

REVIEW ARTICLE

The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations

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Palmitoylethanolamide (PEA) has emerged as a potential nutraceutical, because this compound is naturally produced in many plant and animal food sources, as well as in cells and tissues of mammals, and endowed with important neuroprotective, anti-inflammatory and analgesic actions. Several efforts have been made to identify the molecular mechanism of action of PEA and explain its multiple effects both in the central and the peripheral nervous system. Here, we provide an overview of the pharmacology, efficacy and safety of PEA in neurodegenerative disorders, pain perception and inflammatory diseases. The current knowledge of new formulations of PEA with smaller particle size (i.e. micronized and ultra-micronized) when given alone or in combination with antioxidant flavonoids (i.e. luteolin) and stilbenes (i.e. polydatin) is also reviewed.

LINKED ARTICLES

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Abbreviations

2-AG, 2-arachidonoyl-glycerol; AD, Alzheimer's disease; AEA, anandamide (*N*-arachidonoyl-ethanolamine); ALIA, Autocoid Local Inflammation Antagonism; ALS, amyotrophic lateral sclerosis; CAD, contact allergic dermatitis; CCI, chronic constriction injury; FAAH, fatty acid amide hydrolase; GPR55, orphan GPCR 55; Lut, luteolin; m-PEA, micronized PEA; MS, multiple sclerosis; NAAA, *N*-acylethanolamine acid amidase; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-specific phospholipase D; PD, Parkinson's disease; PEA, palmitoylethanolamide; SCI, spinal cord injury; TBI, traumatic brain injury; TRPV1, transient receptor potential vanilloid type-1 channel; um-PEA, ultra-micronized PEA

Tables of Links

TARGETS	
GPCRs^a	Enzymes^d
CB ₁ receptor	DAGL, diacylglycerol lipase
CB ₂ receptor	FAAH, fatty acid amide hydrolase
GPR55	NAAA, N-acylethanolamine acid amidase
Voltage-gated ion channels^b	NAPE-PLD, N-acyl-phosphatidylethanolamine-specific phospholipase D
TRPV1 channel	
Nuclear hormone receptors^c	
PPAR- α	

LIGANDS
2-AG, 2-arachidonoyl-glycerol
AEA, anandamide
OEA, oleoylethanolamide
PEA, palmitoylethanolamide

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (^{a,b,c,d}Alexander *et al.*, 2015a,b,c,d).

Introduction

Palmitoylethanolamide (PEA) is a lipid mediator used in the clinic for its neuroprotective, anti-neuroinflammatory and analgesic properties (Re *et al.*, 2007; Esposito and Cuzzocrea, 2013; Mattace Raso *et al.*, 2014; Skaper *et al.*, 2015; Iannotti *et al.*, 2016). It was isolated for the first time from purified lipid fractions of soybeans, egg yolk and peanut meal (Coburn *et al.*, 1954; Ganley *et al.*, 1958) and was then found in a wide variety of food sources (Schuel *et al.*, 2002; Venables *et al.*, 2005; Kilaru *et al.*, 2007; Gouveia-Figueira and Nording, 2014) (Table 1), cells (Berdyshev *et al.*, 2000; Stella and Piomelli, 2001; Walter *et al.*, 2002; Muccioli and Stella, 2008; De Filippis

et al., 2010; Petrosino *et al.*, 2010a), tissues (Epps *et al.*, 1979; Baker *et al.*, 2001; Capasso *et al.*, 2001; Chen *et al.*, 2005; Murillo-Rodriguez *et al.*, 2006; Petrosino *et al.*, 2007; Abramo *et al.*, 2014) and body fluids (Giuffrida and Piomelli, 1998; Schuel *et al.*, 2002; Schreiber *et al.*, 2007; Richardson *et al.*, 2008; Lam *et al.*, 2010) of several animal species and human subjects. In animals, the biosynthesis of PEA occurs through the hydrolysis of its direct phospholipid precursor, N-palmitoyl-phosphatidyl-ethanolamine, by the action of N-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD) (Okamoto *et al.*, 2004) (Figure 1A). The degradation of PEA to palmitic acid and ethanolamine occurs by the action of two different hydrolytic enzymes, that is,

Table 1

Food sources that contain PEA

Food source	Concentration of PEA (ng·g ⁻¹ fresh weight)	Reference
Bovine milk	0.25	Gouveia-Figueira and Nording, 2014
Elk milk	1.81	Gouveia-Figueira and Nording, 2014
Human breast milk	8.98 ± 3.35 nmol·L ⁻¹	Lam <i>et al.</i> , 2010
Human breast milk (110 ± 32.3 lactation days)	23.4 ± 7.2 nmol·L ⁻¹	Schuel <i>et al.</i> , 2002
Common bean (<i>Phaseolus vulgaris</i>)	53.5	Venables <i>et al.</i> , 2005
Garden pea (<i>Pisum sativum</i>)	100	Venables <i>et al.</i> , 2005; Kilaru <i>et al.</i> , 2007
Southern or blackeyed peas (<i>Vigna unguiculata</i>)	138	Venables <i>et al.</i> , 2005
Tomato	100	Kilaru <i>et al.</i> , 2007
<i>Medicago sativa</i>	1150	Venables <i>et al.</i> , 2005
Corn	200	Kilaru <i>et al.</i> , 2007
Soybean (<i>Glycine max</i>)	6700	Venables <i>et al.</i> , 2005; Kilaru <i>et al.</i> , 2007
Soy lecithin	950 000	Kilaru <i>et al.</i> , 2007
Peanut (<i>Arachis hypogaea</i>)	3730	Venables <i>et al.</i> , 2005; Kilaru <i>et al.</i> , 2007

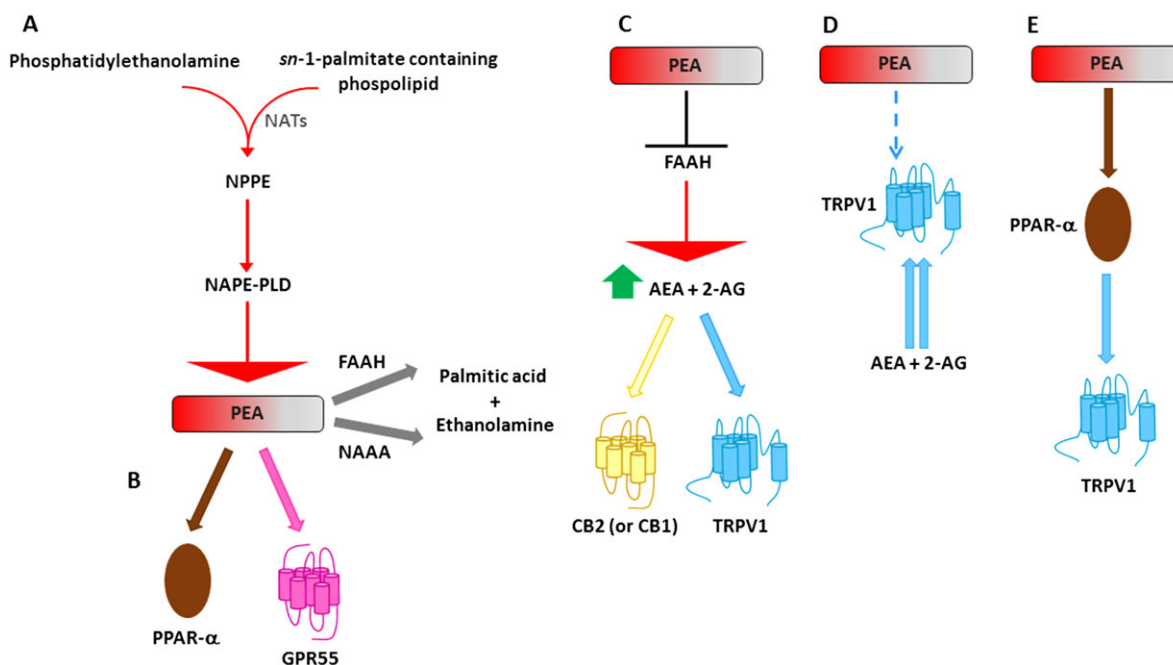


Figure 1

Metabolic pathways and molecular targets of PEA. (A) PEA is biosynthesized from a membrane phospholipid, *N*-palmitoylphosphatidylethanolamine (NPPE), via several routes, the most investigated of which is through the direct hydrolysis by NAPE-PLD. PEA can be then degraded to palmitic acid and ethanolamine by either FAAH or NAAA (Iannotti *et al.*, 2016). (B) PEA can directly activate PPAR- α (Lo Verme *et al.*, 2005b) or, more controversially, GPR55 (Ryberg *et al.*, 2007). (C) PEA, for example through the inhibition of the expression of FAAH, may increase the endogenous levels of AEA and 2-AG, which directly activate CB₂ (or CB₁) receptors and TRPV1 channels (entourage effect) (Di Marzo *et al.*, 2001; Petrosino *et al.*, 2016a). (D) PEA, possibly through an allosteric modulation of TRPV1 channels, potentiates the activation and desensitization by AEA and 2-AG of TRPV1 channels (entourage effect) (De Petrocellis *et al.*, 2001; Di Marzo *et al.*, 2001; Ho *et al.*, 2008; Petrosino *et al.*, 2016a). (E) PEA may also activate TRPV1 channels via PPAR- α (Ambrosino *et al.*, 2013, 2014). NAT, *N*-acyl-transferase.

fatty acid amide hydrolase (FAAH) (Cravatt *et al.*, 1996) and, more specifically, *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (Ueda *et al.*, 2001) (Figure 1A). Interestingly, the biosynthesis and degradation of PEA, as well as other *N*-acylethanolamines, in plants, where these compounds exert quite different physiological functions, seem to occur via identical routes and often similar enzymes (Blancaflor *et al.*, 2014).

Following the discovery of PEA as a naturally occurring anti-allergic and anti-inflammatory compound (Kuehl *et al.*, 1957) and of endogenous metabolic pathways for PEA (Di Marzo *et al.*, 1996; Bisogno *et al.*, 1997; Tsuboi *et al.*, 2005), investigations have been carried out to identify the molecular mechanism of action through which PEA exerts its pharmacological effects. This research has revealed that PEA can act via multiple mechanisms (Iannotti *et al.*, 2016) (Figure 1B–E). The first mechanism of action for PEA was proposed by Rita Levi-Montalcini's research group, who suggested that PEA acts via 'Autacoid Local Injury Antagonism (ALIA)' to down-regulate mast cell activation (Aloe *et al.*, 1993; Levi-Montalcini *et al.*, 1996). Later, the existence of a 'direct receptor-mediated mechanism' was proposed, and several studies demonstrated that PEA can act via direct activation of at least two different receptors: the PPAR- α (Lo Verme *et al.*, 2005a) and the orphan GPCR 55 (GPR55) (Ryberg *et al.*, 2007). It was originally

thought that PEA could also be a CB₂ receptor agonist (Facci *et al.*, 1995), but subsequent studies revealed that PEA has only very weak affinity for this receptor (Sugiura *et al.*, 2000), explaining why some of its anti-inflammatory effects are not blocked by CB₂ receptor antagonists (Costa *et al.*, 2002). As a result, the theory of the 'entourage' effect was put forward to raise the possibility that PEA could produce indirect receptor-mediated effects (De Petrocellis *et al.*, 2001; Di Marzo *et al.*, 2001; Ho *et al.*, 2008). For example, PEA, through the inhibition of the expression of FAAH, the enzyme responsible for the degradation of the endogenous cannabinoid receptor ligand (or endocannabinoid), anandamide (AEA) (Di Marzo *et al.*, 2001), may indirectly activate CB₂ and CB₁ receptors (Di Marzo *et al.*, 2001; Petrosino *et al.*, 2016a). Likewise, PEA can indirectly activate the transient receptor potential vanilloid receptor type 1 (TRPV1) channels, which are also targets for the endocannabinoids (Zygmunt *et al.*, 1999, 2013). In addition, PEA is also able to increase AEA- or 2-AG-induced TRPV1 activation and desensitization (De Petrocellis *et al.*, 2001; Di Marzo *et al.*, 2001; Ho *et al.*, 2008; Petrosino *et al.*, 2016a). More recently, it has also been demonstrated that PEA can activate TRPV1 channels or increase the expression of CB₂ receptors via PPAR- α receptors (Ambrosino *et al.*, 2013; Ambrosino *et al.*, 2014; Guida F, Maione S and Di Marzo V, personal communication). In

summary, these results suggest that PEA does not operate through just one main mechanism of action. Instead, synergistic interactions among several mechanisms often seem necessary so that PEA can produce its important therapeutic effects, both in the central and the peripheral nervous system.

In this review, we discuss the molecular targets of PEA, its pharmacological activity in neurological disorders, pain perception and inflammatory diseases, as well as the current evidence on the effectiveness of micronized and ultra-micronized formulations of PEA when given alone or in combination with the antioxidant flavonoids, such as luteolin, or stilbenes, such as polydatin.

Direct molecular targets of PEA

PPAR- α

PPAR- α is a nuclear receptor protein that belongs to the family of PPARs and acts as transcription factor regulating gene expression (Issemann and Green, 1990). PPAR- α is expressed in many organs and tissues, such as the intestine, heart, liver, kidney, muscle and adipose tissue, and also in several cells of the immune system (Braissant *et al.*, 1996; Daynes and Jones, 2002). Its presence in the latter cells allows it to be implicated in the control of inflammatory processes (Daynes and Jones, 2002; Sheu *et al.*, 2002). The first evidence of PEA as a potential agonist of PPAR- α was reported by Lo Verme *et al.* (2005a), who demonstrated that the lipid directly activates the nuclear receptor with an EC₅₀ of 3 μ M (Lo Verme *et al.*, 2005a), a potency comparable to that of the synthetic PPAR- α agonist Wy-14643, which produced strong anti-inflammatory actions (Sheu *et al.*, 2002). Accordingly, it was hypothesized that, like all PPAR agonists, the binding of PEA to PPAR- α also induces a heterodimerization event with the retinoic acid receptor (RXR), thereby forming the activated receptor complex, which translocates to the nucleus to bind to a peroxisome proliferator response element and reduce the transcription of pro-inflammatory genes (Lo Verme *et al.*, 2005b).

GPR55

The orphan GPR55 receptor belongs to the large family of GPCRs and, although showing a low homology with CB₁ and CB₂ receptors, has been suggested to be activated by the main psychoactive constituent of *Cannabis sativa*, Δ^9 -tetrahydrocannabinol, and by the endocannabinoids AEA and 2-AG (Pertwee, 2007; Sharir *et al.*, 2012). Low concentrations of PEA also seem to directly activate GPR55 with an EC₅₀ of 4 nM (Ryberg *et al.*, 2007), although this issue is still controversial. Accordingly, GPR55 has recently emerged as a putative target for the treatment of inflammation (Yang *et al.*, 2016). It is expressed in almost all brain areas, including the cortex, cerebellum, forebrain, hippocampus and striatum, whereas at the peripheral level, it is amply expressed in the gastrointestinal tract (Ryberg *et al.*, 2007). Although its pharmacology is not completely clear yet, it has been reported that GPR55 utilizes a variety of downstream signalling events including the increase in intracellular calcium via Gq, G12, RhoA, actin, PLC and IP(3)R-gated stores (Lauckner *et al.*, 2008). ERK1/2, MAPK and the induction of transcriptional

regulators such as nuclear factor of activated T-cells (NFAT), NF- κ B and cAMP response element binding protein have also been shown to be coupled to GPR55 activation (Henstridge *et al.*, 2010).

Indirect molecular targets of PEA

CB₁ and CB₂ receptors

The CB₁ and CB₂ receptors, similar to the orphan GPR55 receptor, also belong to the large family of GPCRs (Matsuda *et al.*, 1990; Munro *et al.*, 1993). In the brain, the CB₁ receptor is often expressed in presynaptic terminals, and thanks to this localization, its activation usually inhibits neurotransmitter release (Katona *et al.*, 1999). The CB₁ receptor is also found in the peripheral nervous system and in almost all mammalian tissue and organs such as adipose tissue, skeletal muscle, bone, skin, heart, liver, gastrointestinal tract, lungs and male and female reproductive systems (Pertwee, 1997). It is usually coupled to Gi/o proteins, whereby its activation inhibits adenylate cyclase activity with the subsequent reduction of intracellular levels of cAMP, or stimulates MAPK activity (Turu and Hunyady, 2010). However, the CB₁ receptor can be also be coupled to Gs or Gq proteins (Turu and Hunyady, 2010), as well as to other types of intracellular signals, including the PKB (Akt), phosphoinositide 3-kinase and PLC/inositol 1,4,5-trisphosphate/PKC (PLC β /IP3/PKC) pathways (Gómez del Pulgar *et al.*, 2000; Sanchez *et al.*, 2003).

The CB₂ receptor is largely expressed in cells (such as monocytes, macrophages, B- and T-cells, mast cells and keratinocytes) and peripheral organs (such as the spleen, tonsils, thymus gland, gastrointestinal tract and skin) that play a role in the immune response (Izzo, 2004; Pertwee, 2007; Campora *et al.*, 2012; Iannotti *et al.*, 2016). Instead, the expression of CB₂ receptors in the brain is very low and is observed particularly in activated astrocytes and microglia (Stella, 2010). Accordingly, the main function of the CB₂ receptor seems to be the control of inflammatory and nociceptive responses (Whiteside *et al.*, 2007; Basu and Dittel, 2011). Similar to the CB₁ receptor, the CB₂ receptor is also coupled to Gi/Go proteins, and as a result, its activation inhibits adenylate cyclase activity and promotes MAPK activity (Demuth and Molleman, 2006).

CB₁ and CB₂ receptors are not direct targets of PEA, but they can be indirectly activated by PEA through the aforementioned mechanisms of the entourage effect (Sugiura *et al.*, 2000; Di Marzo *et al.*, 2001; Petrosino *et al.*, 2010b, 2016a).

TRPV1

The TRPV1 channel, also known as the capsaicin receptor, belongs to a subfamily of TRP channels, that is, the TRPV channels, with six transmembrane domains and an intramembrane loop linking the fifth and sixth transmembrane domain and forming the pore channel region (Caterina *et al.*, 1997). TRPV1 is a non-selective ion channel, permeable to mono- and divalent cations (i.e. Mg²⁺, Ca²⁺, Na⁺), and activated by both physical and mechanical stimuli (i.e. high temperatures, low pH, osmotic changes) as well as by

exogenous or endogenous chemical compounds (i.e. capsaicin, AEA, cannabinoids) (Caterina *et al.*, 1997; Di Marzo and De Petrocellis, 2010; Iannotti *et al.*, 2014). TRPV1 is mainly found in dorsal root ganglia and sensory nerve fibres of the A δ and C-type. However, it is also expressed in brain neurons, keratinocytes and other cell types (Cristino *et al.*, 2006; Starowicz *et al.*, 2008; Petrosino *et al.*, 2010a; Julius, 2013; Edwards, 2014). The function of TRPV1 is dependent on changes in its phosphorylation state induced by regulatory proteins, including ATP, PKA, PKC, phosphoinositide-binding protein (PIRT) and phosphatidylinositol 4,5-bisphosphate (PIP2) (Cortright and Szallasi, 2004; Iannotti *et al.*, 2016). Phosphorylation seems to be a requisite for the activation/sensitization of TRPV1, contributing to pain transmission, inflammation and neurotoxicity (Julius, 2013; Edwards, 2014; Nagy *et al.*, 2014). Conversely, the increase in intracellular Ca²⁺ following the stimulation of TRPV1 channels activates: (a) proteins, such as calmodulin, that render the channel stable in a locked conformational state; or (b) Ca²⁺-dependent phosphatases, such as calcineurin, which dephosphorylate the TRPV1 channel and again inactivate it (Cortright and Szallasi, 2004; Iannotti *et al.*, 2016). This process of TRPV1 inactivation, also known as 'desensitization', contributes to the analgesic and anti-inflammatory actions of TRPV1 agonists (Nagy *et al.*, 2014; Iannotti *et al.*, 2016).

Two different mechanisms have been suggested for the action of PEA at TRPV1 channels. The first mechanism proposes that PEA can indirectly activate TRPV1 through the so-called entourage effect. In particular, PEA, possibly through allosteric effects, is able to increase AEA- or 2-AG-induced activation and desensitization at TRPV1 channels (De Petrocellis *et al.*, 2001; Ho *et al.*, 2008; Petrosino *et al.*, 2016a). The second mechanism proposes that PEA can indirectly activate TRPV1 channels via PPAR- α . In particular, because the TRPV1 and PPAR- α antagonists inhibited PEA-induced intracellular Ca²⁺ increase (Ambrosino *et al.*, 2013), a direct biochemical interaction was hypothesized to occur between TRPV1 channels and PPAR- α . In fact, in cells co-transfected with TRPV1 and PPAR- α , the latter receptors were detected in TRPV1-immunoprecipitated fractions, and PPAR- α agonists activated and desensitized closely-associated TRPV1 channels (Ambrosino *et al.*, 2014).

Pharmacokinetics of PEA

There are currently only few data available in the literature on the pharmacokinetics and bioavailability of PEA. The first study was published by Zhukov (1999), who investigated the distribution of N-[1-¹⁴C]-PEA in rat tissues after i.p. administration. The results indicated the following rank order of radioactivity: adrenal >> diaphragm > spleen > kidney > testis > lung > liver > heart > brain > plasma > erythrocytes (Zhukov, 1999). The regional distribution in the rat brain of orally administered PEA (~100 mg·kg⁻¹) has been investigated by the use of N-[9,10-³H]-PEA by Artamonov *et al.* (2005) and recently revised by Gabrielsson *et al.* (2016). The authors found that N-[9,10-³H]-PEA mainly accumulated in the hypothalamus, pituitary and adrenal glands, 20 min after oral administration (Artamonov *et al.*, 2005). The presence of the labelled PEA in the brain

(~98 ng·mg⁻¹ of brain tissue) demonstrated the ability of the compound to penetrate, although in small amounts, through the blood-brain barrier (Artamonov *et al.*, 2005). These results were not surprising if we consider that PEA is a poorly water-soluble substance, which can limit its oral absorption and bioavailability, but they may also suggest a short-lived action of PEA, in agreement with the fact that this compound is degraded by two different hydrolases, that is, NAAA and FAAH. Later, Grillo *et al.* (2013) investigated the tissue distribution of PEA formulated as an emulsion in corn oil and administered s.c. to young DBA/2 mice (10 mg·kg⁻¹) (Grillo *et al.*, 2013; Gabrielsson *et al.*, 2016). The authors found that PEA could be successfully emulsified into an oil depot injection through which it effectively reaches tissues such as the retina, heart, brain and blood, both 24 and 48 h after administration (Grillo *et al.*, 2013). More recently, it has been found that after oral administration of PEA (in a corn oil suspension administered to rats by gastric gavage, at dose of 100 mg·kg⁻¹), the highest plasma concentration was achieved after 15 min corresponding to a 20-fold increase in its basal values. PEA plasma levels dropped 2 h after administration to concentrations very close to the basal ones (Vacondio *et al.*, 2015). Finally, we recently published preliminary data on the bioavailability of two new formulations of PEA, that is, micronized and ultra-micronized (m-PEA and um-PEA), in human volunteers and beagle dogs, respectively (Petrosino *et al.*, 2016a). Our results showed that, in beagle dogs, plasma PEA levels were increased up to sixfold 1 and 2 h after the oral administration of um-PEA (30 mg·kg⁻¹), and at the same time points, the plasma 2-AG levels were also increased by up to ~20-fold (Petrosino *et al.*, 2016a). In human volunteers, following a ~twofold peak in plasma PEA levels at 2 h, the plasma 2-AG levels were increased by up to ~twofold 4 and 6 h after the oral administration of m-PEA (300 mg) (Petrosino *et al.*, 2016a). This smaller increase was proportionate to the smaller peak of PEA levels detected in human volunteers compared with beagle dogs. This difference, in turn, can be caused by the lower total amount of PEA acutely administered to human volunteers (about ~5 vs. 30 mg·kg⁻¹ in dogs) and to the fact that this was a micronized formulation as opposed to the ultra-micronized formulation administered to dogs. Furthermore, the human volunteers engaged for this study were healthy, whereas the dogs were allergic subjects, and the bioavailability of PEA might change during pathological conditions. In summary, these results suggest that, although their bioavailability has not, so far, been compared in the same study with that of 'normal' PEA, formulations of this compound with smaller particle size might be a useful alternative to overcome its solubility problems encountered particularly following oral administration. It is well known, in fact, that the solubility of a drug is intrinsically related to the particle size. Particle size reduction by various means, such as jet mill, leads to an increase in the specific surface area with enhanced solubility and potentially higher bioavailability (Rasenack and Müller, 2004). While m-PEA and um-PEA seem to have a reasonably good oral bioavailability, complete pharmacokinetics studies are necessary to assess the exact tissue exposure and site of metabolism of PEA when administered through these formulations.

PEA and neurological disorders

Neurodegenerative diseases

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) are characterized by gradual and selective neuronal cell death that causes the slow and progressive loss of one or more functions of the nervous system. Depending on the type of disease, neuronal damage can lead to cognitive deficits, dementia, behavioural disorders, motor abnormalities or paralysis.

The potential neuroprotective effects of PEA have been demonstrated in several experimental models of AD. In a mouse model, the s.c. administration of the compound reduced the behavioural impairments, lipid peroxidation, inducible NO synthase (iNOS) induction and caspase-3 activation induced by i.c.v. injection of amyloid- β 25-35 (A β 25-35) peptide (D'Agostino *et al.*, 2012). In addition, GW7647, an agonist of PPAR- α , produced similar effects to PEA, whereas PEA did not protect against A β 25-35-induced memory deficits in PPAR- α KO mice (D'Agostino *et al.*, 2012). In a different *in vivo* model, performed in adult male rats and consisting of the intrahippocampal injection of amyloid- β 1-42 (A β 1-42) peptide, the systemic administration of PEA counteracted the increased transcription and expression of proteins typical of activated astrocytes (GFAP and S100 β), as well as the increased expression of amyloidogenic (BACE1 and APP) and phosphorylated τ proteins (Scuderi *et al.*, 2014). PEA also restored the altered expression of microtubule-associated protein (MAP-2) and cognitive functions induced by A β 1-42 peptide (Scuderi *et al.*, 2014). Also in this case, the involvement of PPAR- α was confirmed by co-administration of PEA with GW6471, a selective antagonist of PPAR- α , which completely abolished PEA-induced effects (Scuderi *et al.*, 2014). These data are in agreement with previous *in vitro* studies on rat mixed neuroglial cultures and organotypic hippocampal slices, challenged with A β 1-42 peptide, where PEA treatment blunted A β 1-42-induced astrocyte activation and improved neuronal survival, and with the effects of PEA being reversed by another PPAR- α antagonist, MK886 (Scuderi *et al.*, 2011, 2012). The neuroprotective benefits of the new composite co-ultraPEALut, consisting of a mixture of PEA and the antioxidant flavonoid, luteolin, in a mass ratio of 10:1, subjected to ultra-micronization, were first shown both in *in vitro* and *ex vivo* organotypic models of AD by using differentiated SH-SY5Y neuroblastoma cells and hippocampal slice cultures, respectively, stimulated with A β 1-42 peptide (Paterniti *et al.*, 2014). Pretreatment with co-ultraPEALut significantly reduced iNOS, glial fibrillary acidic protein expression and apoptosis, and restored neuronal NO synthase as well as brain-derived neurotrophic factor (BDNF; Paterniti *et al.*, 2014).

Neuroprotective actions of PEA were also demonstrated in an animal PD model that consists of injecting i.p. the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Esposito *et al.*, 2012). In particular, chronic treatment with PEA (i.p.) counteracted the loss of nigrostriatal neurons, altered expression of microtubule-associated proteins (MAP-2 and S100 β), activation of astrocytes and expression of iNOS protein induced by MPTP in mice (Esposito *et al.*, 2012). In addition, PEA reduced MPTP-associated

behavioural impairments and motor dysfunctions (Esposito *et al.*, 2012). These effects were dependent on the activation of PPAR- α because genetic ablation of this receptor exacerbated MPTP-induced neurotoxicity (Esposito *et al.*, 2012). The same beneficial effects were recently observed in this PD model after the administration of the aforementioned composite co-ultraPEALut (Siracusa *et al.*, 2015a). Treatment with co-ultraPEALut was able both to reduce the neuroinflammatory response and to increase the autophagy process caused by MPTP intoxication in mice (Siracusa *et al.*, 2015a).

These findings, taken together, help identify the molecular mechanism through which PEA is able to modulate the altered expression of proteins associated with AD or PD and to down-regulate activation of pro-apoptotic markers and pro-inflammatory factors, which lead to the loss of neurons in the cerebral cortex and hippocampus for AD or in the substantia nigra for PD. Thus, PPAR- α emerged as the primary target for the neuroprotective effects of PEA in the central nervous system, and this evidence is supported by the use of PPAR- α agonists and antagonists, which mimic or block PEA effects, respectively.

Therapeutic effects of PEA have also been reported in several chronic models of MS, such as chronic relapsing experimental autoimmune encephalomyelitis (CREAE), Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) and myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE). Initially, it was observed that the endogenous levels of PEA increased in the spinal cord of mice with CREAE or TMEV-IDD (Baker *et al.*, 2001; Loria *et al.*, 2008) and also in the plasma of relapsing–remitting and secondary-progressive MS patients (Jean-Gilles *et al.*, 2009) (Table 2). In addition, it was demonstrated that the exogenous administration of PEA (i.p.) ameliorated spasticity or motor deficits in mice with CREAE or TMEV-IDD, respectively, (Baker *et al.*, 2001; Loria *et al.*, 2008) and reduced the severity of neurobehavioral scores in mice with MOG-induced EAE (Rahimi *et al.*, 2015). Furthermore, the expression of inflammatory cytokines was greatly reduced by PEA both in TMEV-IDD and MOG-induced EAE mice (Loria *et al.*, 2008; Rahimi *et al.*, 2015), and these effects were accompanied by decreased demyelination and axonal damage in the latter model (Rahimi *et al.*, 2015). These data suggest that exogenous PEA might be helpful to compensate or amplify the endogenous defence mechanism deployed by the cells or tissues to counteract neurodegenerative and neuro-inflammatory processes. However, the molecular target(s) through which PEA exerts these effects has(have) not been investigated yet.

The first clinical experience on the efficacy of ultra-micronized PEA (um-PEA) was obtained in a single patient with ALS, who received 600 mg um-PEA p.o. twice daily (Clemente, 2012). The study showed that um-PEA improved respiration, as noticed by the patient, and the clinical condition as measured by electromyography analysis (Clemente, 2012). More recently, a second clinical study was conducted in 64 patients with ALS randomly assigned to one of two groups: 28 patients received 50 mg riluzole (a drug used to treat ALS and delay the onset of ventilator-dependence or tracheotomy) plus 600 mg um-PEA (PEA-treated patients) twice daily, and 36 patients received riluzole only (untreated

Table 2

Some examples of altered endogenous PEA levels during pathological conditions

Disease	Type of study	Area	PEA levels	Reference
Multiple sclerosis	CREAE in mice	Spinal cord	↑	Baker <i>et al.</i> , 2001
	TMEV-IDD in mice	Spinal cord	↑	Loria <i>et al.</i> , 2008
	RR-SP in patients	Plasma	↑	Jean-Gilles <i>et al.</i> , 2009
Ischaemic stroke	HS in a patient	Ischaemic lesion	↑	Schabitz <i>et al.</i> , 2002
	FCI in mice	Cerebral cortex	↑	Franklin <i>et al.</i> , 2003
	MCAO in rats	Infarcted brain areas	↑	Berger <i>et al.</i> , 2004
Pain	CCI in rats	Spinal cord	↓	Petrosino <i>et al.</i> , 2007
		Rostral ventral medulla	↓	
		Dorsal raphe magnus	↓	
	Carrageenan-induced inflammatory pain in rats	Hind paw	↓	Jhaveri <i>et al.</i> , 2008
		Blood	↑	Sanchez <i>et al.</i> , 2016
	Chronic neck shoulder pain in women	Trapezius muscle (microdialysis)	↑	Ghafouri <i>et al.</i> , 2011, 2013, 2014
Inflammatory nature	Croton oil-induced chronic intestinal inflammation in mice	Small intestine	↓	Capasso <i>et al.</i> , 2001
	Chronic inflammation in mice (implant of sterile polyethylene sponges instilled with carrageenan under the dorsal skin)	Infiltrating leukocytes	↓	Solorzano <i>et al.</i> , 2009
	Chronic inflammation in rats (implantation of carrageenan-soaked sponges on the back)	Newly formed granuloma	↓	De Filippis <i>et al.</i> , 2010
	Ulcerative colitis in human patients	Colon	↑	Darmani <i>et al.</i> , 2005
	CdCl ₂ -administered rat testis	Testis	↑	Kondo <i>et al.</i> , 1998
	DNFB-induced CAD in mice	Ear	↑	Petrosino <i>et al.</i> , 2010
	Atopic dermatitis in dogs	Skin	↑	Abramo <i>et al.</i> , 2014
	Cyclophosphamide-induced cystitis in female rats	Bladder	↑	Pessina <i>et al.</i> , 2015
	Acrolein-induced cystitis in female rats	Bladder	↑	Merriam <i>et al.</i> , 2011
	Allergy in rats (experimentally induced airway hyper-responsiveness)	Brainstem (nucleus solitary tract)	↑	Spaziano <i>et al.</i> , 2015
	Osteoarthritis and rheumatoid arthritis in human patients	Knee synovial fluid	↓	Richardson <i>et al.</i> , 2008
	Glaucoma in human patients	Ciliary and choroid body	↓	Chen <i>et al.</i> , 2005
	Cirrhotic human patients	Blood and liver	↑	Caraceni <i>et al.</i> , 2010
	Cancer	Local and metastatic syngeneic melanoma in mouse	Blood, paw and metastasis sites	↓

RR-SP, relapsing–remitting and secondary–progressive; HS, hemispheric stroke; FCI, focal cerebral ischaemia; MCAO, middle cerebral artery occlusion; CdCl₂, cadmium chloride; ↑, increase; ↓, decrease.

patients) (Palma *et al.*, 2016). ALS patients treated with um-PEA showed a slowdown in the worsening of respiratory function, as measured by a lower reduction in their forced

vital capacity over time compared with untreated ALS patients (Palma *et al.*, 2016). Moreover, death and tracheotomy occurred more frequently in untreated than in

PEA-treated patients, suggesting that the disease progressed more slowly in subjects receiving um-PEA (Palma *et al.*, 2016). The authors also demonstrated that um-PEA reduced the desensitization of acetylcholine-evoked currents after repetitive neurotransmitter application, in *Xenopus* oocytes transplanted with muscle membranes from selected ALS patients (Palma *et al.*, 2016). These results, taken together, suggest that PEA might contribute to the conservation of muscle excitability and be beneficial to ALS as an add-on treatment (Palma *et al.*, 2016).

Ischaemic stroke and traumatic brain injury

PEA also plays a protective role in neurological disorders caused by ischaemic stroke and traumatic brain injury (TBI). Ischaemic stroke is a condition characterized by reduced blood flow in the brain leading to limited oxygen supply and, as a result, to the death of brain tissue. Depending on the region of the brain affected, a stroke can cause paralysis, speech impairment and loss of memory. As previously described in patients with MS, it was observed that PEA levels also increased in tissue surrounding the primary ischaemic lesion, in a patient with hemispheric stroke (Schabitz *et al.*, 2002), as well as in the mouse cerebral cortex after focal cerebral ischaemia (Franklin *et al.*, 2003) (Table 2). Moreover, it was later reported that, in the blood of acute stroke patients, PEA levels significantly correlate with NIH stroke scale scores (Naccarato *et al.*, 2010). In consideration of these findings, the neuroprotective potential of PEA has been investigated in ischaemic stroke experimental models. In particular, it was observed that when PEA was exogenously administered (i.p.) after transient middle cerebral artery occlusion (tMCAO), an animal model of acute stroke, it reduced infarct size in cortical and total infarct areas compared with controls (Schomacher *et al.*, 2008; Ahmad *et al.*, 2012a), blocked infiltration and activation of astrocytes, reduced pro-inflammatory marker expression and amended neurobehavioral functions as determined by monitoring motor deficits (Ahmad *et al.*, 2012a). More recently, it was reported that the co-ultraPEALut, at a lower dose compared with PEA alone, was able to produce the same neuroprotective effects after tMCAO (Caltagirone *et al.*, 2016). Importantly, the administration of co-ultraPEALut to a cohort of 250 stroke patients was able to improve all clinical indices (such as neurological status, the degree of spasticity, cognitive abilities, pain and independence in daily living activities) after 30 days of treatment (Caltagirone *et al.*, 2016).

These studies suggest that the higher levels of PEA often associated with neurological impairments may represent an adaptive protective mechanism and that the exogenous administration of PEA, alone or in combination with luteolin, might provide a therapeutic alternative to counteract such impairments through as yet uninvestigated molecular mechanisms.

TBI is a condition produced by a violent trauma to the head that causes damage to the brain. The effects on an individual can be balance problems, headache, dizziness, behavioural impairments and loss of memory. In a controlled cortical impact, an adult mouse model of TBI, it was shown that PEA treatment (i.p.) reduced oedema and the size of lesion, blocked the infiltration of astrocytes and decreased the expression of chymase, tryptase and iNOS (Ahmad *et al.*,

2012b). PEA also improved neurobehavioral functions as evaluated by behavioural tests (Ahmad *et al.*, 2012b). Lately, it has been observed that co-ultraPEALut produces similar effects in the TBI model, but at a lower dose compared with PEA alone (Cordaro *et al.*, 2016), confirming, as suggested above, that the new composite might improve their ability to counteract neurodegeneration and neuroinflammation.

The effects of PEA and co-ultraPEALut have also been evaluated on secondary damage induced by experimental spinal cord injury (SCI) in mice. In particular, in mice with SCI induced by the application of vascular clips to the dura mater via a four-level T(5)–T(8) laminectomy, repeated PEA administration (i.p.) reduced the degree of spinal cord inflammation and tissue injury, the infiltration of neutrophils, the expression of pro-inflammatory cytokines and iNOS, as well as the activation of NF- κ B (Genovese *et al.*, 2008). Moreover, PEA significantly ameliorated the recovery of motor limb function (Genovese *et al.*, 2008). All these neuroprotective effects were absent in PPAR- α KO mice (Paterniti *et al.*, 2013a). The administration of co-ultraPEALut (i.p.), at a lower dose compared with PEA alone, also reduced the severity of trauma induced by compression, improved locomotor activity (Paterniti *et al.*, 2013b) and reduced the expression of protein promoter of autophagy (Siracusa *et al.*, 2015b). Interestingly, co-ultraPEALut stimulated the expression of neurotrophic factors such as BDNF, glial cell-derived neurotrophic factor, nerve growth factor and neurotrophin-3, suggesting that this composite exerts a prominent effect on the management of survival and differentiation of new neurons and spine maturation (Crupi *et al.*, 2016). Nevertheless, the exact mechanism by which the composite co-ultraPEALut seems more efficacious than PEA has not yet been investigated. A possible explanation may be that the administration of this composite, which consists of a mixture of an anti-inflammatory mediator (i.e. PEA) and an antioxidant compound (i.e. luteolin), produces complementary and synergistic effects by acting simultaneously on two phenomena – inflammation and formation of reactive oxygen species – that independently feed neuronal death. Moreover, chemical–physical considerations argue for higher activity of the composite compared with the single physical mixture. Observation by scanning electron microscopy, in fact, has showed an intimate intermixing of the two components of the composite, and data obtained from differential scanning calorimeter and X-ray diffraction documented the transformation into a new crystalline form different from the original two, possibly representing a ‘higher energy content’ form (Paterniti *et al.*, 2013b). Based on such findings, one could hypothesize that co-micronization results in decreased particle-particle agglomeration and electrostatic attraction compared with PEA in its micronized state, in agreement with data obtained following co-micronization of different compounds (Spence *et al.*, 2005). This would in turn result in enhanced substance solubility and/or dispersion, a crucial factor for the absorption from the gastrointestinal fluids.

PEA and pain perception

The first studies showing the ability of PEA to produce analgesia and anti-nociceptive effects date back to 1998, when it was

demonstrated that the local administration of PEA is able to inhibit nociception evoked in mice by intraplantar injection of formalin, acetic acid, kaolin or magnesium sulfate (Calignano *et al.*, 1998, 2001), as well as hyperalgesia following turpentine-induced urinary bladder inflammation in the rat (Jaggar *et al.*, 1998). The analgesia produced by PEA was thought to be mediated by peripheral CB₂ receptors because it was reversed by administration of the CB₂ receptor antagonist, SR144528 (Calignano *et al.*, 1998, 2001; Farquhar-Smith and Rice, 2001).

Later, PPAR- α agonists were proposed as a new class of analgesics because GW7647 was found to be efficacious, like PEA, at reducing pain behaviours elicited in mice by intraplantar injection of formalin or magnesium sulfate, as well as hyperalgesic responses in the chronic constriction injury (CCI) model of neuropathic pain or in the complete Freund's adjuvant and carrageenan models of inflammatory pain (Lo Verme *et al.*, 2006; D'Agostino *et al.*, 2009; Di Cesare Mannelli *et al.*, 2013). The role of PPAR- α in mediating the actions of PEA (after both i.c.v. and s.c. administration) was suggested by the lack of anti-hyperalgesic effects of PEA in mutant mice lacking PPAR- α (Lo Verme *et al.*, 2006; D'Agostino *et al.*, 2009; Di Cesare Mannelli *et al.*, 2013). Importantly, it was also demonstrated that the i.c.v. administration of PEA was effective at preventing inhibitory κ B- α degradation and NF- κ B nuclear translocation, in dorsal root ganglia after carrageenan-induced inflammatory pain, suggesting the involvement of this transcriptional factor in the control of hyperalgesia (D'Agostino *et al.*, 2009). Nevertheless, because the antinociceptive effects occurred within minutes of agonist administration in wild-type mice, it was considered that they could be mediated via a transcription-independent mechanism (Lo Verme *et al.*, 2006). Thus, it was demonstrated that blockade of large-conductance KCa channels (BKCa) and intermediate-conductance KCa channels (IKCa) prevented the antinociceptive actions of GW7647 and PEA in the formalin test, suggesting that PPAR- α agonists can also modulate nociception through a nongenomic mechanism (Lo Verme *et al.*, 2006). In addition, it was also shown that blockade of PPAR- α and large-conductance Ca²⁺-activated K⁺ channels, by using selective antagonists, such as GW6471 and charybdotoxin, respectively, also prevented PEA effects, which after intraperiaqueductal grey microinjection reduced the ongoing activity of ON and OFF cells and produced an increase in the latency of the nociceptive reaction (De Novellis *et al.*, 2012). Finally, it has recently been demonstrated that PEA, by acting at PPAR- α , may also be responsible, at least in part, for NMDA-NR2b subunit down-regulation, contributing to reduce pain-related behaviours (Guida *et al.*, 2015).

CB₁ receptors, PPAR- γ and TRPV1 channels have also been suggested as potential targets for the analgesic actions of PEA, for example in the CCI model of neuropathic pain (Costa *et al.*, 2008). In fact, antagonists of these receptors blocked the anti-allodynic and anti-hyperalgesic effects induced by i.p. administration of PEA, suggesting that the most likely mechanism of action of PEA was through entourage effects (Costa *et al.*, 2008), due to the enhancement of tissue levels of AEA (Di Marzo *et al.*, 2001), which in turn is able to produce analgesia by activating CB₁ receptors (Guindon and Hohmann, 2009) or by desensitizing TRPV1 channels

(Starowicz *et al.*, 2012). The conclusions put forward by Costa *et al.* (2008) were supported by previous findings in rats with CCI, where thermal hyperalgesia and mechanical allodynia were accompanied by increased AEA and 2-AG levels in three brain areas involved in nociception (the dorsal raphe, periaqueductal grey and rostral ventral medulla), while PEA levels were significantly decreased (Petrosino *et al.*, 2007) (Table 2). These two studies, taken together, suggest that, although the endocannabinoids are up-regulated during pain conditions, their elevation may not be sufficient to exert an analgesic action because of reduced PEA levels (Petrosino *et al.*, 2007) and that exogenously administered PEA could be an effective alternative to potentiate the endogenous anti-nociceptive mechanism exerted by endocannabinoids (Costa *et al.*, 2008). On the other hand, Jhaveri *et al.* (2008) demonstrated that the levels of AEA and PEA are decreased in the hind paw after carrageenan-induced inflammatory pain (Table 2) and that the administration of a FAAH inhibitor, URB597, significantly elevated the levels of the two mediators, while causing anti-nociceptive effects that were blocked by the PPAR- α antagonist, GW6471 (Jhaveri *et al.*, 2008). Thus, a synergistic interaction among the several mechanisms of actions of PEA may occur, thereby allowing PEA to exert its analgesic and anti-nociceptive effects.

The analgesic properties of micronized and ultra-micronized formulations of PEA, that is, m-PEA and um-PEA, respectively, were initially shown in a rat model of carrageenan-induced inflammatory pain, where carrageenan-induced paw oedema and thermal hyperalgesia were markedly and significantly reduced by oral treatment with either formulation (Impellizzeri *et al.*, 2014). Later, clinical studies have demonstrated the efficacy of m-PEA and um-PEA, both when administered alone and in combination with the antioxidant stilbene, polydatin. In particular, it was shown that the administration of m-PEA (300 mg p.o. twice daily) reduced pain scores in diabetic patients suffering from peripheral neuropathy (Schifilliti *et al.*, 2014). Importantly, no alterations related to m-PEA treatment were revealed from haematological and urine analyses, and no adverse events were observed (Schifilliti *et al.*, 2014). Interestingly, in a mouse model of streptozotocin-induced type 1 diabetes, it has been recently observed that PEA treatment (i.p.) was not only able to relieve mechanical allodynia but also to improve insulin levels and preserve Langerhans islet morphology by reducing the development of insulinitis (Donvito *et al.*, 2015). The effectiveness of um-PEA has instead been evaluated both in patients with neuropathic pain due to lumbosciatalgia and in patients with chronic pain caused by different etiopathogenesis (Dominguez *et al.*, 2012; Gatti *et al.*, 2012). In the former study, the addition of um-PEA (600 mg·day⁻¹ p.o.) to a standard treatment was well tolerated and showed an improvement in pain relief (Dominguez *et al.*, 2012). In the latter study conducted on 610 patients, um-PEA (600 mg p.o. twice daily) was administered in addition to standard analgesic therapies or as a single therapy (Gatti *et al.*, 2012). The results demonstrated that the decrease in pain intensity induced by um-PEA was also present in patients without concomitant analgesic therapy and that PEA produced no adverse effects (Gatti *et al.*, 2012). Furthermore, short-term

efficacy of um-PEA was also demonstrated in patients with diabetic or traumatic neuropathic pain, where the administration of the formulation (1200 mg·day⁻¹ p.o.) in addition to standard therapies improved both the visual analogue scale and neuropathic pain symptom inventory scores within the first 10 days (Cocito *et al.*, 2014). Importantly, a pooled data meta-analysis has recently been performed to evaluate the efficacy and safety of m-PEA and um-PEA on pain intensity in patients suffering from chronic and/or neuropathic pain (Paladini *et al.*, 2016). This study confirmed that PEA-induced pain relief is progressive, age- and gender-independent and not related to the aetiopathogenesis of chronic pain (Paladini *et al.*, 2016). Moreover, PEA administration lacked acute and chronic toxicity and was not associated with gastric mucosal lesions (Paladini *et al.*, 2016). In contrast to these promising actions, a more recent report showed that um-PEA as add-on therapy was ineffective on neuropathic pain in individuals with SCI (Andresen *et al.*, 2016). This negative outcome is not surprising considering that the study included patients (i) with different causes and severities of SCI and, more importantly, with an average time since injury of 10 years; (ii) receiving concomitant analgesic medication (dosing and length of treatment not specified); and (iii) with high pain scores at entrance (possibly indicating they were refractory to pain treatment) (Andresen *et al.*, 2016). All the aforementioned factors could have synergistically contributed to the lack of effect. Accordingly, the study might suggest that the administration of um-PEA may be beneficial if administered in early stages of SCI, as observed in experimental studies. Furthermore, it has been reported that the new combination m-PEA-polydatin (one tablet twice daily), constituted of m-PEA (400 mg) and the antioxidant stilbene polydatin (40 mg), is effective at reducing pelvic pain in women with endometriosis (Indraco and Barbieri, 2010; Cobellis *et al.*, 2011; Giugliano *et al.*, 2013). Likewise, it has been demonstrated that the oral administration of um-PEA is effective at reducing viscerovisceral hyperalgesia in a rat model of endometriosis plus ureteral calculus (Iuvone *et al.*, 2016). These findings are in agreement with the recently reported elevation of the plasma levels of PEA (and AEA) in patients with moderate-to-severe dysmenorrhea and dyspareunia compared with those with low-to-moderate pain symptoms (Sanchez *et al.*, 2016).

In summary, these findings suggest that PEA, alone or in combination with polydatin, represents a new promising and well-tolerated therapeutic strategy for the management of chronic pain in different pathological conditions.

Inflammatory diseases

The anti-inflammatory effects of PEA seem to be mainly related to its ability to modulate mast cell activation and degranulation, and this action is also known as the ALIA (autacoid local inflammation antagonism) mechanism (Aloe *et al.*, 1993; Facci *et al.*, 1995). The first evidence of the anti-inflammatory effects of PEA in animal models was reported by Mazzari *et al.* (1996), who demonstrated that orally administered PEA is able to decrease the amount of degranulated

mast cells and plasma extravasation induced by substance P injection in the mouse ear pinna (Mazzari *et al.*, 1996). Oral PEA also reduced paw oedema induced by carrageenan, dextran and formalin, suggesting that the compound directly down-modulates mast cell activation *in vivo* and suppresses pathological consequences initiated by mast cell activation regardless of the activating stimuli (Mazzari *et al.*, 1996). Later, the involvement of CB₂ receptors in these effects was suggested, based on the finding that the CB₂ receptor antagonist, SR144528, prevented the anti-oedema effect produced by PEA (Conti *et al.*, 2002) but did not reverse its curative effect after carrageenan-induced acute inflammation (Costa *et al.*, 2002).

Indeed, it was later shown that PPAR- α also mediates the anti-inflammatory effects of PEA, since both after carrageenan-induced paw oedema and phorbol ester-induced ear oedema, the topically applied compound attenuated inflammation in wild-type mice but had no effect in mice deficient in PPAR- α , whereas the PPAR- α agonist, GW7647, mimicked the effects of PEA (Lo Verme *et al.*, 2005a). Importantly, acute i.c.v. administration of PEA reduced carrageenan-induced paw oedema, restored carrageenan-induced PPAR- α reduction, prevented I κ B- α degradation and NF- κ B nuclear translocation in the spinal cord, suggesting the involvement of this transcriptional factor also in the central control of peripheral inflammation (D'Agostino *et al.*, 2007). These anti-inflammatory effects of PEA were again mimicked by a PPAR- α agonist, GW7647, and absent in mutant mice lacking PPAR- α (D'Agostino *et al.*, 2007).

The anti-inflammatory effects of PEA have also been investigated in numerous inflammatory diseases. In particular, the efficacy of this lipid compound was demonstrated in several animal models of inflammatory bowel diseases, such as croton oil-induced chronic intestinal inflammation, dextran sodium sulphate (DSS)-induced ulcerative colitis, oil of mustard (OM)-induced accelerated transit and dinitrobenzene sulfonic acid (DNBS)-induced colitis. Initially, it was observed that endogenous PEA levels were decreased in the small intestine of mice with chronic intestinal inflammation induced by croton oil and were associated with increased intestinal transit (Capasso *et al.*, 2001) (Table 2). Accordingly, exogenous PEA administration (i.p.) decreased intestinal transit and motility, and these effects were not blocked by the CB₁ and CB₂ receptor antagonists, SR141716A and SR144528, respectively, suggesting that they were independent from cannabinoid receptor activation (Capasso *et al.*, 2001). Therefore, the role of PPAR- α in inflammatory bowel diseases was also studied, and in a mouse model of DSS-induced ulcerative colitis as well as in cultured human biopsies deriving from patients with ulcerative colitis, PEA treatment improved the macroscopic signs of ulcerative colitis, decreased the expression and release of pro-inflammatory cytokines as well as neutrophil infiltration (Esposito *et al.*, 2014), all these anti-inflammatory effects being abolished by PPAR- α antagonists (Esposito *et al.*, 2014). More recently, the effects of PEA on inflammation-associated angiogenesis in mice with DSS-induced ulcerative colitis and in patients with ulcerative colitis were also studied (Sarnelli *et al.*, 2016). PEA treatment, in a PPAR- α -dependent manner, inhibited colitis-associated angiogenesis by decreasing VEGF release and new vessel formation via the mammalian target of rapamycin/PKB (mTOR/Akt) axis (Sarnelli *et al.*, 2016).

These results suggest that PEA may exert its protective effects, in both inflammation and cancer, by reducing mucosal damage, disease progression and the shift towards carcinogenesis (Sarnelli *et al.*, 2016). On the other hand, in a mouse model of delayed accelerated transit – a pathological condition that persists after the resolution of colonic inflammation and is known as post-inflammatory irritable bowel syndrome – induced by OM, the CB₁ receptor antagonist, SR141716A, blocked the inhibitory effect of PEA on accelerated transit, whereas the TRPV1 antagonist, 5'-iodo-resiniferatoxin (I-RTX), increased it (Capasso *et al.*, 2014). Likewise, in a mouse model of DNBS-induced colitis, the TRPV1 antagonist, capsazepine, increased the anti-inflammatory effects of PEA, which instead were reversed not only by a CB₂ antagonist but also by GPR55 and PPAR- α antagonists (Borrelli *et al.*, 2015). These studies, taken together, suggest that, although a direct activation of GPR55 or PPAR- α occurs, PEA can produce its anti-inflammatory action in the gut also via indirect activation of CB₁ and CB₂ receptors, probably due to the ability of this compound to potentiate the action of endocannabinoids at these receptors. Indeed, elevated levels of AEA and 2-AG were found both in the intestine of mice with accelerated transit induced by OM and in the colon of mice with colitis induced by DNBS (Capasso *et al.*, 2014; Borrelli *et al.*, 2015).

The anti-inflammatory effects of PEA have also been demonstrated in *in vitro* and *in vivo* models of contact allergic dermatitis (CAD). Specifically, in human keratinocyte (HaCaT) cells stimulated with polyinosinic polycytidylic-acid (poly-(I:C)) and treated with PEA, the TRPV1 antagonist, I-RTX, reversed PEA inhibitory effects on the expression and release of chemokine monocyte chemoattractant protein 2 (MCP-2 also known as CCL8) (Petrosino *et al.*, 2010a). Moreover, in dinitrofluorobenzene (DNFB)-sensitized mice, PEA anti-inflammatory effects (after *i.p.* administration) were counteracted by another TRPV1 antagonist, capsazepine (Petrosino *et al.*, 2010a). Also in this case, the TRPV1-mediated effects of PEA were attributed to the elevated levels of AEA and oleoylethanolamide (another endogenous TRPV1 agonist) detected both in poly-(I:C)-HaCaT cells and in the DNFB *in vivo* model of CAD (Karsak *et al.*, 2007; Petrosino *et al.*, 2010a) (Table 2) and hence to an entourage effect of PEA on the 'endovanilloid' desensitizing action over this channel (De Petrocellis *et al.*, 2001; Ho *et al.*, 2008). The ability of PEA to down-regulate canine mast cell activation by inhibiting the release of histamine, PGD₂ and TNF- α has also been reported (Cerrato *et al.*, 2010), and an increase in endocannabinoid and PEA levels was found in the skin of dogs with atopic dermatitis (Abramo *et al.*, 2014) (Table 2). Therefore, the efficacy of um-PEA was recently investigated in canine atopic dermatitis (Noli *et al.*, 2015). Oral administration of um-PEA to 160 dogs with atopic dermatitis and moderate pruritus was effective and safe in reducing pruritus and skin lesions in dogs (Noli *et al.*, 2015). PEA also strongly reduces the cutaneous allergic inflammatory reaction induced by different immunological and non-immunological stimuli in *Ascaris suum* hypersensitive Beagle dogs (Cerrato *et al.*, 2012), and this action is likely to be due to an entourage effect on the skin levels of the endocannabinoid 2-AG, which were dramatically elevated by PEA following oral administration (Petrosino *et al.*, 2016a). Interestingly, an entourage effect on

the skin levels of PEA has also been demonstrated (Petrosino *et al.*, 2016b). In fact, a synthetic PEA analogue, adelmidrol, was able to increase the endogenous levels of PEA in human and canine keratinocytes and reduce the skin inflammatory response, that is, chemokine production following allergic stimulation (Petrosino *et al.*, 2016b). These results suggested that the increase in endogenous concentrations of PEA might partially mediate the anti-inflammatory effect of adelmidrol (Petrosino *et al.*, 2016b).

Recently, the anti-inflammatory effects of PEA have also been investigated in animal models of uveitis, retinal inflammation and cystitis. In rats subjected to endotoxin-induced uveitis, PEA treatment (*i.p.*) was effective at reducing the degree of ocular inflammation, because it decreased inflammatory cell infiltration, TNF- α and intercellular adhesion molecule 1 (ICAM-1) levels, and inhibited iNOS expression as well as nuclear NF- κ B translocation (Impellizzeri *et al.*, 2015). Likewise, oral PEA attenuated the degree of ocular inflammation in rats with experimental diabetic retinopathy and preserved the blood-retinal barrier (Paterniti *et al.*, 2015). Finally, in a female rat model of cyclophosphamide-induced cystitis, it has been reported that pain behaviour, bladder inflammation and voiding dysfunction were associated with increased bladder levels of PEA, up-regulation of CB₁ receptor expression and down-regulation of PPAR- α expression (Pessina *et al.*, 2015) (Table 2). Oral administration of um-PEA produced both anti-inflammatory and analgesic effects, which were counteracted both by CB₁ receptor and PPAR- α antagonists (Pessina *et al.*, 2015).

The effectiveness of the combinations co-ultraPEALut and m-PEA-polydatin has also been shown in animal models of inflammation. In mice subjected to collagen-induced arthritis, treatment with co-ultraPEALut (*i.p.*) reduced periarticular erythema and paw oedema, nitrotyrosine and malondialdehyde levels, as well as plasma levels of cytokines and chemokines (Impellizzeri *et al.*, 2013). In rats subjected to carrageenan-induced acute inflammation, the efficacy of an oral combination of m-PEA and polydatin was compared with that of a new co-micronized composite containing PEA and polydatin, given by the same route, with the latter showing stronger anti-inflammatory and anti-hyperalgesic effects compared with the simple association of two compounds (Esposito *et al.*, 2016).

Conclusions

Although PPAR- α is the molecular target that directly mediates some of the neuroprotective, anti-(neuro)inflammatory and analgesic effects of PEA (Mattace Raso *et al.*, 2014), the existence of indirect mechanisms of action for this compound has also often been demonstrated. In particular, endocannabinoid-mediated mechanisms of action following the activation of CB₁, CB₂ receptors or TRPV1 channels, known as the entourage effect (Di Marzo *et al.*, 2001; Petrosino *et al.*, 2016a), and a CB₂ receptor-TRPV1-mediated mechanism of action via PPAR- α (Ambrosino *et al.*, 2013, 2014; Guida F, Maione S and Di Marzo V, personal communication), have been identified. These findings suggest that the existence of the 'direct or via PPAR- α mechanisms' does not exclude the entourage effect, and in fact, a synergistic

interaction can occur between the various mechanisms and explain why PEA has multiple effects and the ability to act on different cell types. Indeed, while the ALIA mechanism (Aloe *et al.*, 1993), that is, the ability of PEA to inhibit mast cell degranulation, has been widely confirmed (Facci *et al.*, 1995; Cerrato *et al.*, 2010; De Filippis *et al.*, 2011), the participation of astrocytes, microglia and keratinocytes in PEA anti-inflammatory actions has also been revealed (Petrosino *et al.*, 2007; Bettoni *et al.*, 2013; Luongo *et al.*, 2013; Guida *et al.*, 2015). These features distinguish PEA from classical steroidal and non-steroidal anti-inflammatory drugs that act by inhibiting the cascade of arachidonic acid. Preclinical and human studies indicate that PEA, especially when co-micronized together with antioxidants, such as luteolin and polydatin, and in micronized or ultra-micronized forms, is a therapeutic tool with high potential for the effective treatment of different pathologies characterized by neurodegeneration, (neuro)inflammation and pain. Likewise, PEA also shows high safety, as recently reported by Nestmann (2016). While it is intuitive why, for a particularly water insoluble compound such as PEA, any formulation aimed at enhancing its specific surface, such as m-PEA and um-PEA, is likely to increase tissue exposure to this compound following oral administration, or why co-administration of PEA with antioxidants should enhance its efficacy, it must be emphasized that full pharmacokinetic/pharmacodynamic comparisons between normal and m-PEA or um-PEA, or between PEA composites and the single components thereof, in the same study, have not been reported yet.

Author contributions

S.P. and V.D. drafted the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

S.P. is an employee of Epitech Group.

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