The Palmitoylethanolamide Family: A New Class of Anti-Inflammatory Agents?

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Abstract: The discovery of anandamide as an endogenous ligand for the cannabinoid receptors has led to a resurgence of interest in the fatty acid amides. However, *N*-palmitoylethanolamine (PEA), a shorter and fully saturated analogue of anandamide, has been known since the fifties. This endogenous compound is a member of the *N*-acylethanolamines, found in most mammalian tissues. PEA is accumulated during inflammation and has been demonstrated to have a number of anti-inflammatory effects, including beneficial effects in clinically relevant animal models of inflammatory pain. It is now engaged in phase II clinical development, and two studies regarding the treatment of chronic lumbosciatalgia and multiple sclerosis are in progress. However, its precise mechanism of action remains debated. In the present review, the biochemical and pharmacological properties of PEA are discussed, in particular with respect to its analgesic and anti-inflammatory properties.

1. INTRODUCTION

When William Devane isolated *N*-arachidonoylethanolamine (**1**) from brain lipid extracts, christened anandamide [1], in the early nineties and proposed it as an endogenous ligand for cannabinoid receptors, there was a dramatic upsurge in interest in the pharmacology, biochemistry and physiology of the fatty acid amides. However, several fatty acid amides (*N*-acylethanolamines, abbreviated here as NAEs) had been known for more than 40 years. Among the NAEs, *N*-palmitoylethanolamine (PEA, **2**), a shorter and fully saturated analogue of anandamide, is the most-studied compound and the subject of the present review. The purpose of this paper is:

1) To present briefly the origin, occurrence, biosynthesis and cellular removal of PEA

2) To describe its pharmacological effects, in particular with respect to inflammation and pain.

3) To review the few structure-activity relationship data available in the literature

2. ORIGIN AND OCCURRENCE OF PEA

PEA, also called palmitoylethanolamide or palmidrol by some authors, is a naturally occurring C16:0 fatty acid

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derivative where the carboxylate function is amidated by the primary amine of ethanolamine (Fig. **1**). Its chemical name is *N*-(2-hydroxyethyl)hexadecanamide. PEA was first synthesized by refluxing ethanolamine with palmitic acid [2] giving white crystals melting at 98-99°C. Due to the simplicity of structure, various syntheses of PEA have been described : the acyl chloride is the most common, but activating agents such as dicyclohexylcarbodiimide and carbonyldiimidazole allow the condensation between the acid and the ethanolamine in very good yields (> 80 %).
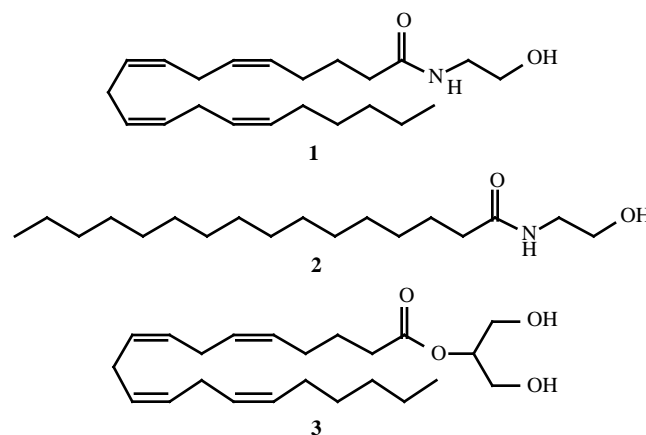
Fig. (1). Structures of *N*-palmitoylethanolamine (PEA, **2**) compared to anandamide (**1**) and 2-arachidonoylglycerol (**3**), the two main endocannabinoids proposed as endogenous ligands of cannabinoid receptors.

The evidence that PEA is a natural compound came from Kuehl *et al.* [3] who isolated PEA from soybean lecithin, egg yolk and peanut meal. In 1965 Bachur and co-workers

[4] reported the presence of NAEs, including PEA in the lipid fraction of rat brain, liver and skeletal muscle. Since then, the presence of PEA (as well in some cases as its higher homologue *N*-stearoylethanolamine) has been found in the mouse brain and spinal cord [5], canine heart extracts [6], degenerating tissues [7], testis [8], paw skin [9] and in peritoneal macrophages [10]. NAEs are even present in the blood. By using isotope dilution GC/MS determinations of anandamide, PEA and *N*-oleoylethanolamine, Giuffrida *et al.* [11] found small amounts of these compounds in rat blood plasma, the most abundant being PEA at a concentration of 16.7 ± 2.7 pmol/ml. PEA is also found in human cerebrospinal fluid, and the levels are increased in fluid obtained from patients with schizophrenia [12], although the importance of this finding is at present unclear.

In addition to mammalians, there is evidence of the presence of PEA as well as other NAEs in marine species. The fatty acid amides were found in lipid extracts of five bivalve mollusks, *Mytilus galloprovincialis*, *Venus verrucosa, Tapes decussatus, Callista chione, Crassostrea* sp. [13], and from sea urchin ovaries [14]. It seems that PEA is present throughout animal evolution, as it was recently

detected in the central nervous system of the leech *Hirudo medicinalis* [15].

3. THE PEA "LIFE CYCLE"

3.1. Biosynthesis

Two biosynthetic pathways have been proposed in the 1970's-80's for PEA and extensively reviewed elsewhere [7, 16, 17, 18]. In principle, the pathways are analogous to those for anandamide, namely a) the "in reverse activity" of an enzyme that normally catalyzes PEA hydrolysis, in this case producing PEA by the condensation of ethanolamine and palmitic acid, through an ATP- and CoA-independent process [19] (presumably fatty acid amide hydrolase acting in reverse); and b) the hydrolysis of a phospholipid precursor, *N*-palmitoyl-phosphatidylethanolamine by a phosphodiesterase of the D type [20] (see Fig. **2**). The second biosynthetic route is probably the major one.

One of the key features of palmitoylethanolamide is that this endogenous substance accumulates during inflam-
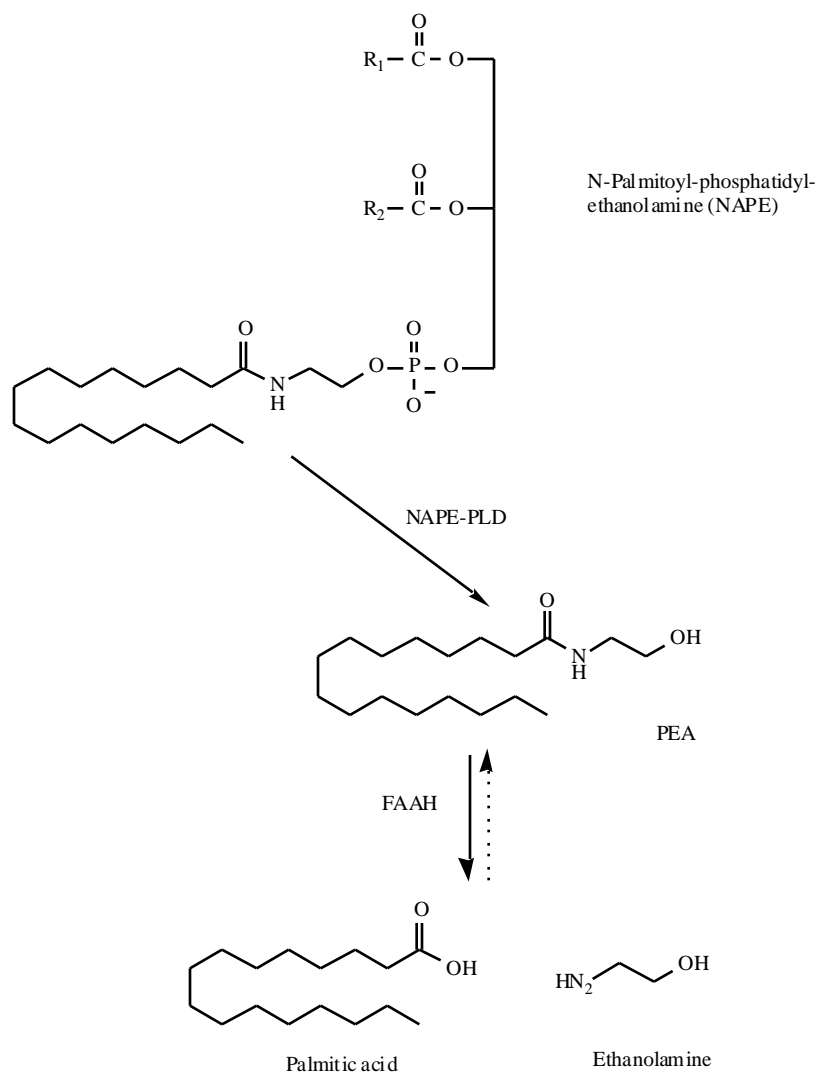
Fig. (2). Schematic representation of the synthesis and metabolism of PEA. FAAH, fatty acid amide hydrolase.

mation. Kondo and co-workers [8] noted that PEA was the major component (44.7 %) of the NAE family of compounds in rat testis. When inflammation and degeneration was induced by the injection of cadmium chloride, the total amount of NAEs in rat testis increased up to 25-fold with a preponderance of PEA. Cell death, as in post-mortem tissues, or, more simply, cell damage, as during ischemic conditions or in glutamate-induced neurotoxicity, also caused a several-fold increase of PEA and NAE levels [7, 21, 22, 23]. Recently, Baker *et al.* [5] found an increased level of PEA in the spinal cord of spastic (but not non-spastic) mice suffering from chronic relapsing experimental allergic encephalomyelitis. However, not all inflammatory stimuli are sufficient to produce an increased concentration of PEA: paw skin PEA concentrations do not increase 1 hour following local injection of formalin (1-5%) [24]. Nevertheless, the finding that during certain types of inflammation, the local synthesis of a compound with documented anti-inflammatory properties (see below) increases, raises the possibility that compounds affecting the metabolism of PEA may be useful therapeutically.

3.2. Cellular Removal And Degradation

In vivo, the actions of PEA (summarised in Section 3 below) and anandamide are relatively short-lived, due to their rapid metabolism. As with PEA biosynthesis, the mechanism of cellular removal is at first sight similar to that for anandamide, namely cellular uptake followed by fatty acid amide hydrolase (FAAH) -catalysed hydrolysis to form palmitic acid and ethanolamine (see Fig. **2**). Whilst there is good evidence to surmise that the FAAH responsible for the hydrolysis of PEA is the same enzyme as that metabolising anandamide [14, 25, 26, 27], the uptake processes for the two fatty acid amides are different. Anandamide is taken up into cells predominantly by an energy-independent mechanism of facilitated transport that at least in part is driven by the intracellular FAAH-catalysed removal of accumulated anandamide [28, 29]. This means that FAAH inhibition per se can reduce the rate of anandamide uptake. In contrast, at least 50% of cellular PEA uptake is brought about by passive diffusion [30], and although the remaining uptake can be inhibited by anandamide, 2-arachidonoylglycerol and related compounds [30, 31], the presence of such a large uptake component due to passive diffusion means that inhibition of active PEA uptake is unlikely to be a viable pharmacological strategy for prolonging the pharmacological actions of this compound. In contrast, there is good evidence to show that the pharmacological effects of exogenous anandamide in vivo are potentiated following inhibition of FAAH [32, 33], and it is thus reasonable to suggest that inhibition of this enzyme may also potentiate the pharmacological effects of PEA (discussed in section 5).

4. INTERACTION OF PEA WITH CANNABINOID AND VANILLOID RECEPTORS

4.1 Cannabinoid (CB) Receptors

The initial report that anandamide was capable of activating CB receptors [1] has naturally stimulated considerable research into the ability of other fatty acid amides to interact with these receptors. To date, two sub-classes of CB receptors have been characterized and cloned: the CB_1 receptor [34] expressed in the brain and some peripheral tissues and the CB_2 receptor, predominantly found in the immune system [35] (although there is some evidence for central expression of the CB_2 receptor in embryonic tissue [36]). An alternatively spliced form of CB_1, christened CB_{1A}, has also been described [37] but so far, no peculiar property in terms of ligand recognition and receptor activation has been shown for this variant. Anandamide acts as a partial agonist at both CB_1 and CB_2 receptors whereas the related compound 2-arachidonoyl glycerol acts as a full agonist [38, 39, 40, 41].

The ability of PEA to activate CB receptors has long been a matter of debate. In general, there is a consensus that PEA does not interact with CB_1 receptors at physiologically relevant concentrations, a result first reported by Devane *et al.* [1; see also 42]. However, it has been suggested that PEA acts as an endogenous ligand for CB_2 receptors. This suggestion was based upon the report by Facci *et al.* [43] that PEA was able to inhibit the binding of the non-selective CB receptor agonist [^{3}H]WIN 55,212-2 to RBL-2H3 basophilic leukaemia cell membranes with a potency (IC_{50} 1 nM) >30-fold greater than for anandamide. These cells, which have good homology to mucosal mast cells, express mRNA for the CB_2 receptor [43, 44]. More recent studies, however, have failed to demonstrate an interaction between PEA and CB_2 receptors unless very high (100 μM) concentrations are used [41, 44, 45]. It is unclear as to whether the RBL-2H3 [^{3}H]WIN 55,212-2 binding reported by Facci *et al.* [43] reflects a CB_2 receptor or some novel related receptor. Expression of this binding site is dependent upon the RBL-2H3 cell passage number [16] and in our hands, no binding of either this ligand or of the agonist [^{3}H]CP-55,940 has been found [30, 45]. Ross *et al.* [44], however, were able to measure [^{3}H]CP-55,940 binding in RBL-2H3 cell membranes that was inhibited by low affinity by the CB_1 receptor antagonist SR141716A (K_i 264 nM) and by anandamide (K_i 405 nM) but not by PEA (<25% inhibition at 10 μM). It can thus be concluded with reasonable confidence that the physiological effects of PEA (discussed in Section 4) are not the result of direct actions at CB receptors.

4.2 Other Receptor Systems

Anandamide is by no means a specific cannabinoid receptor agonist, and actions upon vanilloid receptors and an acid- and anaesthetic-sensitive background K^+ channel (TASK-1) that is found in the brain, have recently been reported at concentrations ~1 μM [46, 47, 48]. Anandamide also binds with low affinity (K_i 40 μM) to the dihydropyridine binding site of the brain L-type calcium channel [49]. In contrast, PEA, at a concentration of 10 μM, had no effect on TASK-1 currents or upon the binding to L-type calcium channels [48, 49] and at best only partially activated recombinant human vanilloid VR_1 receptors [46, 47]. Other receptor-modulatory effects of anandamide found at concentrations ranging from ~1 to >100 μM [see e.g. 50,

51, 52] have not, to our knowledge, been investigated for PEA.

5. PHARMACOLOGICAL ACTIONS OF PEA

5.1. Inflammation

The first report of an anti-inflammatory activity of PEA was made quite early by Coburn *et al.* in 1954 [53]. They found that egg yolk and alcohol-soluble fraction of egg-yolk protected the guinea pigs from anaphylactic arthritis. This work led to the isolation of the purification of PEA from egg yolk and peanut meal by Kuehl et al [3]. Various analogues of PEA were synthesized by these authors [3] and were found to be active on inflammation (anaphylaxis assay), and Kuehl *et al.* attributed the anti-inflammatory activity to the ethanolamine moiety. However, PEA was the most active compound in the series. In 1993, Aloe *et al.* [54] reported that PEA, when administered subcutaneously to rats, could reduce within a relatively short time after administration (20 min) the degranulation of mast cells produced by the local injection of substance P into the ear pinna, although the shorter C4:0 ethanolamine (*N*-butanoylethanolamine, **4**) was more potent in this respect (Fig. **3A** and Fig. **4**). A subsequent study using a 60 min interval between PEA and substance P administrations demonstrated that orally administered PEA inhibited almost completely both substance P-induced mast cell activation and plasma extravasation produced either by substance P or passive cutaneous anaphylaxis [55]. The maximum inhibition of substance P-induced mast cell degranulation and plasma extravasation was ~80-90%, and doses producing 50% of the maximal possible effects towards substance P-induced mast cell degranulation and plasma extravasation were calculated to be 0.65 ands 0.85 mg/kg, respectively. In contrast, plasma extravasation in response to substance P was not prevented by a combination of the products of the hydrolysis of PEA, i.e. palmitic acid and ethanolamine, suggesting that the parent molecule and not a metabolic fragment is necessary for the activity [55]. As PEA is produced during inflammation (see Section 3.1 above), it was proposed that PEA acted as an "ALIAmide" (Autocoid Local Inflammation Antagonist Amide). A more recent study has demonstrated positive effects of PEA towards mast cell degranulation in 10 of 15 cats with eosinophilic granuloma or eosinophilic plaque [56]. Thus PEA may represent the basis of a new approach to the treatment of inflammation [57].

In contrast to the robust effects found in vivo, experiments conducted on the ability of PEA and PEA analogues to affect mast cell degranulation in vitro have given somewhat contradictory results (as illustrated in Fig. **3**). The first study published in 1962 reported that PEA (0.1 mg/ml, corresponding to ~300 µM, an impressive concentration given the limited solubility of this compound) blocked histamine release in response to treatment of rat peritoneal mast cell suspensions with Russell viper venom, whereas the response to treatment with either brain or lung thromboplastin was not affected [58]. In 1995, Facci *et al.* [43] reported that PEA reduced the immunogenic activation of serotonin release from granules of both RBL-2H3 basophilic leukaemia cells and isolated rat peritoneal mast cells.
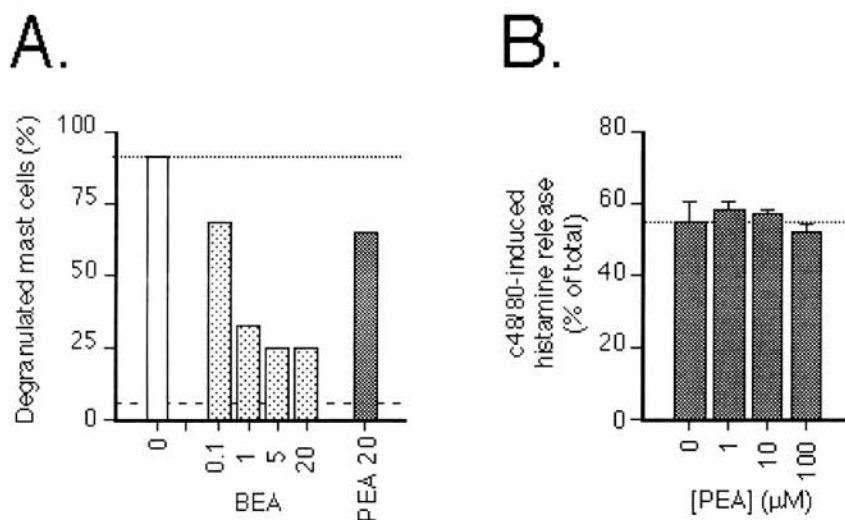
Fig. (3). Discrepancy between *in vivo* and *in vitro* effects of PEA upon mast cell degranulation. *Panel A*: Effects of PEA ("LG 2110/1" in original paper) and *N*-butanoylethanolamine (BEA; "LG 2130/2" in original paper) upon mast cell degranulation in the rat ear pinna following s.c. administration of 1 pmol substance P (injection volume 1 µl). The fatty acid amides were administered s.c. 20 min prior to substance P (doses in mg/kg), and mast cell degranulation was assessed histologically 5 min after the substance P injection. Data are means of duplicate experiments, and are taken from Table **1** of Aloe *et al.* [54]. The dotted line indicates the level of mast cell degranulation for animals given substance P alone.The dashed line indicates the level of mast cell degranulation for animals given saline instead of substance P. *Panel B.* Lack of effect of PEA upon the degranulation of isolated rat peritoneal mast cells induced by compound 48/80 (0.2 µg/ml). The cells were pretreated with PEA for 10 min prior to addition of compound 48/80 and assay of histamine release 5 min later. Data are means ± s.e.m., N=3, and are taken from Table **2** of Bueb *et al.* [60]. The dotted line indicates the histamine release in the absence of PEA. Spontaneous histamine release was <8%..

More recent data, however, have not shown so positive results. In rat isolated peritoneal mast cells, Bueb *et al.* [59, 60] investigated the properties of both plant-derived cannabinoids and PEA and PEA analogues and for their capacity either to induce histamine release per se or to prime the release response to a non-immunogenic stimulus (compound 48/80). Only ^{9}-THC and ^{8}-THC at µM range were able to induce a non-lytic, energy- and concentration-dependent histamine release from the peritoneal mast cells that was not blocked by the CB_1 antagonist SR141716A (up to 10 µM), but was reduced by at least 20 % by pertussis toxin (10-1000 nM). PEA neither induced histamine secretion, nor primed the secretion induced by compound 48/80 (Fig. **3B**).
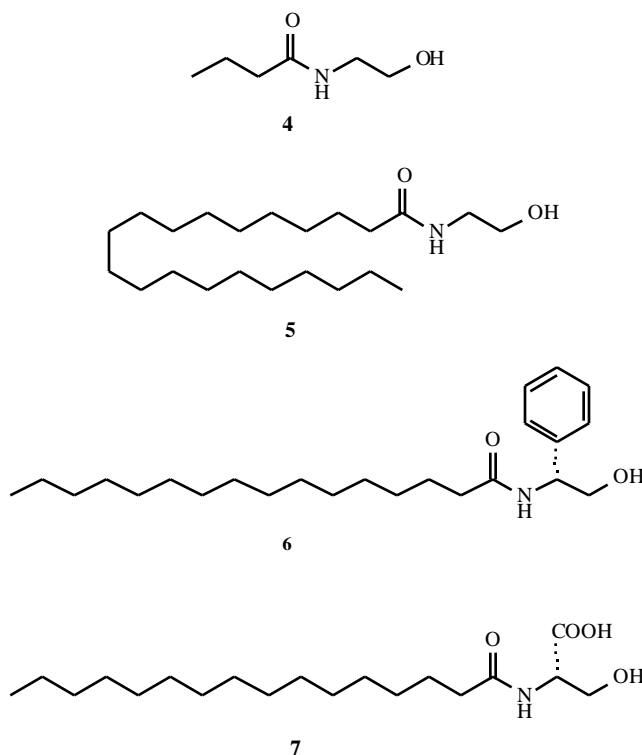
Fig. (4). Structures of *N*-butanoylethanolamine (**4**), *N*-eicosanoylethanolamine(**5**) and the palmitoylamides of (*R*)-2-aminophenylethanol (**6**) and of L-serine (**7**).

In a human mast cell line (HMC-1 cells), degranulation as measured by release of tryptase in response to the calcium ionophore A23187 was not affected by PEA [61]. These authors concluded that rat and human mast cells present major difference in the cell activation, and that the ALIA mechanism does not seem to be plausible in humans [61]. A more recent study found that PEA has at best modest effects (and only at unphysiologically high concentrations) upon antigen-induced RBL-2H3 cell degranulation over and above that produced by the solvent system needed to dissolve the compound [62]. It should be pointed out, however, that a number of factors, such as mast cell heterogeneity and changes in the properties of the mast cells upon isolation should be taken into consideration, since these may account for the lack of consistent effect of PEA upon mast cell degranulation in vitro.

Anti-inflammatory effects of PEA may not be restricted to mast cells alone. PEA, as well as anandamide, exhibited moderate anti-inflammatory properties against aerolized lipopolysaccharide (LPS)-induced pulmonary inflammation in mice. PEA decreased the levels of the cytokine TNF-but did not influence macrophage recruitment [63]. The same authors observed a similar inhibitory effect of PEA on interleukins 4, 6 and 8 release from human peripheral blood mononuclear cells [66]. More recently, Ross *et al.* [64] reported that PEA (5-30 µM, in the presence of an inhibitor of FAAH) reduced NO release from RAW264.7 macrophage cells in response to lipopolysaccharide by a cannabinoid-receptor independent mechanism. However, this finding could not be reproduced in our (S.V., D.M.L.) hands [65].

5.2. Inflammatory Pain

Plant-derived, synthetic and endogenous cannabinoids have well-documented analgesic effects [see 67]. Given the beneficial effects of PEA upon mast cell activation and inflammatory responses in vivo (see above), positive analgesic effects towards inflammatory pain are also to be expected. In 1996, Mazzari *et al.* [55] reported that PEA, when administered orally in 1.5- % carboxymethylcellulose, reduced carrageenan-induced hyperalgesia, as well as carrageenin-formalin- and dextran-induced edema. The doses used were 10 mg/kg in the rat, and ranged from 0.1 to 10 mg/kg in mice. The compound was found to be inactive towards phospholipase A2-induced edema.

In 1998, two important papers investigating in detail the effects of PEA upon inflammatory pain were published [9, 68]. Calignano *et al.* [9] investigated the effects of PEA and anandamide using the formalin test. When PEA and anandamide were given to mice by intraplantar injection at the same dose (50 µg), the authors noticed different pharmacological patterns of efficacy for the two compounds. PEA suppressed both the early and late phase responses to formalin. This effect was reversed by the administration of SR144528, a CB_2 antagonist, suggesting the intervention of CB_2 receptors or at least CB_2-like receptors. Interestingly, the CB_1 cannabinoid receptor antagonist SR141716 had no effect on the analgesic actions of PEA. In contrast, anandamide was only effective in the early phase response of formalin, and this effect was suppressed by SR141716 and not by SR144528. Combined administration of PEA and anandamide gave higher antinociception in the two phases, both suppressed by the cannabinoid receptor antagonist. The co-administration of PEA and anandamide led to a synergistic potentiation of analgesic effects, which, according to the authors, did not involve the CNS.

Jaggar and co-workers [68] compared the effects of anandamide and PEA in visceral and somatic inflammatory pain, two models of persistent inflammatory pain that are relevant to clinical pain. At a dose between 10 to 30 mg/kg, PEA attenuated the behavioral response during the second (but not the first) phase of the formalin test (Fig. **5**) and reversed the reduced micturition threshold produced by instillation of turpentine into the urinary bladder [68]. However, unlike anandamide, PEA did not prevent the viscero-visceral hyper-reflexia associated with turpentine

inflammation of the rat urinary bladder [69]. The authors suggested that PEA becomes effective as an analgesic only when an inflammatory state is established This is supported by the fact that nerve growth factor (NGF) has been identified as a pivotal molecule in visceral inflammatory hyperalgesia and that both PEA and anandamide prevent NGF (as opposed to turpentine) induced bladder hyper-reflexia, the effects of anandamide being totally reversed by SR141716 and partially reversed by SR144528, whilst the actions of PEA were sensitive to SR144528 alone [70]. Furthermore, turpentine-induced inflammation of the urinary bladder results in an NGF-dependent referred hyperalgesia, and it was found that this could be prevented by PEA in a manner that could, at least in part, be reversed by SR144528 (but not SR141716) [71]. A possible interpretation of these findings is that PEA attenuates only the mast cell-mediated amplification of the NGF signal during inflammation, as opposed to the direct interaction of NGF with primary afferent neurones [72]. More recently, this group has suggested that PEA reduces thermal hyperalgesia produced by NGF administration into the paw by reducing neutrophil infiltration into the affected area. Anandamide also reduces NGF-induced thermal hyperalgesia [73] but in contrast does not significantly affect neutrophil accumulation (although there was a trend towards such an effect), suggesting a different mechanism of analgesic action for the two endocannabinoids [74].
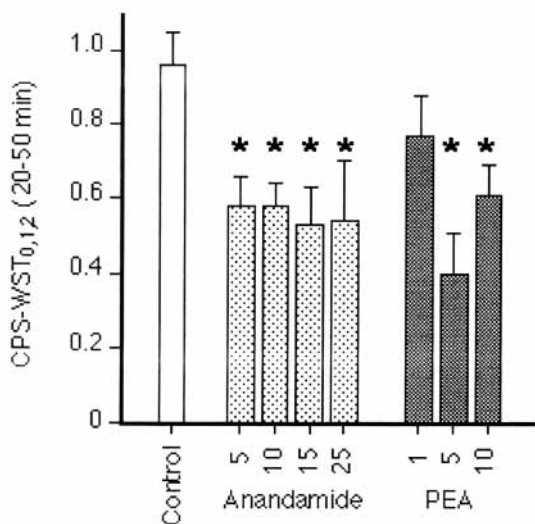

Fig. (5). Effects of anandamide and PEA given i.p. on the late phase response to formalin. $CPS\text{-}WST_{0,1,2}$ is the composite pain score measured 20-50 min after administration of formalin (2.5%, 50 μl) into the dorsum of the right paw. The figure is drawn from data (means ± s.e.m., N=5-6; *, P<0.05 *vs.* formalin alone, Dunnett's *post hoc* test following ANOVA) published as Table **1** of Jaggar *et al.* [68].

The findings above [9, 70, 71] that the antinociceptive effects of intraplantar PEA are prevented by the CB_2 receptor antagonist / inverse agonist SR144528 is intriguing, given that PEA does not interact with these receptors in vitro unless very high concentrations are used (see Section 3). Two explanations for this discrepancy between in vivo and in vitro data can be considered:

1. PEA acts as a "pro-drug" for a metabolite capable of interacting with CB_2 receptors. Little is known about the metabolism of PEA in vivo. However, upon incubation of neuroblastoma cells with [^{14}C]PEA, the reduction in radiolabel associated with PEA was paralleled by an increase in labelled free fatty acid, followed by labelling of esterified fatty acids [75]. 2-Palmitoylglycerol can potentiate the ability of 2-arachidonoylglycerol to bind to and activate CB receptors [76] (described as an "entourage effect" by the authors of this paper) and it is thus in theory possible that the administration of PEA in vivo leads to sufficient accumulation of an "entourage compound" to allow endogenous compounds effectively to activate the CB_2 receptor. It is, however, unclear why the effects of 2-arachidonoylglycerol should be mediated by CB_2 receptors alone when the compound acts as a full agonist at both CB_1 and CB_2 receptors [39, 40, 41]. Whatever the explanation, if PEA acts as a prodrug, blockade of PEA metabolism would be expected to reduce rather than enhance its analgesic efficacy in vivo.

2. PEA itself, rather than a metabolite, acts as an "entourage" compound (see Fig. **6**), whereby it potentiates the action of another endogenous compound, either by increasing the sensitivity of CB_2 receptors or by preventing its breakdown [for discussion, see Ref. 16]. Again, it is difficult to envisage anandamide and 2-arachidonoylglycerol as the endogenous compounds affected in this way, due to their efficacies at CB_1 receptors – indeed the analgesic effects of intraplantally administered anandamide in the formalin test are mediated by CB_1 receptors [9]. Nevertheless, if the PEA "entourage" hypothesis is correct, blockade of PEA metabolism would be expected either to result in a retained activity (if the PEA effect per se is maximal) or alternatively to potentiate the effects of PEA.

The suggestion by Jaggar *et al.* [69] that PEA becomes effective as an analgesic only when an inflammatory state is established is borne out in studies of acute pain. Calignano *et al.* [9] investigated the effects of anandamide and PEA on the behavioral response (escape or hind-paw licking) to an acute thermal stimuli (hot plate heated at 55.5°C). At 10 μg administered intracerebroventricularly, only anandamide was able to exhibit antinociception 20 and 30 min after injection. PEA, at the same dose, was ineffective, an expected result given that unlike anandamide, PEA does not interact with brain CB_1 cannabinoid receptors. Interestingly, PEA did not potentiate (i.e. had no "entourage" effect) the antinociceptive effect of 10 μg i.c.v. anandamide in this model [9]. In another model of acute pain, the tail flick model, Adams *et al.* [78] found an analgesic effect of the C20 saturated analogue of anandamide (i.e. the C20 homologue of PEA, Fig. **4**, compound **5**) when administered iv to mice in a 1:1:18 mixture of ethanol, emulphor and saline. The dose required for the anti-nociceptive effect was, however, 5-fold higher (ED_{50} = 261.5 μmol/kg) than that needed to decrease spontaneous activity in mice (ED_{50} = 50.3 μmol/kg, roughly equipotent to anandamide). It is hard to know whether the anti-nociceptive effect at such a high dose is a specific effect or not. In this respect, PEA displays anticonvulsive activity in the mouse (ED_{50} 8.9 mg/kg i.p., corresponding to 30 μmol/kg) [79], and 10 mg/kg i.v.
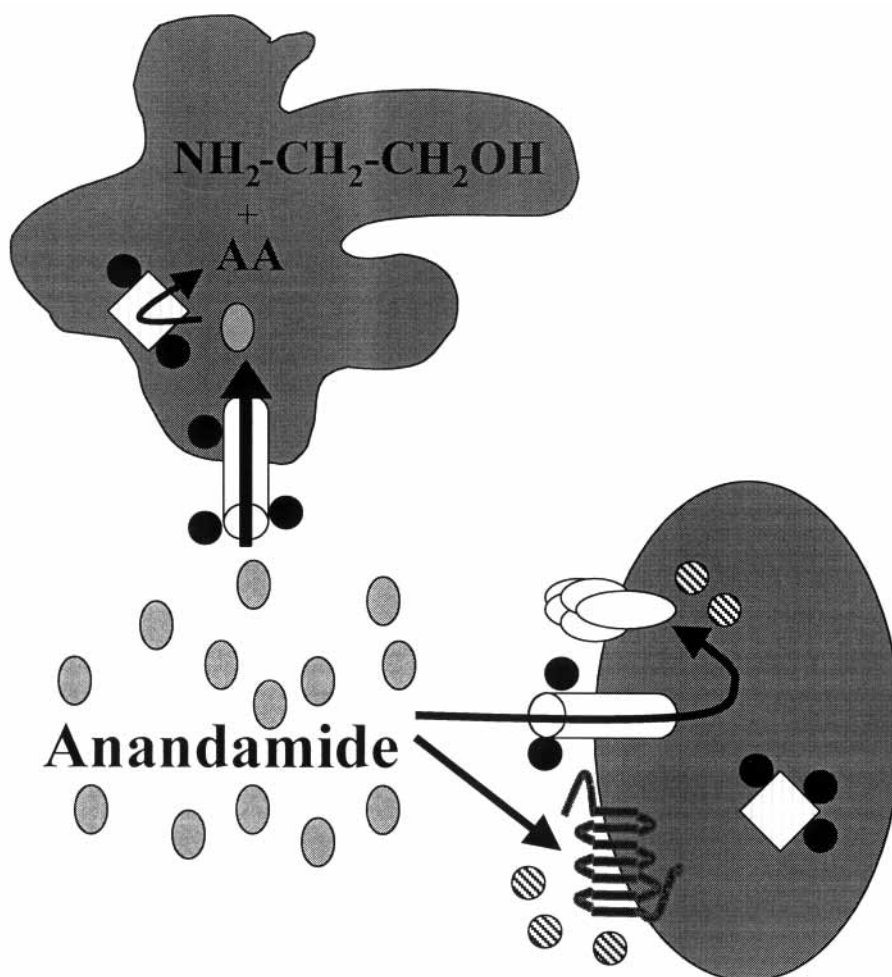
Fig. (6). The "entourage" effect hypothesis. Compounds (depicted as black filled circles) interfering with the uptake of anandamide (depicted as grey filled circles) and/or the FAAH located inside the cells and anchored to the membrane (depicted as striped diamonds) may, by enhancing the levels of endocannabinoids such as anandamide, strengthen their pharmacological actions on receptors : CB_1 / CB_2 receptors (represented as a 7 TM receptor in the picture) and vanilloid receptors (represented here as a ligand gated receptor channel with a pentameric structure). In the case of the vanilloid receptors, the binding site for anandamide appears to be intracellular (as it is for capsaicin) and thus the endocannabinoid has to be transported into the cell via its uptake mechanism before it can activate these receptors [77]. PEA will reduce the metabolism of AEA by acting as a competing substrate for fatty acid amide [see 26]. PEA does not, however, interact with the AEA transport site [see 30]. Entourage effects could also be found if the compounds (in this case shown as hatched circles), despite having no effect on the CB_1 / CB_2 (or vanilloid) receptors *per se*, are able in some way directly to enhance the efficacy of AEA at these receptors.

(corresponding to 33 µmol/kg) of PEA prolongs barbiturate sleeping time but neither induces catalepsy nor reduces body temperature in mice [33].

5.3. Ischaemia

CB_1 recepors are induced in experimental stroke [80] and the synthetic cannabinoid receptor agonist WIN 55,212-2 is neuroprotective in vivo in models of global and focal ischaemia as a result effects upon CB_1 receptors [see 81]. In 1996, Skaper *et al.* [82] reported that in cerebellar granule cells in primary culture, PEA treatment reduced the neurotoxic effects of a short incubation with glutamate. Since PEA is produced by the brain following ischaemic insult [22], the authors suggested that this compound may act as an endogenous neuroprotective agent. The authors further reported that anandamide not only afforded no

protection against the glutamate neurotoxicity, but antagonised the protection produced by PEA [82]. Other authors have, however, found anandamide to be neuroprotective in vitro in hypoxia models by a CB-receptor independent mechanism [81, 83]. Information as to the efficacy of PEA in these models was not provided. No neuroprotective actions of either PEA or anandamide were found in vitro in chick neurons in primary culture following a prolonged glutamate exposure [84]. There is thus a need for further experiments delineating whether or not PEA has a robust neuroprotective action following ischaemic insult, and to determine how such neuroprotection is brought about. One possibility is that antioxidant properties of fatty acid amides may be of importance for their neuroprotective actions in vitro, akin to the situation found for plant-derived cannabinoids [85]. In this respect, oleoylethanolamide (admittedly at high concentrations, 50-150 µM) can reduce the level of lipid peroxidation produced by treatment of

isolated rat heart mitochondria with $FeSO_4$ or $FeCl_3$ / ADP [86]. Another possibility is that protective effects of AEA and possibly PEA that are more relevant to the situation in vivo may be seen if cAMP levels are raised during the exposure periods [87].

5.4. Spasticity Associated with Multiple Sclerosis

An important area where PEA may have clinical utility is in the treatment with spasticity associated with multiple sclerosis. In an animal model of multiple sclerosis (chronic relapsing experimental allergic encephalomyelitis induced by repeated administration to mice of syngenic spinal cord homogenate emulsified in Freund's complete adjuvant), PEA was found to alleviate the spasticity found in the hind limbs [5]. Given that similar alleviation was found with both plant-derived and endogenous cannabinoids [5, 88] and by inhibitors of anandamide uptake and metabolism [5], the most likely explanation for the positive effect of PEA is that it is acting in this model as an "entourage" compound to prevent anandamide breakdown by competing for FAAH.

6. CLINICAL STUDIES WITH PEA

In contrast to the increasing body of experimental data in inflammatory conditions, clinical data of PEA is sparse. In 1960, Coburn and Rich [89] conducted a limited clinical evaluation as to the ability of PEA to prevent rheumatic occurrences in children with rheumatic fever. The results could not be interpreted, due to the low incidence of rheumatic occurrences in all the children possibly as a result of a better general diet (not the least eggs) during the test period. Other early clinical trials with PEA conducted between 1973-1975 suggested that this compound reduced the incidence of acute respiratory infections in soldiers [90]. Two studies regarding the treatment of chronic lumbosciatalgia and multiple sclerosis are reportedly in progress [91], and, although no results have yet been published, the wide spectrum of anti-inflammatory actions found in experimental animals clearly distinguishes PEA from other anti-inflammatory agents such as NSAIDs and corticosteroids. In this respect, patents were taken out in 1996 for PEA and its derivatives (fatty acid amides of aminoalcohols or aminoethers [92] and fatty acid amides of amino acids or glycosamines such as the palmitoylamides of (R)-2-aminophenylethanol (**6**) and of L-serine (**7**) [93]). These two last molecules are depicted in Fig. **4**. The possible therapeutic applications suggested in the patents include immune disorders such as treatment of multiple sclerosis and inflammatory conditions such as rheumatoid arthritis, viral and bacterial meningitis [91].

7. STRUCTURE-ACTIVITY RELATIONSHIPS OF PEA ANALOGUES AVAILABLE IN THE LITERATURE

In contrast to the wealth of data concerning the pharmacological properties of anandamide analogues in the literature [see e.g. Refs. 78, 94 as examples], relatively few studies have devoted themselves to the structure-activity relationships of PEA analogues. However, there is evidence that such an approach could be rather fruitful. In this respect, a simple substitution of a fluoride atom for the hydroxy group in the ethanolamine moiety of the C20 homologue of PEA resulted in 10- and 26-fold increased potencies towards inhibition of the tail-flick acute pain response and reduction in spontaneous activity, respectively [78].

One approach has been the design of a series of PEA homologues and analogues [45] with a view to exploring the "entourage" properties of such compounds. This approach is based on the finding discussed in section 5.2 above that the naturally-occuring PEA analogue, 2-palmitoylglycerol (**8**), does not bind to cannabinoid receptors, but does act as an "entourage" compound for 2-arachidonoylglycerol [76]. Among the analogues so far investigated, palmitoylisopropylamide (**9**) looks particularly promising, since it does not interact with CB receptors per se [45] but is able to prevent both the uptake of anandamide and its subsequent metabolism by FAAH [95].

The PEA structure has also formed the basis for the design of "transition state" inhibitors of FAAH. Thus, Deutsch *et al.* [96] reported that palmitylsulfonyl fluoride (AM374, **10**) potently inhibited the metabolism of anandamide by rat brain homogenates (IC_{50} 7nM at a substrate concentration of 30 µM, 13 nM at a substrate concentration of 100 nM) whereas it was a weaker inhibitor of the binding of [^{3}H]CR 55,940 to rat brain CB_1 receptors (IC_{50} 520 nM). The potency of palmitylsulfonyl fluoride towards FAAH was shared by the shorter homologues lauryl - and myristylsulfonyl fluoride (C12 and C14, respectively **11** and **12,** Fig. **7**) and by stearylsulfonyl fluoride (C18, **13,** Fig. **7**), whereas arachidylsulfonyl fluoride (C20) was less potent [96]. Trifluorometyl ketones are also known inhibitors of FAAH [97], and palmitoyl trifluoromethyl ketone (**14**, Fig. **7**), originally designed as an inhibitor of phospholipase A_2 (IC_{50} 3.8 µM for inhibition of phospholipase A2 in murine P388D1 macrophage-like cells) [98] has been found in our hands also to be a potent inhibitor of FAAH, with an IC_{50} value (79 nM) similar to that found under the same assay conditions for oleoyl trifluromethylketone (57 nM [27],**15,** Fig. **7**) [95].

8. CONCLUSIONS

It is now clear that PEA is a compound with documented anti-nociceptive and anti-inflammatory effects. The mechanism of action of PEA, however, remains unclear. It is to be hoped that future studies using new PEA analogues, selective CB receptor antagonists as well as compounds preventing the metabolism of PEA will provide useful information concerning this elusive mechanism of action.

NOTE ADDED IN PROOF

Since this review was written, several papers on PEA have been published, reflecting the upsurge of interest in this compound. We have chosen (with apologies to the other
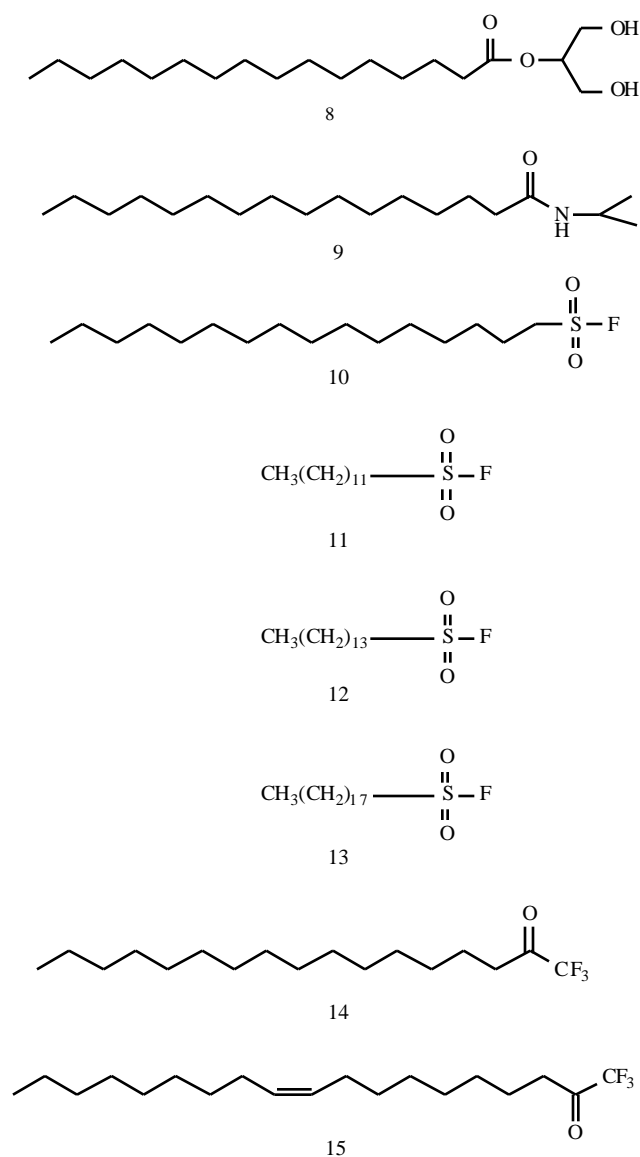
Fig. (7). Structures of compounds related to *N*-palmitoylehanolamine interfering with the metabolism of anandamide, i.e. either with the cellular transport and/or the FAAH-catalysed metabolism of this endocanabinoid.

authors) three of these papers which have a direct bearing on the present review. Calignano *et al.* (Eur. J. Pharmacol. 419 [2001] 191-8) confirmed that CB2-receptor-mediated effects of PEA using the formalin test of nociception, and found further that oleoyl-, myristoyl-, palmitoleyl- and palmitelaidyl-ethanolamide analogues did not share this action of PEA. De Petrocellis *et al.* (FEBS Letts. 506 [2001] 213-6) reported that PEA possessed an entourage action towards the stimulation of vanilloid VR1 receptors by anandamide. PEA exerted this effect by increasing the affinity of the receptor for anandamide rather than by reducing the rate of anandamide metabolism. Finally, Ueda *et al.* (J. Biol. Chem. 276 [2001] 3552-7) reported the purification of an amidase distinct from FAAH that was expressed in the lung and spleen (but not to any great extent in the brain or liver) and that showed selectivity for PEA over oleoyolethanolamide and anandamide as substrates.

ABBREVIATIONS

ALIAmide	=	Autocoid Local Inflammation Antagonist Amide
CB	=	Cannabinoid
FAAH	=	Fatty Acid Amide Hydrolase
HMC-1	=	Human Mast Cell line
NAEs	=	*N*-acylethanolamines
NGF	=	Nerve growth factor
PEA	=	*N*-palmitoylethanolamine
THC	=	Tetrahydrocannabinol

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