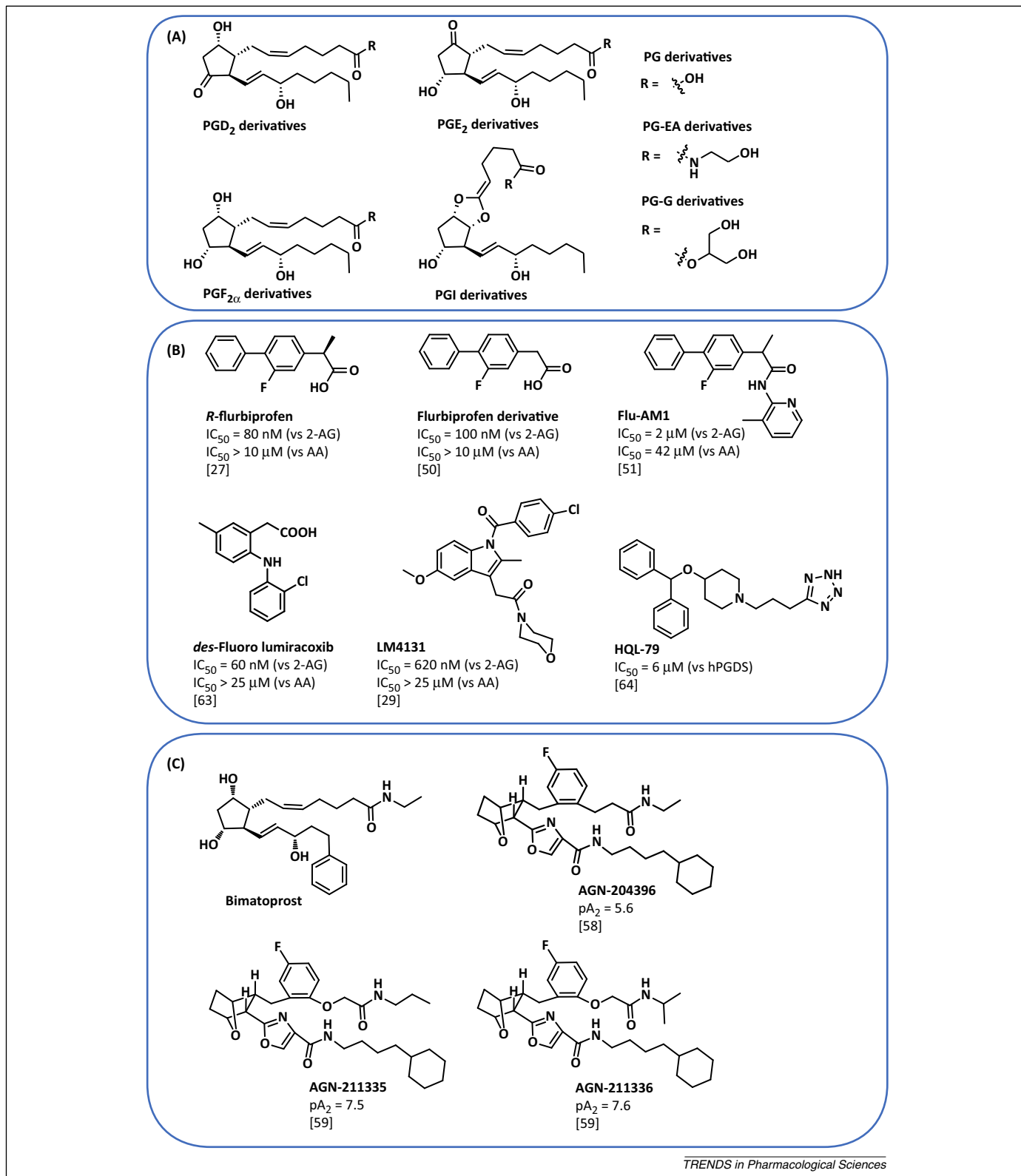


Figure 1. Cyclooxygenase-2 (COX-2)-derived endocannabinoid metabolites and the tools to interact with their signaling. **(A)** Structure of the prostaglandins (PGs) PGD₂, PGE₂, PGF_{2α}, and PGI₂ and of the corresponding PG ethanolamide (PG-EA) and PG glycerol ester (PG-G) derivatives. The key for the R substituent is shown on the right. **(B)** Structures of the substrate-selective COX-2 inhibitors. The IC₅₀ values refer to the inhibition of COX-2 activity using either 2-arachidonoylglycerol (2-AG) or arachidonic acid (AA) as substrate. The structure of the PGD synthase inhibitor HQL-79 is also shown. **(C)** Structure of three antagonists of the PGF_{2α}-EA receptor (AGN-204396, AGN-211335, and AGN-211336) together with their potency. The structure of the PGF_{2α}-EA analog, bimatoprost, is also illustrated. Abbreviations: hPGDS, human prostaglandin D synthase; pA₂, affinity value [63,64].

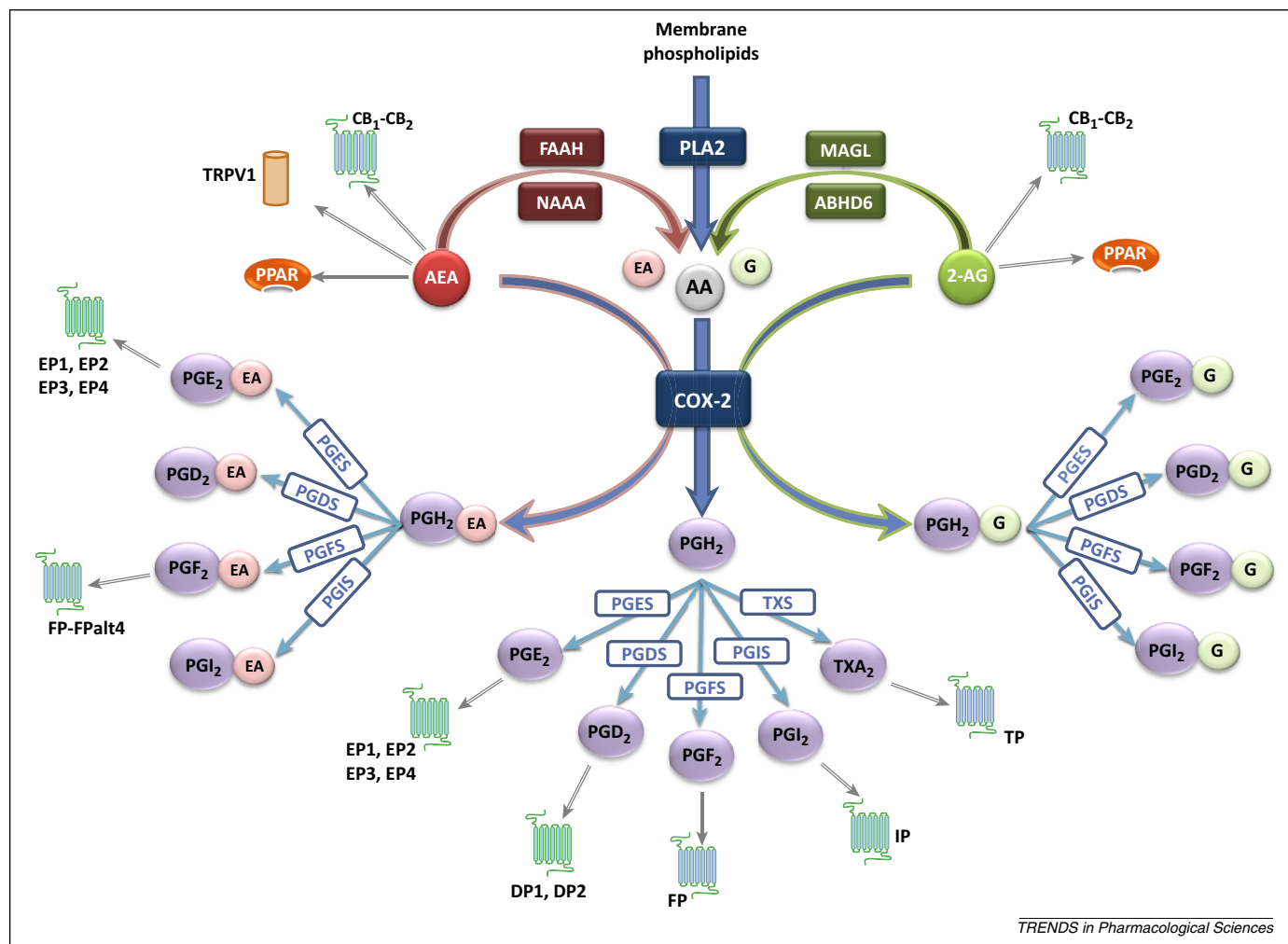


Figure 2. COX-2 at the interface of the eicosanoid and endocannabinoid systems. Two enzymes are responsible for the hydrolysis of 2-arachidonoylglycerol (2-AG) into arachidonic acid (AA) and glycerol (G): the monoacylglycerol lipase (MAGL) and α/β hydrolase domain 6 (ABHD6). Fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine hydrolyzing acid amidase (NAAA) degrade *N*-arachidonylethanolamine (AEA) into AA and ethanolamine (EA). During inflammation, AA is produced from membrane phospholipids by phospholipase A₂ (PLA₂). AA is metabolized by cyclooxygenase-2 (COX-2) to give PGH₂, which in turn is taken up by specific PG synthases (PGS) to give the PGs (PGD₂, PGE₂, PGF₂, PGI₂). PGs act through specific G protein-coupled receptors. Classically, during inflammatory conditions, COX-2 is upregulated leading to increased formation of PGs. 2-AG and AEA can be metabolized by COX-2, similarly to AA, to give, *in fine*, PG-glycerol esters (PG-Gs) and PG-ethanolamides (PG-EAs), respectively. The molecular targets of PG-Gs and PG-EAs are not well elucidated. PGE₂-EA is a ligand of the PGE₂ receptors, whereas PGF₂-EA activates a heterodimer of the PGF₂ receptor and one of its splicing variants. Other metabolic pathways responsible for endocannabinoid oxygenation (summarized in Box 2) are not shown here for simplicity. Abbreviations: DP, PGD₂ receptor; EP, PGE₂ receptor; FP, PGF₂ receptor; IP, PGI₂ receptor; TP, TXA₂ receptor.

anti-inflammatory effects, then increasing their levels along with COX-2 inhibition would lead to reducing the doses of NSAIDs and therefore their side effects [30]. Studies combining NSAIDs with a FAAH inhibitor show a synergistic effect, and compounds with dual inhibitory activity on FAAH and COX are being identified [30].

Although this is a very interesting approach, it is centered on increasing endocannabinoid levels and does not take into account the potential biological effects of the COX-2-derived metabolites of endocannabinoids (Figure 2). Indeed, if these metabolites exert proinflammatory or hyperalgesic effects, then blocking their production is a goal; however, if they have anti-inflammatory effects, then blocking their production might impair the resolution of inflammation. It is also noteworthy that, although endocannabinoids are generally considered anti-inflammatory compounds, proinflammatory effects have been reported in some settings. For instance, 2-AG induced swelling and neutrophils recruitment when applied topically to the mouse ear

[31]. 2-AG and AEA were also shown to induce ileitis following an intraluminal administration into isolated ileal segments in rats [32].

COX-2 metabolites of endocannabinoids

The metabolism of AEA and 2-AG by COX-2 leads to the production of PGH₂-ethanolamide (PGH₂-EA) and PGH₂-glycerol ester (PGH₂-G) respectively. Following the action of the PG synthases, the amides and esters of the corresponding PGs are produced (Figure 2). PGH₂-EA and PGH₂-G are however poor substrates of thromboxane synthase [7]. Therefore COX-2-mediated metabolism of endocannabinoids leads to the production of PGD₂-EA, PGE₂-EA, PGF_{2 α} -EA and PGI₂-EA from AEA and PGD₂-G, PGE₂-G, PGF_{2 α} -G and PGI₂-G from 2-AG (Figures 1A and 2).

Concerning the degradation of these products, PG-EAs are stable to hydrolysis in rat plasma, human plasma, and whole blood [33], therefore failure to detect them *in vivo* is

Box 2. Additional oxidative pathways for AA and endocannabinoids

Arachidonic acid (AA) can also be metabolized by the lipoxygenase (LOX) enzymes to give hydroperoxyeicosatetraenoic acids (HPETEs). HPETEs can be reduced into hydroxyeicosatetraenoic acids (HETEs) or further metabolized to give leukotrienes. Multiple lipoxygenations produce lipoxins with pro-resolution properties. AA can also be oxidized by CYP450 enzymes to give HETEs or epoxyeicosatrienoic acids (EETs) [65].

Although LOX enzymes accept multiple free fatty acids as substrates, they exhibit high regioselectivity and stereospecificity regarding the site and orientation of oxygen addition. Thus, for most mammalian LOXs the resulting product is an *S*-hydroperoxide, and the AA-metabolizing LOXs are named by designating the number of the carbon atom where oxygen addition occurs [7].

Several LOX enzymes have been shown to also metabolize AA derivatives, such as AEA, 2-AG, and *N*-arachidonoyltaurine. These include human 12-LOX and 15-LOX, although platelet 12-LOX showed some loss of regioselectivity, producing derivatives of both 12-HETE and 15-HETE. CYP450 enzymes also oxidize AEA and 2-AG to give EET-EA and EET-G respectively [7].

In contrast to PG-EAs, which are generally not ligands for the corresponding PG or cannabinoid receptors, some LOX-derived metabolites of AEA are comparable in affinity to AEA in binding to CB₁ or CB₂, and even to TRPV1 [7,66]. Anti-allodynic effects of FAAH inhibition in neuropathic rats were accompanied by a surprising decrease in AEA levels and were blocked by TRPV1 antagonism and 12/15-LOX inhibition. This suggests that FAAH inhibition provided a pool of AEA for LOX enzymes leading to the formation of a 12/15-LOX metabolite of AEA with activity at TRPV1 receptors [67]. 15-HETE-G, the 15-LOX hydroperoxidation product of 2-AG, acts as a PPAR- α agonist [68]. Because PPAR- α agonists are known anti-inflammatory compounds, this LOX-derived product of 2-AG could be interesting in an inflammatory setting.

CYP450-derived metabolites of AEA and 2-AG exhibit high affinity at cannabinoid receptors, and can be induced by inflammatory stimuli in microglia, suggesting their involvement in inflammatory signaling in these cells [69,70]. Interestingly, these endocannabinoid metabolites were detected *in vivo*, in higher quantities than the PG-Gs, before being enzymatically characterized *in vitro* [7].

likely not due to their hydrolysis. By contrast, PG-Gs were much less stable and were subject to very rapid hydrolysis in rat plasma, although they were somewhat more stable in human plasma and whole blood. Hydrolysis products of PG-Gs *in vivo* are the corresponding PGs [34], thus failure to detect them *in vivo* could be due to their rapid hydrolysis [35]. The endocannabinoid hydrolyzing enzymes MAGL and FAAH were proposed to mediate PG-G hydrolysis. However, convincing data show that these enzymes are probably not the best candidates because PG-Gs (i) are very poor substrates for the purified enzymes [36], (ii) do not block FAAH and MAGL activity [37], and (iii) FAAH and MAGL inhibitors only partially block PGE₂-G hydrolysis [36]. Carboxylesterases (CES) 1 and 2 are more likely candidates because they can efficiently metabolize PG-Gs and are found in rodent but not human plasma, and this could explain the difference in stability of PG-Gs between rodent and human plasma. PG-EAs and PG-Gs can also be metabolized by 15-hydroxyprostaglandin dehydrogenase, the enzyme responsible for PGs oxidation into keto-PGs, although less efficiently [7].

Role of PG-G and PG-EA in inflammatory settings

Although the formation of these endocannabinoid-derived metabolites, first in assays with purified enzymes, then

from cells and tissues, was put forth a few years ago (Table 1) – along with the endocannabinoid metabolites of lipoxygenases (Box 2) – their effects *in vivo* have only been recently investigated. It was initially hypothesized that these endocannabinoid-derived PG-EAs and PG-Gs are subsequently hydrolyzed into PGs that mediate their effects. However, there is little evidence to support this pathway.

The first evidence for biological effects of oxygenated endocannabinoids came from the therapeutic efficacy of the PGF_{2 α} -EA analog bimatoprost in the treatment of glaucoma [38]. Concerning PG-Gs, PGE₂-G was first shown to induce Ca²⁺ mobilization in RAW264.7 cells, an effect which was not reproduced by PGE₂ and therefore not due to PG-G metabolism into PG. This was confirmed in another cell line, the non-small cell lung cancer line H1819, along with PGF_{2 α} -G [39,40]. Subsequent studies investigating the implication of their oxygenated metabolites in the effects of endocannabinoids and COX-2 in the central nervous system suggested that PG-EAs and PG-Gs are not ligands for the traditional eicosanoid receptors, and that unknown receptors mediate the effects of PG-EAs and PG-Gs [7]. PGF_{2 α} -EA and bimatoprost shed some light on the potential receptors implicated. These compounds exert the same effects as PGF_{2 α} on intraocular pressure, but the fact that some antagonists block the effects of PGF_{2 α} -EA and bimatoprost, without affecting the effects of PGF_{2 α} , supports the notion that they have distinct receptors. Accordingly, the receptor of PGF_{2 α} -EA and bimatoprost was identified as a heterodimer of the wild type FP receptor (i.e., one of PGF_{2 α} receptors) and one of its splice variants (Figure 2) [38]. Whether a similar picture will arise for other PG-EAs and PG-Gs remains to be seen.

PGF_{2 α} -EA was also implicated in inflammatory settings. In a model of inflammatory pain in rats, spinal PGF_{2 α} -EA levels were increased and shown to exert proalgesic effects. Selective inhibitors of COX-1 and COX-2 and a non-selective COX inhibitor reduced the inflammation-induced increase in PGF_{2 α} -EA levels [41]. However, PGE₂-EA suppresses the expression of interleukin-12 and interleukin-23 in microglial cells, although this effect was blocked by an EP2 antagonist [42]. PGE₂-EA also reduces lipopolysaccharide (LPS)-induced tumor necrosis factor- α production in human blood, monocytes and the THP-1 monocytic cell line, again possibly through EP2 activation [43]. Indeed, PGE₂-EA was shown to bind all the PGE₂ receptors [44]. The only reported effect for PGD₂-EA is induction of apoptosis in colorectal carcinoma cells [45].

Concerning PG-Gs, PGE₂-G was shown to induce hyperalgesia and modulate NF- κ B activation in carrageenan-induced inflammation in the rat paw [34] and exert proinflammatory, and neurotoxic effects in a rat model of Huntington's disease [46]. Moreover, PGE₂-G and PGF_{2 α} -G increase LPS-induced proinflammatory macrophage activation *in vitro* [28]. However, not all PG-Gs exert proinflammatory effects because PGD₂-G was shown to reduce macrophage activation and exert anti-inflammatory effects in LPS-induced inflammation in mice [28]. The PGD₂-G metabolite, 15-deoxy- Δ ^{12,14}prostaglandin J₂-glycerol ester (15d-PGJ₂-G) was shown to activate PPAR- γ and reduce T cells activation [47,48]. If the effects of PGD₂-G are medi-

Table 1. Detection of PG-Gs and PG-EAs *in vitro* and *in vivo*^{a,b}

Substrate	Product	Purified enzyme	<i>In vitro</i> detection	<i>In vivo</i> detection
AEA	12(S)-HETE-EA	12-LOX (rat, porcine) [71,72]	<i>Human platelets</i> [73]	
	15(S)-HETE-EA	15-LOX (soybean, rabbit) [71,72]	<i>Human PMNs</i> [73]	
	PGE ₂ -EA	COX-2 [8]/mPGES1 [74]	<i>HCA-7 cells</i> [74] Stimulated dorsal root ganglions [27]	<i>Lung and kidney in WT mice</i> [75] <i>Higher levels in FAAH^{-/-} mice</i> [75]
	PGD ₂ -EA		<i>LPS/IFN-γ-stimulated RAW264.7 cells</i> [74] <i>HCA-7 cells</i> [74]	<i>Lung and kidney in WT mice</i> [75] <i>Higher levels (lung, kidney, liver, intestine) in FAAH^{-/-} mice</i> [75]
	PGF _{2α} -EA	PGFS [76]	<i>HCA-7 cells</i> [74] Stimulated dorsal root ganglions [27] 3T3-L1 murine pre-adipocytes [77]	<i>Lung, kidney, liver, intestine in FAAH^{-/-} mice</i> [75] Spine of rats with carrageenan-induced knee inflammation [41] Epididymal fat of mice [77]
	PGI ₂ -EA	COX-2/PGIS [74]		
2-AG	12(S)-HETE-G	12-LOX (porcine) [78]	<i>Transfected COS-7 cells</i> [78]	
	15(S)-HETE-G	15-LOX (soybean, rabbit, human) [68]	<i>Transfected COS-7 cells</i> [68] <i>Human keratinocytes</i> [68]	
	PGE ₂ -G	COX-2 [9]/mPGES1 [74]	<i>HCA-7 cells</i> [74] <i>LPS-stimulated J774 cells</i> [28] <i>Zymosan/LPS-stimulated RPMs</i> [79] Stimulated dorsal root ganglions [27] M-213 cells exposed to malonate and a MAGL inhibitor [46]	Rat hindpaw Higher levels with MAGL inhibitor, lower levels with COX inhibitors [34]
	PGD ₂ -G	COX-2 [9]/hPGDS [74]	<i>LPS/IFN-γ-stimulated RAW264.7 cells</i> [9] <i>LPS-stimulated J774 cells</i> [28]	
	PGF _{2α} -G		<i>HCA-7 cells</i> [74] Stimulated dorsal root ganglions [27]	
	PGI ₂ -G	COX-2/PGIS [74]	<i>Zymosan/LPS-stimulated RPMs</i> [79]	

^aEntries in italic font denote experiments with exogenous substrate added, i.e., in the presence of AEA or 2-AG.

^bFurther abbreviations: hPGDS, human prostaglandin D synthase; IFN, interferon; mPGES1, mouse prostaglandin E synthase 1; PGIS, prostaglandin I synthase; RPM, resident peritoneal macrophage; WT, wild type.

ated, fully or in part, through its non-enzymatic metabolism to 15-d-PGJ₂-G, as is the case with PGD₂ and 15-d-PGJ₂, remains speculative because production of this putative metabolite has not been put forth *in vivo* or in a cell model.

Finally it is worth mentioning that, as for 2-AG, the 2-regioisomer of PG-G derivatives undergo isomerization to the more stable 1,3-regioisomers. For this reason the PG-G derivatives usually used in these studies are the 1,3-regioisomers, and it cannot be excluded that in some models they could behave slightly differently than the corresponding 2-regioisomer.

In summary, the biological activities of oxygenated endocannabinoids are distinct from those of the corresponding PGs and are probably mediated by distinct receptors. Moreover, all PG-Gs and PG-EAs do not exert the same effects with PGE₂-EA and PGD₂-G being anti-inflammatory and PGF_{2α}-EA and PGE₂-G being proinflammatory and hyperalgesic.

Pharmacological modulation of the COX-endocannabinoid axis

Further studies are warranted to dissect the extent of the production of these PG-EAs and PG-Gs *in vivo* in various inflammatory settings, as well as their effects and the receptors that mediate them. Different PG-EAs or PG-Gs can be produced, depending on the tissue or condition studied, due to different expression of PG synthases between tissues. Pharmacological modulation of the levels or receptors of these oxidized metabolites of endocannabinoids could open up new therapeutic paths.

Modulation of the COX-endocannabinoid axis by interfering with PG-Gs and PG-EAs levels

COX inhibitors, such as NSAIDs block the production of PGs as well as PG-EAs and PG-Gs. Inhibition of the production of those PG-EAs and PG-Gs that exert proinflammatory and algescic effects, such as PGF_{2α}-EA, PGF₂-G and PGE₂-G, could be part of the therapeutic efficacy of NSAIDs. However, not all oxygenated endocannabinoids are deleterious as PGD₂-G and PGE₂-EA exert anti-inflammatory effects (see above).

Besides its role in initiating inflammation, COX-2 has also been implicated in the resolution of inflammation. Indeed, COX-2^{-/-} mice exhibit an inflammatory phenotype with impaired resolution and administration of COX inhibitors in the late stages of an inflammation also leads to impaired resolution of inflammation. Along this line, the COX-2/PGD synthase axis has been put forth as generating pro-resolution mediators. This was imputed to PGD₂ itself and to its metabolite PGJ₂ [4]. In light of the new findings on PG-Gs, PGD₂-G could be considered as a mediator of the pro-resolution effects of COX-2. However, this is quite difficult to assess *in vivo* because classical COX inhibition blocks both the production of PGs and oxygenated endocannabinoids. Thus, one way to differentiate between them is the substrate selective inhibition of COX-2.

The first notion of COX-inhibitors that inhibit metabolism of endocannabinoids, but not AA came from rapid, reversible inhibitors of COX-2, such as ibuprofen and mefenamic acid, which inhibit endocannabinoid metabolism by COX-2 at lower doses than those required to inhibit AA metabolism (Box 1) [49]. Further studies identified

R-profens, the 'inactive' enantiomers of *S*-profens, as substrate-selective inhibitors of COX-2 that only inhibit endocannabinoid metabolism by COX-2, without affecting AA metabolism [27]. However, these *R*-profens can undergo unidirectional inversion to the *S*-enantiomers *in vivo* [50], prompting the development of achiral profens to increase *in vivo* stability. Inhibitors based on the flurbiprofen scaffold showed the higher selectivity and potency (Figure 1B) [50]. Tertiary amide derivatives of indomethacin (Figure 1B) were also synthesized and shown to increase endocannabinoid levels *in vitro* and *in vivo*, without affecting AA metabolism [29]. Compounds derived from flurbiprofen and naproxen that combine COX-2 substrate selectivity with a FAAH inhibitory properties are also under investigation (e.g., Flu-AM1, Figure 1B) [51].

COX-2 substrate-selective inhibition has been reported to increase the levels of endocannabinoids [25,26,28,29], and therefore, the observed effects could be due to simply increasing endocannabinoid levels, or decreasing PG-EAs and PG-Gs levels, or the result of inhibiting the formation of proinflammatory PG-EAs and PG-Gs and increasing the levels of anti-inflammatory cannabinoids. This would be an interesting approach in conditions such as Huntington's disease, in which 2-AG is known to be neuroprotective, whereas PGE₂-G exerts neurotoxic and proinflammatory effects [46]. The question remains as to why increasing 2-AG levels by inhibiting MAGL in the study by Valdeolivas *et al.* led to the production of the proinflammatory PGE₂-G and not to cannabinoid-mediated neuroprotection [46]. One hypothesis raised by the authors pertained to the time-course of COX-2 induction and increasing 2-AG levels following the injury. Indeed, in other studies, 2-AG is increased following a neurotoxic stimuli such as traumatic brain injury [52], which decreases COX-2 levels through a CB₁ and PPAR-γ dependent mechanism [53,54] and exerts neuroprotective effects. In this case of malonate-induced neurotoxicity, 2-AG levels were not increased by the injurious stimulus, whereas COX-2 was strongly upregulated [46]. Therefore, the subsequent increase in 2-AG due to MAGL inhibition provided a pool of substrate for the highly expressed COX-2 and led to the potential production of PGE₂-G. However, this remains speculative as the authors do not provide a time-course for the increase in 2-AG levels. Moreover, PPAR-γ was shown to be decreased following malonate-induced neurotoxicity [46] and a loss of cannabinoid receptors has been reported in the substantia nigra in Huntington's disease [55] potentially explaining the lack of effect of 2-AG via these receptors.

Further supporting the notion of using substrate-selective COX-2 inhibitors as potential therapeutic tools is the dichotomy between the anti-hyperalgesic effects of AEA in the spinal cord, and the hyperalgesic effects of PGF_{2α}-EA [41]. Indeed, inhibiting COX-2 in this setting would prevent oxygenation of the analgesic AEA, potentially increasing its levels, and inhibit the production of the hyperalgesic PGF_{2α}-EA, thus leading to an overall analgesic effect.

A caveat to COX-2 substrate-selective inhibition is that all PG-EAs and PG-Gs do not exert the same effects. Thus, inhibitors of PG synthases could be used in order to assess the specific contributions of these PG-EAs or PG-Gs. For instance, we showed that in LPS-activated macrophages,

the effects of 2-AG are not mediated by classical cannabinoid receptors activation, but rather by its COX-2 metabolism. *R*-flurbiprofen blocks the 2-AG-induced decrease of macrophage activation. Using the selective PGD synthase inhibitor, HQL79 (Figure 1B), we show that this effect is due to the production of the anti-inflammatory PGD₂-G [28].

Therefore, in such cases where the anti-inflammatory endocannabinoid-derived metabolites are mainly formed (i.e., PGD₂-G or PGE₂-EA), COX inhibition would not be the right therapeutic approach. In this case, inhibiting a given PG synthase could prove more beneficial. For instance, because PGE₂ and PGE₂-G are proinflammatory and PGD₂-G and PGD₂ are anti-inflammatory in several models [28,46,56,57], inhibiting PGE synthase could be the way to go, thus reducing production of the proinflammatory mediators. Moreover, because PG synthases compete for the same substrate, inhibiting PGE synthase could lead to increased PGD synthase products. However, the pharmacology of PGs *in vivo* is complex and this would certainly not be the right approach in asthma or allergic reactions where PGD₂ is implicated in disease pathogenesis. Further complicating the use of PG synthase inhibitors to dissect the contribution of PG-EAs and PG-Gs to inflammatory processes is the possibility that PGs and their endocannabinoid-derived counterparts could exert opposing effects. Therefore, a more thorough knowledge of which oxygenated products of the endocannabinoids are formed in a specific tissue and following specific stimuli is necessary to establish potential therapeutic targets.

Modulation of the COX-endocannabinoid axis by blocking/activating PG-EA or PG-G receptors

Using agonists or antagonists of the receptors mediating the effects of these oxygenated cannabinoids could be a useful approach to dissect selectively their impact on a given pathology. However, the receptors for these PG-EAs and PG-Gs remain largely unidentified, except for PGF_{2α}-EA which signals through a heterodimer of the PGF_{2α} receptor and one of its splice variants, FP-FPalt4 (Figure 2). Several antagonists of the PGF_{2α}-EA receptor (or bimatoprost receptor), such as AGN 204396, have been developed and were shown to block selectively the effects of bimatoprost (pA₂ = 5.64), but not PGF_{2α}, or PGE₂-G (Figure 1C) [58].

Because AEA exerts analgesic effects, whereas its COX-2 metabolite PGF_{2α}-EA is hyperalgesic [41], the idea of PGF_{2α}-EA receptor antagonists which could also inhibit AEA degradation seemed interesting. AGN 211335 and AGN 211336 (Figure 1C) are two such compounds that antagonize the bimatoprost receptor (affinity pA₂ values of 7.5 and 7.6, respectively) and inhibit FAAH, the primary enzyme responsible for AEA hydrolysis (IC₅₀ values of 3 and 3.6 μM, respectively). These compounds were shown to increase AEA levels *in vitro* and exert antinociceptive effects in the formalin-induced pain model in mice. Although these antagonists allow distinguishing between PGF_{2α} and PGF_{2α}-EA mediated effects, it has to be noted that they do bind to the TP and CB₁ receptors in the submicromolar and low micromolar ranges, respectively [59]. Therefore, at high doses, some of the effects of these antagonists could be due to off-target action at the TP

Box 3. Outstanding questions

Among the points that are not fully elucidated yet, the following questions are of particular interest.

- What are the receptors mediating the effects of PG-Gs and PG-EAs?
- Will it be possible to modulate one PG-G level without affecting PGs levels (i.e., substrate-specific inhibition of PG synthases)?
- To what extent do PG-Gs and PG-EAs contribute to the reported effects of NSAIDs in inflammation?
- Are some of these PG-Gs or PG-EAs pro-resolution lipids?

or CB₁ receptors and not to antagonism of PGF_{2α}-EA signaling.

Concluding remarks

The involvement of COX-2 in the metabolism of endocannabinoids increases the complexity of controlling inflammatory settings with COX inhibition. The new tools that are being developed and the recent studies depicting the biological actions of oxygenated endocannabinoids have increased our understanding of this system; however, several questions remain unanswered (Box 3) and require further investigation. Indeed, in a setting where these endocannabinoid-derived products are proinflammatory [41,46], whereas endocannabinoids exert anti-inflammatory effects, COX inhibitors would be very beneficial. However, some endocannabinoid metabolites such as PGD₂-G and PGE₂-EA exert beneficial effects [28], and thus their production is part of the anti-inflammatory effects exerted by endocannabinoids [28,43]. COX inhibition in this situation would decrease the production of PGs and also the production of these anti-inflammatory lipid mediators. Moreover, all PG-EAs or PG-Gs do not exert the same effects, therefore it would be interesting to see, depending on the tissue or the pathology, which PG synthase is more expressed and which PG-EA or PG-G would be preferentially formed. The knowledge of which products are more abundantly produced in a disease-specific setting should help to tailor the therapeutic approach.

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