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Harnessing the anti-inflammatory potential of palmitoylethanolamide

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Palmitoylethanolamide (PEA) is a peroxisome proliferator-activated receptor alpha (PPAR-α) ligand that exerts anti-inflammatory, analgesic and neuroprotective actions. PEA is synthetized from phospholipids through the sequential actions of N-acyltransferase and N-acylphosphatidylethanolamine-preferring phospholipase D (NAPE-PLD), and its actions are terminated by its hydrolysis by two enzymes, fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolysing acid amidase (NAAA). Here, we review the impact of PEA administration in inflammatory and neurodegenerative settings and the differential role of FAAH and NAAA in controlling PEA levels. Recent studies with NAAA inhibitors put forth this enzyme as capable of increasing PEA levels in vivo in inflammatory processes, and identified it as an interesting target for drug discovery research. Thus, PEA hydrolysis inhibitors could constitute potential therapeutic alternatives in chronic inflammatory and neurodegenerative diseases.

A brief overview of N-palmitoylethanolamine

N-Acylethanolamines (NAEs) constitute a family of endogenous bioactive lipids implicated in the regulation of several processes, from the modulation of food intake to pain and inflammation. Perhaps the most-studied NAEs are the endocannabinoid N-arachidonoylethanolamine (AEA), the anorexigenic compound N-oleoylethanolamine (OEA) and the anti-inflammatory and/or analgesic compound PEA.

NAEs share the same biosynthetic and degradation pathways (Fig. 1). They are synthetized on demand from membrane phospholipids by the sequential actions of an N-acyltransferase (to generate NAPEs) and a NAPE-PLD. Their actions are terminated by their hydrolysis by two enzymes, FAAH and the more recently described NAAA [1]. FAAH and NAAA have different catalytic properties and substrate specificity [2]. FAAH is a serine hydrolases that is active at neutral and alkaline pH, and has the highest reactivity against AEA, whereas NAAA is a cysteine amidase active at acidic pH (4.5–5), inactive at alkaline pH and is more efficient at hydrolyzing PEA [2].

Despite their shared metabolic pathways, NAEs constitute a diverse set of endogenous mediators, acting at different receptors and exerting a variety of effects. For instance, AEA is an endocannabinoid, classically exerting its actions by activation of two G protein-coupled receptors (GPCR), the CB₁ and CB₂ cannabinoid receptors. It is also a known ligand for the ion channel transient receptor potential vanilloid receptor (TRPV1) and the PPAR nuclear receptors. OEA is an anorexigenic compound, known to exert its actions through activation of PPAR-α, GPR119 and TRPV1. Our focus here is on PEA, which is a known anti-inflammatory compound with analgesic, neuroprotective and antiallergic properties.

The receptors mediating the effects of PEA had been elusive until recently, with increasing evidence that the anti-inflammatory and nociceptive effects of PEA are mediated, at least in part, by PPAR-α activation [3–7] (Box 1). Moreover, PPAR-α expression is downregulated in inflammatory settings and restored by PEA treatment [4].

It was postulated that PEA could bind to the CB₂ receptor [8]. However, this is not the case in other studies [9,10]. In addition, although the CB₂ antagonist SR144528 prevented the antinociceptive effects of PEA [11], it did not block its anti-inflammatory effects [12,13]. One explanation of these discrepancies is the possibility that SR144528 binds to a CB₂-like receptor [11]. Another explanation is that PEA could compete with AEA for FAAH-mediated hydrolysis, thus causing an increase in AEA levels, which would then activate the CB₂ receptors. Moreover, PEA was shown

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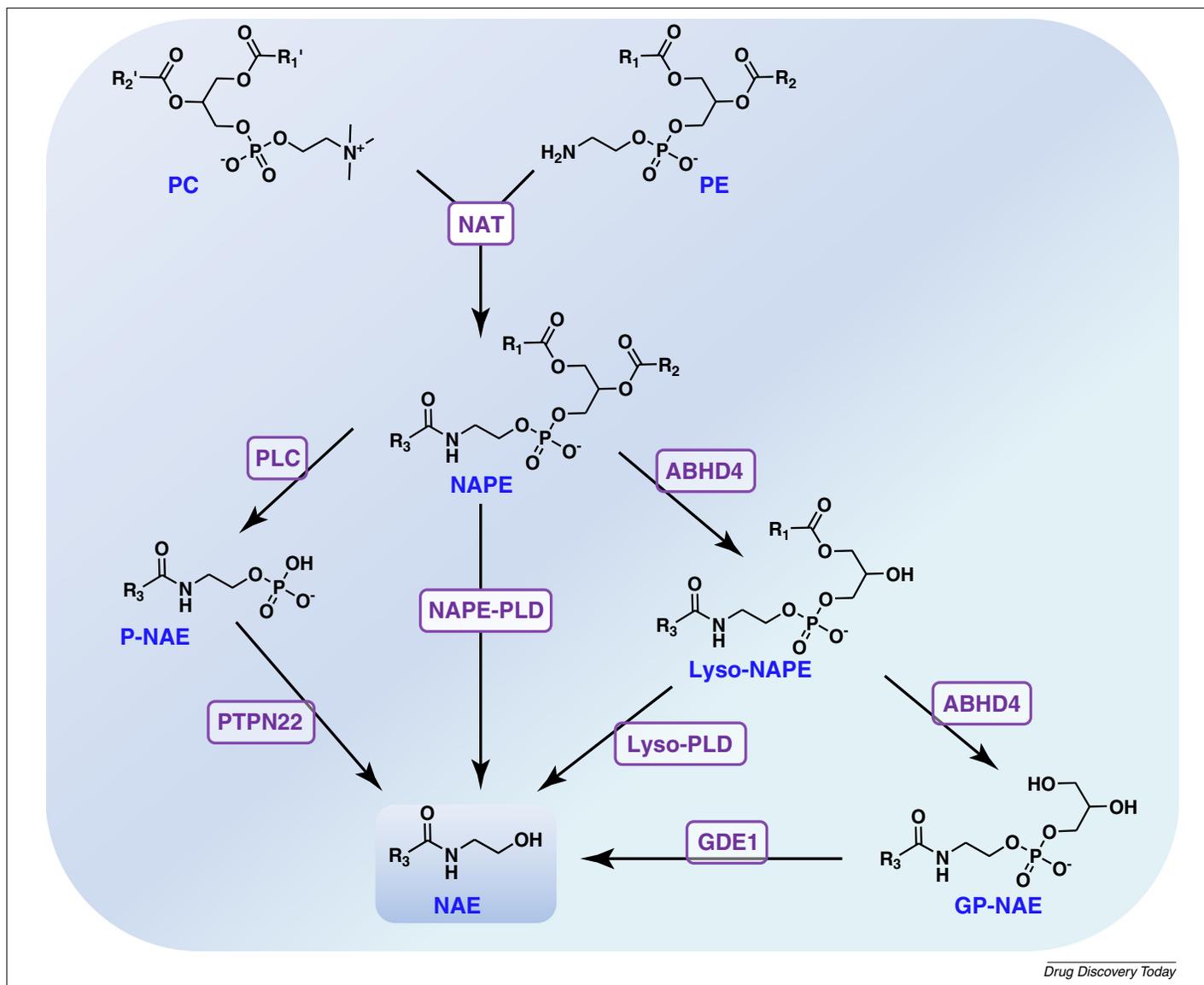
FIGURE 1

N-acylethanolamines biosynthesis. N-acylphosphatidylethanolamines (NAPE), the key precursors in the synthesis of N-acylethanolamines (NAE), are obtained from phosphatidylethanolamines (PE) and phosphatidylcholines (PC) via the action of a Ca^{2+}-dependent N-acyl transferase (NAT) [R'1 = R3]. Several routes have been described for the synthesis of NAE from NAPE. A N-acylphosphatidylethanolamine-preferring phospholipase D (NAPE-PLD) can directly release NAE from NAPE. Alternatively, phospho-N-acylethanolamine (P-NAE) can be obtained from NAPE via a phospholipase C activity (PLC), and the phosphate group subsequently removed by phosphatases, such as PTPN22. Lyso-NAPE, another intermediate in the synthesis of NAE, is obtained by the removal of one acyl chain from NAPE by the α/β-hydrolase domain 4 (ABHD4). Lyso-NAPE can be directly hydrolyzed by lyso-phospholipase D (lyso-PLD) to yield NAE, or by ABHD4 to yield a glycerophospho-N-acetylethanolamine (GP-NAE), which is then converted to NAE by the glycerophosphodiesterase GDE1.

to potentiate the affinity and potency of AEA for TRPV1. This was independent from the inhibition of its hydrolysis, because PEA was also able to potentiate the effect of low concentrations of resiniferatoxin and capsaicin, two other TRPV1 agonists [14]. This phenomenon was coined the 'entourage effect'. Interestingly, in one study, PEA administration decreased rather than increased AEA levels [3]. However, this was in an inflammatory setting and the authors did not report the effect of inflammation on AEA levels; therefore, the decrease in AEA levels could be the result of the anti-inflammatory effects of PEA.

Although it is now clear that PEA is a ligand for PPAR-α, some of its effects occur through as yet unidentified receptors. PEA was proposed as a putative ligand for the recently deorphanized GPR55 in contradicting pharmacological assays; however, there are few

data so far to support this claim. In a human mastocytic cell line, HMC-1, the inhibitory effect of PEA on nerve growth factor release was abolished in the presence of GPR55 RNAi [15]. There is also evidence for an effect of PEA on cAMP accumulation in microglial cells through a $G_{i/o}$-coupled GPCR, distinct from CB_1 and CB_2 [16]. Further studies are warranted for a better understanding of the receptors mediating the effects of PEA.

Impact of inflammation on PEA metabolism

Evidence suggests that PEA metabolism is disturbed during inflammation, and that a decrease in PEA levels contributes to the inflammatory response. For instance, stimulation with pro-inflammatory agents, such as the endotoxin lipopolysaccharide (LPS) or the irritants carrageenan and croton oil, led to decreased PEA levels

in cells and tissues [13,17–19]. PEA levels also decreased following sciatic nerve constriction injury or ligation of the sciatic nerve in spinal cord and brain areas involved in nociception [20].

In some instances, this decrease results from decreased biosynthesis, as is the case in the murine macrophage cell line RAW264.7, in which activation with the endotoxin LPS suppressed transcription of NAPE-PLD. This suppression of NAPE-PLD transcription was accompanied by a decrease in NAPE-PLD activity, with no effect on FAAH or NAAA activity [19]. Interestingly, the authors showed in the same study that NAPE-PLD$^{-/-}$ mice were able to synthetize PEA through an alternative pathway that is not regulated by pro-inflammatory stimuli. Accordingly, pro-inflammatory stimuli did not affect PEA levels in these mice and elicited a limited response from macrophages [19]. This would support the hypothesis that PEA has a role in dampening an inflammatory response and that blocking its hydrolysis could be a means to re-establish PEA levels that could then exert homeostatic and anti-inflammatory functions. In other cases where PEA levels were decreased, an increase in FAAH protein levels was observed [13]; however, PEA levels were decreased by carrageenan injection into the rat hind paw, with no variation in FAAH activity in another study [21]. In this latter study, PEA levels were restored following treatment with a cyclooxygenase 2 (COX-2) inhibitor [21]. The fact that an anti-inflammatory treatment restores PEA levels reinforces the role of PEA as an anti-inflammatory mediator.

More studies implicate a dysregulation in NAE metabolism in inflammatory settings. In biopsies of patients with ulcerative colitis, NAPE-PLD immunoreactivity in the colonic epithelium seems to be decreased during acute flares and returns to normal following treatment. This would suggest a potentially reduced production of NAEs during colon inflammation. By contrast, FAAH and NAAA immunoreactivity were greatly increased in infiltrating immune cells during acute colitis [22]. Taken together, these changes suggest that we could expect a decrease in PEA levels during colon inflammation; however, although some researchers found that colon inflammation did not affect PEA levels in two models of murine colitis and biopsies of patients with Crohn's disease, others showed increased PEA levels in dextran sulfate sodium (DSS)-induced colitis and biopsies of patients with ulcerative colitis [7,23,24]. This discrepancy might be the result of a difference in infiltrating cells between the models and in the expression of the enzymes responsible for the biosynthesis and hydrolysis of PEA, or perhaps a difference in the severity of inflammation and tissue destruction [24].

In the brain, PEA levels seem to be increased following injurious stimuli, and this has been proposed as a homeostatic mechanism aimed at counteracting inflammation and blunting the inflammatory response. PEA levels are increased in the brain after focal cerebral ischemia [16], in areas of nerve damage in multiple sclerosis (MS) models and in astrocytes stimulated with beta amyloid (Aß)

BOX 1

Peroxisome proliferator-activated receptors and inflammation

The PPARs subfamily, which comprises three members PPAR-α, PPAR-γ and PPAR-δ, is part of the nuclear hormone receptor superfamily of ligand-activated transcription factors. PPARs form heterodimers with retinoid X receptors (RXRs), which are also members of the nuclear hormone receptor superfamily, and regulate target gene expression by binding to specific peroxisome proliferator response elements in the promoter region of target genes, resulting in their activation or suppression [73].

In the absence of a ligand, transcriptional activation is prevented by the formation of high-affinity complexes between the PPAR–RXR heterodimer and nuclear receptor co-repressor proteins. Binding of a ligand to the heterodimer results in the release of the co-repressor from the complex and binding of the activated heterodimer to the response element on the DNA.

PPAR-α has been implicated in the regulation of inflammatory responses in vitro and in vivo and recognizes several structurally heterogeneous molecules because of its large multifunctional ligand-binding pocket. Among natural PPAR-α agonists are nonesterified fatty acids, oxygenated fatty acids and fatty-acid ethanolamides, such as PEA (EC$_{50}$ = 3.1 MM) [3] and OEA (EC$_{50}$ = 120 nM) [74]. PEA activates the nuclear receptor PPAR-α with potency comparable to the synthetic agonist, WY-14643 (EC$_{50}$ = 1.4 MM) [3].

Synthetic PPAR-α agonists comprise ligands that are potent at reducing hyperlipidemia, atherosclerosis and inflammation in animal models, such as the fibrates, which are used in the clinic to treat dyslipidemia [73].

PPAR-α reduces inflammation by inducing the expression of anti-inflammatory proteins, such as IκB-α, which prevents the nuclear translocation of NF-κB, repressing the expression of pro-inflammatory proteins such as TNF-α, and limiting the recruitment of immune cells [75]. These transcription-dependent effects occur over a period of hours or even days [3,39]. However, some of the

effects of PPAR-α agonists, such as the antinociceptive effects of PEA, occur rapidly after agonist administration through transcription-independent mechanisms [11,76] probably through activation of large- and intermediate-conductance calcium-operated potassium channels [76].

To support the fact that the effects of PEA are PPAR-α dependent, they were tested in PPAR$^{-/-}$ mice or in the presence of the PPAR-α antagonist GW6471, and reproduced by the synthetic PPAR-α agonists WY-14643 and GW7647 (Fig. I).

GW6471
(IC$_{50}$ = 240nM)

WY-14643
(EC$_{50}$ = 630nM)

GW7647
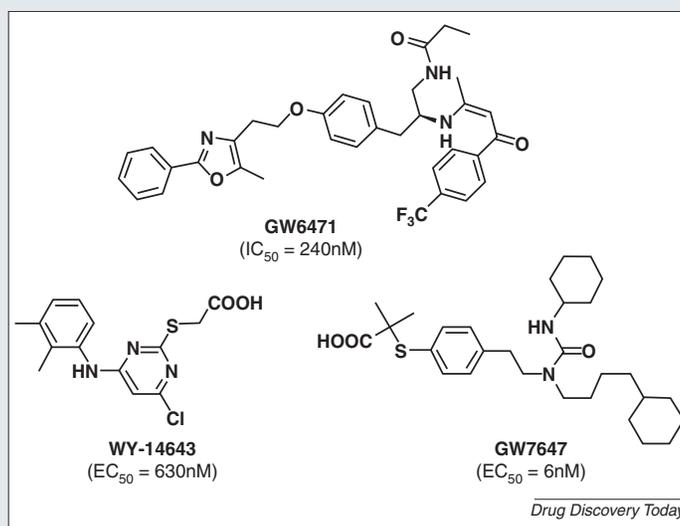(EC$_{50}$ = 6nM)

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FIGURE I

Selected PPAR-α ligands used in the studies with PEA. Structures and potency values for GW6471, a PPAR-α antagonist and WY-14643 and GW7647, two PPAR-α agonists.

peptide [25–27]. This 'pro-homeostatic' increase, although probably slowing disease progression, seems insufficient to exert anti-inflammatory effects in itself, and should be further amplified by the use of PEA hydrolysis inhibitors to attain a therapeutic effect.

It is also interesting to consider the fact that PEA and AEA levels vary differently in some settings. For instance, whereas PEA levels are increased in the brain in a murine model of MS, there is no change in AEA levels [27]. Conversely, in astrocytes in primary culture, ionomycin increased AEA levels, without affecting PEA [28]. The same effect was observed following activation of α7 nicotinic receptors in neurons in primary culture, whereas activation of muscarinic receptors increased PEA, but not AEA levels [29]. Activation of RAW264.7 macrophages in culture with LPS led to decreased PEA levels and increased AEA levels [18], whereas in peritoneal macrophages, AEA levels increased with no change in PEA levels [30]. Taken together, these observations suggest that, although these two NAEs share the same metabolic pathways, different biosynthetic pathways have a predominant role for each of them. This is reinforced by the finding that PEA levels were decreased in the brain of NAPE-PLD$^{-/-}$ mice under physiological conditions, whereas AEA levels were unchanged [31]. Conversely, mice deficient in glycerophosphodiester phosphodiesterase 1 (GDE1), an enzyme responsible for a NAPE-PLD independent synthesis of NAEs, or deficient in GDE1 and NAPE-PLD did not exhibit reduced NAEs levels in the brain under physiological conditions [32]. However, both PEA and AEA levels were decreased in another NAPE-PLD$^{-/-}$ strain [33]. Altogether, these results suggest yet another biosynthetic pathway distinct from NAPE-PLD and GDE1.

By contrast, the fact that PEA levels are not systematically increased in FAAH$^{-/-}$ mice [18,34] also suggests that PEA and AEA are hydrolyzed by different enzymes depending on the tissue or condition. Indeed, PEA and AEA levels are increased in the brain of FAAH$^{-/-}$ mice [35], but there is no increase in PEA levels in the duodenum or immune cells [18,34]. This is reinforced by the fact that FAAH inhibitors do not always increase PEA levels [18,21,36]. Moreover, although both FAAH and NAAA were shown to be responsible for NAE hydrolysis in macrophages, AEA hydrolysis in the brain was shown to be mostly FAAH dependent [37]. Additionally, an enzyme distinct from FAAH and NAAA and responsible for PEA hydrolysis in microglial-like cells has been suggested [38]. Therefore, it would be interesting to dissect these differences further in a tissue- and disease-specific manner.

The role of PEA in inflammation-related pathologies

Although the first identification of PEA as an anti-inflammatory compound occurred more than 50 years ago, general interest in its anti-inflammatory and analgesic properties was not sparked again until the mid-1990s [11,39]. In these early studies, the anti-inflammatory effects of PEA were investigated in various settings, in vivo or in vitro. PEA was shown to reduce tumor necrosis factor alpha (TNF-α) release in LPS-induced pulmonary inflammation in mice as well as mast cell degranulation and edema formation in various inflammatory models [39].

The mechanisms responsible for this anti-inflammatory effect have been investigated more recently. PEA inhibits phosphorylation of kinases involved in activation of pro-inflammatory pathways, such as mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases (ERK), and the nuclear translocation of the transcription factors nuclear factor (NF)-κB and activator protein 1 (AP-1) and prevents degradation of the inhibitory IκB-α, which when associated to NF-κB prevents its nuclear translocation [4,7].

Besides reducing inflammatory cells activation and recruitment [6,18,40,41], PEA modulates the expression of enzymes involved in pro-inflammatory processes, such as COX-2 and inducible nitric oxide synthase (iNOS) and reduces nitric oxide and pro-inflammatory cytokines production in vitro and in vivo, following different pro-inflammatory stimuli, such as carrageenan or LPS [4–6,41].

In a murine model of inflammatory bowel diseases induced by DSS, PEA administration reduced the macroscopic signs of colon inflammation as well as neutrophil infiltration, COX-2 and iNOS expression and splenomegaly in a PPAR-α-dependent manner. The effect of PEA also extended to a decrease in pro-inflammatory cytokines, and prostaglandin E$_2$ (PGE$_2$) release in the plasma [7]. In this same study, PEA was also shown to be effective when applied to cultured human biopsies of patients with ulcerative colitis, again in a PPAR-α-dependent manner [7]. Colon inflammation is accompanied by an increase in activation of enteric glial cells. These enteric glial cells, when isolated from the colons of mice with DSS-induced colitis, expressed high levels of S100B and Toll-like receptor 4 (TLR4), which were reduced by PEA. This led to an inhibition of the TLR4 signaling pathway and downstream inhibition of NF-κB activation [7]. In chronic granulomatous inflammation in rats, PEA reduced granuloma formation and decreased the number of infiltrating mast cells and their degranulation [42].

The neuroprotective effects of PEA are in part the result of its effects on downregulating the inflammatory cascade. Indeed, many neurodegenerative diseases are associated with a strong inflammatory component, such as Alzheimer's disease (AD), Parkinson's disease (PD) or MS [43]. Activation of glial cells and the subsequent inflammatory response are a prominent feature of the pathogenesis of these diseases and can aggravate their course [43]. This neuroinflammation is no longer simply considered as a consequence of neurodegeneration, but might be a primary factor in some cases; therefore, anti-inflammatory treatments might represent interesting therapeutic strategies in these diseases [44], especially when coupled with neuroprotective compounds. In AD, Aβ accumulation leads to overactivation of glial cells. In rat neuronal cultures and hippocampal slices challenged with Aβ, PEA reduced astrocyte activation measured by GFAP, S100B, iNOS and COX-2 expression as well as pro-inflammatory cytokines and PGE$_2$ production, thus blocking the subsequent inflammatory response and exerting neuroprotective effects against Aβ-mediated neurotoxicity [5]. These effects were mediated by inhibition of MAPK phosphorylation and the transcription factors NF-κB and AP-1 and were partly blocked by PPAR-α antagonism [5]. In mice receiving the neurotoxin MPTP to trigger symptoms similar to PD, PEA administration protected against the loss of dopaminergic neurons and reduced behavioral impairment. These effects were accompanied by a decrease in MPTP-induced microglia and astrocytes activation, as measured by GFAP-expressing astrocytes, S100B overexpression and iNOS expression. Some of the effects of PEA were decreased or lost in PPAR-α$^{-/-}$ mice, whereas others were not affected, thus pointing to the presence of additional molecular targets for PEA that are yet to be discovered [45]. In a model of MS induced by Theiler's virus inoculation to mice, PEA administration

reduced microglia activation and pro-inflammatory cytokine expression in the spinal cord [27].

It has been proposed that pain sensitivity could be altered by non-neuronal cells, such as mast cells or microglia, which are activated in neuropathic pain models and participate in chronic pain states; thus, the local post-traumatic inflammatory response amplifies the damage [46]. PEA was shown to reduce mast cell recruitment and degranulation, as well as microglia activation in a model of chronic constriction nerve injury in mice [40], and spinal microglia and astrocytes activation in mice treated with formalin to induce neuropathic-like pain [41]. In the context of inflammatory hyperalgesia induced by carrageenan injection into the paws of rats, PEA reduced paw edema as well as COX-2 and iNOS expression in the paw and nitric oxide production [12]. In another experiment of carrageenan-induced hyperalgesia, intracerebroventricular PEA administration reduced paw edema, COX-2 and iNOS expression in the sciatic nerve in a PPAR-α-dependent manner. PEA also restored PPAR-α expression in dorsal root ganglia [47]. Although in other experiments PEA is administered intraperitoneally, this study shows that PEA acting in the central nervous system is able to reduce peripheral effects.
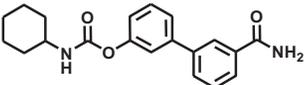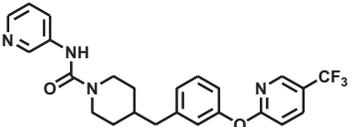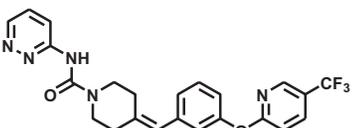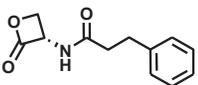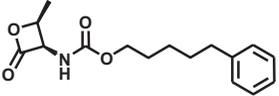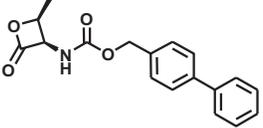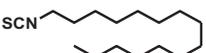
In mice with spinal cord injury, PEA reduced microglia and astrocyte activation as well as edema, inflammatory cell infiltration, pro-inflammatory cytokine production and iNOS expression in the spinal cord, and ultimately reduced motor dysfunction [6,48,49]. This effect was shown to be PPAR-α dependent, but was also blocked in one study by antagonists of PPAR-γ and PPAR-δ [6]. However, PEA did not activate PPAR-γ and PPAR-δ in vitro [3], and administration of ciglitazone, a PPAR-γ agonist, and GW0742, a PPAR-δ agonist, did not reproduce the effects of PEA in another study. Therefore, this is probably an indirect effect of PEA. Indeed, the expression of PPAR receptors is downregulated in this case and in other reports of inflammation-induced hyperalgesia, and this was re-established by PEA treatment [4]. Therefore, PEA could be affecting the activation of PPAR-γ and PPAR-δ by their endogenous ligands.

Overview of available inhibitors of FAAH and NAAA

Although both FAAH and NAAA are able to hydrolyze the amide bond of NAEs, the two enzymes differ in their structure and catalytic mechanisms. These differences allowed the development of selective inhibitors able to inhibit FAAH without affecting NAAA activity and, more recently, of NAAA inhibitors devoid of FAAH affinity (Table 1).

TABLE 1

Selected FAAH and NAAA inhibitors[a]

Name	Structure	FAAH	Refs	NAAA	Refs
FAAH inhibitors					
URB597		IC_{50} = 5 nM; K_{inact}/K_i = 1590 M^{-1} s^{-1}	[52,57]	No inhibition	[37]
PF-3845		K_{inact}/K_i = 12,600 M^{-1} s^{-1}	[57]	ND	
PF-04457845		IC_{50} = 7–50 nM[b]; K_{inact}/K_i = 40,300 M^{-1} s^{-1}	[58]	ND	
NAAA inhibitors					
(S)-OOPP		No inhibition	[18]	IC_{50} = 420 nM	[18]
ARN077 (URB913)		No inhibition	[62]	IC_{50} = 50–130 nM	[62,63]
14q		No inhibition	[62]	IC_{50} = 7 nM	[62]
AM9023		No inhibition	[66]	IC_{50} = 600 nM	[66]

[a] The FAAH and NAAA inhibitors reported here were selected based on their potency and selectivity. For FAAH inhibitors, their wide use in animal models (URB597–PF-3845) or clinical study (PF-04457845) were also a criteria of choice. More comprehensive reviews on FAAH inhibitors are available [50,51]. K_{inact}/K_i values are the more accurate way to characterize irreversible inhibitors potency. Depending on pre-incubation time.

FAAH is a serine hydrolase with an atypical catalytic triad constituted of Lys142–Ser217–Ser241, rather than the typical catalytic triad, Ser–His–Asp, of serine hydrolase, with Ser241 acting as the catalytic nucleophile. Several X-ray crystal structures of the enzyme in complex with various inhibitors were solved and enabled researchers to explore the interactions between inhibitors and the enzyme. The use of activity-based protein profiling technologies also greatly favored the development of highly selective inhibitors. Several chemical motifs have been used to develop FAAH inhibitors, including α-ketoheterocycles, β-lactams, carbamates (URB597) and arylureas (PF-3845 and PF-04457845) [50,51].

URB597, the most widely used FAAH inhibitor based on a carmabate moiety, irreversibly inhibits FAAH activity by carbamylation of the catalytic serine [52,53]. It is a potent and rather selective inhibitor, devoid of affinity for monoacylglycerol lipase (MAGL) and NAAA, although it inhibits several carboxylesterases in the liver [54]. When administered in vivo, URB597 induces a strong increase in AEA levels in the brain, liver and several other tissues [55]. This prompted its use in numerous preclinical models over the past decade. A recent evolution of URB597 is the peripherally restricted URB937, which will enable researchers to distinguish between centrally and peripherally mediated effects of FAAH inhibition [56].

Several urea derivatives were developed as FAAH inhibitors, notably by increasing the reactivity of the urea moiety towards the catalytic triad. Among these derivatives, PF-3845 emerged as one of the most widely used in animal models. PF-3845 is a highly potent and selective inhibitor interacting with FAAH in a covalent and irreversible way [57]. Its administration in vivo results in long-lasting increase in AEA levels (up to 24 h) [57]. Modifications of PF-3845 led to PF-04457845, which combines high potency and selectivity with oral bioavailability [58]. PF-04457845 is currently being investigated in clinical trials with, to date, mitigated results because, despite being able to increase plasma AEA levels, it had no effect on pain levels in patients with osteoarthritis [59].

Regarding NAAA inhibitors, and despite recent progress, the field is less crowded when compared with FAAH inhibitors because of the more recent molecular identification of NAAA. NAAA is a lysosomal cysteine hydrolase of the N-terminal nucleophile hydrolase superfamily. The mature form of the enzyme results from an autocatalytic cleavage taking place at an acidic pH and involving the catalytic nucleophile Cys126. The catalytic triad comprises the Cys126–Arg142–Asp145 residues [60].

Following the seminal studies showing that fatty acid amides were micromolar NAAA inhibitors [61], the first breakthrough came with the description of β-lactone derivatives as potent and selective NAAA inhibitors. The first inhibitor of this series, (S)-OOPP, inhibited NAAA in the submicromolar range and was selective against FAAH [18]. Evolutions of (S)-OOPP led to several 2-methyl-β-lactone derivatives, such as ARN077, which showed improved potency towards NAAA [62]. These compounds inhibit NAAA through a covalent mechanism involving the catalytic Cys126 residue. These inhibitors enabled researchers to demonstrate for the first time that NAAA inhibition could lead to increased PEA levels both in cells and in vivo after local administration. Indeed, the major drawback of this series of compounds is its poor in vivo stability [62–65]. Another series of NAAA inhibitors is represented by AM9023, a lipophilic isothiocyanate that interacts with NAAA in a reversible way [66].

Targeting FAAH and NAAA to control PEA levels in vivo

Given that PEA has been proposed to behave as a local autacoid, it might be beneficial to increase its levels where it is produced. This could be done by inhibiting the enzymes responsible for its hydrolysis. However, the question remains whether FAAH or NAAA would be the right target in a given pathology or tissue.

There are relatively few NAAA inhibitors that can be used to study the effects of increasing PEA levels in pathophysiological settings. By contrast, several potent FAAH inhibitors can be used as a means to increase AEA levels and dissect the role of this endocannabinoid. However, because FAAH does not hydrolyze PEA with the same efficiency as AEA, pharmacological inhibition of this enzyme, or even its genetic deletion, does not always lead to increased PEA levels. Indeed, some data with FAAH knockout mice showed no differences in PEA levels in the small intestine in one study [34], whereas they were increased in the duodenum in another study [55], both under physiological conditions. FAAH inhibition with arachidonoylserotonin (AA-5-HT) led to increased PEA levels in the stomach [67] and small intestine [34] of mice, both under physiological conditions, and no increase in the colon of mice with pre-neoplastic lesions [36]. Another FAAH inhibitor, URB597, increased AEA levels in primary cultures of dorsal root ganglion (DRG) neurons but had no effect on PEA levels [68], whereas the NAAA inhibitor ARN077 increased PEA levels without affecting AEA levels [69]. In the carrageenan-induced inflammatory hyperalgesia model in rats, intraplantar administration of URB597 increased AEA but not PEA levels in the inflamed paw [21], while intraperitoneal administration of URB597 increased PEA and AEA levels in the spinal cord (and brain), but not in the inflamed paw [70]. The NAAA inhibitor (S)-OOPP increased PEA levels in macrophages stimulated with LPS, but had no effect on AEA levels [18].

Here, it is important to consider that the contribution of FAAH and NAAA to NAEs hydrolysis might be different depending on the tissue or pathology. Moreover, the fact that FAAH inhibitors generally increase AEA levels makes it harder to dissect the contribution of PEA to the observed effects, although this is sometimes possible. In an elegant study, Lichtman and colleagues dissected some of the effects resulting from genetic ablation of FAAH. In this study, FAAH$^{-/-}$ mice exhibited less carrageenan-induced paw edema compared with their wild type counterparts and this effect was only reproduced by PEA administration to wild type mice, whereas AEA had no effect [71].

Therapeutic potential of NAAA inhibitors

Among the first-described NAAA inhibitors, the β-lactone derivative (S)-OOPP is a relatively potent inhibitor, although with a limited plasma stability [18]. Therefore, this compound was tested in local administration schemes. Sponges instilled with carrageenan and vehicle or the NAAA inhibitor were implanted subcutaneously in mice and, 3 days later, the leukocytes (neutrophils and macrophages) infiltrating the sponges were collected. PEA levels were decreased in the leukocytes in the presence of carrageenan and restored in presence of the NAAA inhibitor, which also inhibited leukocyte infiltration and plasma extravasation induced by

carrageenan. Whereas PEA levels remained increased by the NAAA inhibitor in PPARα$^{-/-}$ mice, its anti-inflammatory effects were abrogated. This compound also increased PEA levels in RAW264.7 macrophages stimulated with LPS and decreased TNF-α production. In the same experiments, URB597 had no effect on PEA levels. In a model of spinal cord injury, intrathecal administration of (S)-OOPP reduced neutrophil infiltration and iNOS expression [18]. Another NAAA inhibitor [1-(2-biphenyl-4-yl)ethyl-carbonyl pyrrolidine; IC$_{50}$ = 2100 nM], also increased PEA levels in RAW264.7 macrophages stimulated with LPS and decreased iNOS and interleukin (IL)-6 expression in a dose-dependent manner [72].

Concluding remarks and future research directions

As outlined here, the potential of using PEA as a beneficial endogenous bioactive lipid in the setting of inflammation is well established. To make use fully of its properties, several research directions should be further explored. Among these, a better understanding of how NAAA and FAAH share the role of controlling PEA tissue levels is needed, both in control and in disease situations. A related point will be to determine how NAEs levels are affected by NAAA inhibition. FAAH inhibition results in strong increase in NAE levels (AEA, PEA, OEA, N-stearoylethanolamine, etc.), but it remains to be seen whether the marked substrate preference of NAAA for PEA, over the other NAEs, is also present *in vivo*. Another question deserving a closer look is the specificity of NAAA towards NAEs. Indeed, although it is well known that FAAH inhibition results in increased levels of the bioactive *N*-acyltaurines (in addition to the increase in NAE levels), it is less clear whether *in vivo* NAAA inhibition might affect other metabolite levels beside NAE. The development of potent and selective NAAA inhibitors that are active *in vivo* will certainly help to answer these questions. Although great progress has been made over the past few years in that direction (Table 1), improved efficacy *in vivo* might still be necessary to understand NAAA functions fully.

In conclusion, understanding PEA metabolism by FAAH and NAAA in inflammation and inflammation-related pathologies will open up new therapeutic avenues because these two enzymes are the gatekeepers of the beneficial effects of PEA. The future will tell which strategy will most efficiently tackle inflammation between FAAH inhibition, NAAA inhibition or direct PEA administration, depending on the tissue and inflammatory condition.

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