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Palmitoylethanolamide is a New Possible Pharmacological Treatment for the Inflammation Associated with Trauma

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Abstract: The endogenous fatty acid palmitoylethanolamide (PEA) is one of the members of N-acyl-ethanolamines family. PEA was identified more than five decades ago and was shown to reduce allergic reactions and inflammation in animals along with influenza symptoms in humans. Interest in this compound faded, however, until the discovery that one of its structural analogs, anandamide (arachidonoylethanolamide), serves as an endogenous ligand for cannabinoid receptors, the molecular target of Δ^9-tetrahydrocannabinol in marijuana. Since this finding, PEA has been shown to inhibit peripheral inflammation and mast-cell degranulation, as well as to exert neuroprotective and antinociceptive effects in rats and mice. These actions are also mediated by PPAR-α activation and are accompanied by a decrease in nitric oxide production, neutrophil influx, and expression of proinflammatory proteins such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition to the hypothesis that PEA has potent immunoregulatory properties, recent data have demonstrated that PEA may also play a key role in the regulation of complex systems involved in the inflammatory response, pruritus, neurogenic and neuropathic pain. In this review, we discuss briefly the present understanding therapeutic mechanisms of PEA and the novel possible PEA clinical use for the treatment of several inflammatory diseases and trauma.

Keywords: Acylethanolamines, inflammation, PPAR-alpha, SCI.

INTRODUCTION

Palmitoylethanolamide (PEA) is a saturated fatty acid derivative (C16:0) where the carboxylate function is amidated by the primary amine of ethanolamine, the molecular formula being C18H37NO2 and molecular weight 299.49. It is chemically known as N-(2-hydroxyethyl) hexadecanamide or N-(2-hydroxyethyl)-palmitamide and also referred to with the International Nonproprietary Name (INN) Palmidrol. PEA is a naturally occurring N-acyl ethanolamine (NAE) endorsed with pleiotropic effects, collectively considered to play protective and homeodynamic roles, in animals [1,2] and plant kingdoms [3]. It has recently been suggested that NAEs represent an evolutionarily conserved signalling pathway in multicellular eukaryotes, since the machinery for the biosynthesis and degradation of NAEs appears to be functionally conserved in both animal [4] and plant systems [3]. The observation highlights the importance of this lipid regulatory pathway in cell biology.

All NAEs are produced "on demand", they are not stored in vescicles but enzymatically released. When cells are subjected to potentially harmful stimuli, immune cells produce significant amounts of PEA and express a selective phospholipase D (PLD) that releases these endogenous fatty acid from their membrane phospholipid precursor, N-acylphosphatidylethanolamine (NAPE). Like other NAEs, PEA acts locally, and its tissue levels are tightly regulated through a balance between anabolic and catabolic pathways. Inflammatory cells express 2 intracellular amidases that have been implicated in lipid amide degradation: fatty-acid amide hydrolase (FAAH) and N-acylethanolaminehydrolyzing acid amidase (NAAA) [5,6]. The FAAH enzyme displays significant homology with the "amidase signature family" of enzymes and can act as a hydrolytic enzyme not only for fatty-acid ethanolamides such as anandamide, that remain the preferred substrate, but also for primary amides such as oleamide and even for non-cannabinoid fatty-acid ethanolamides, such as (PEA) and oleoylethanolamide (OEA). Recently, N-acylethanolamine-hydrolyzing acid amidase (NAAA) was identified as being able to specifically hydrolyze PEA. NAAA, that belongs to the choloylglycine hydrolase family reveals no sequence homology with FAAH and preferentially recognizes PEA. The most striking catalytic property of NAAA is pH optimum at 4.5–5, which is consistent with its immunocytochemical localization in lysosomes [7]. Thus, NAAA is a novel lysosomal hydrolase, which is structurally and functionally similar to acid ceramidase. Novel and recently results suggest a unique role of NAAA in the degradation of PEA. Since NAAA is an intracellular enzyme, PEA needs to be transported into the cell in order to be inactivated. It has been recognized that both immune and neuronal cells do uptake PEA by a "carrier-mediated" transport [8,9]. In fact, PEA, beside penetrating the cells by passive transfer through the cell

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membrane (due to its high lipophilicity), is uptaken by cells through a facilitated transport, that is apparently similar in neuronal and immune cells (i.e., mast cells) and pharmacologically distinct from that for the PEA analogue anandamide [9].

Several endogenous bioactive lipids, including anandamide (AEA), (PEA), oleamide, and (OEA), participate in many physiological activities such as analgesia, anxiety, sleep modulation, anti inflammatory responses, and appetite suppression. Because FAAH plays an essential role in controlling the tone and activity of these endogenous bioactive lipids, this enzyme has been implicated to be a drug target for the therapeutic management of pain, anxiety, and other disorders. In an effort to discover FAAH inhibitors, many recent studies reported the development of a novel highly potent and selective inhibitor of FAAH enzyme, such as KDS-4103 (URB59), that offer a novel approach with a favorable therapeutic window for the treatment of anxiety, depression and pain elevating PEA levels and enhancing its anti-inflammatory and antinociceptive effects.

Within the last decade, the research on NAEs has proceeded with a novel group of lipid receptors that belong to the superfamily of seven transmembrane receptors and are termed cannabinoid receptors. Two types of cannabinoid receptors are known so far: the CB1 receptor and the CB2 receptor. The endogenous ligands for both receptor subtypes are N-arachidonoylethanolamine also called anandamide (here abbreviated 20:4-NAE), and 2- arachidonoylglycerol (2-AG). However, of these two lipids, 2-AG appears to be a full agonist for both receptors, whereas 20:4-NAE is only a partial agonist for both receptors. 20:4-NAE belongs to a group of N-acyl-ethanolamines (NAEs), which are formed in large amounts in injured neurons, and these bioactive lipids may exert neuroprotective actions, being mediated by several cellular mechanisms [10]. It is well documented that activation of CB1 receptors is neuroprotective, most likely via a dominant presynaptic inhibition of glutamate release. NAEs may exert neuroprotective actions by several mechanisms, e.g., (1) by inhibiting the necrosis of injured cells, (2) by stimulating the injured cell and/or neighboring cells to activate apoptotic mechanisms in order to stop the spreading of necrosis, and (3) by inhibiting release of mediators that promote necrosis and inflammation. In addition at the level of the individual injured cell, a variety of saturated and monounsaturated NAEs may stabilize injured mitochondria, thereby preventing Ca2+ leakage, which is interesting because mitochondrial dysfunction is an important aspect of excitotoxicity [10].

Moreover, also PEA was originally considered to be a *bona fide* eCB, since it was suggested to be a cannabinoid receptor 2 (CB2) agonist [11]. Although these results were not confirmed [12-15], some of the pharmacologic effects of PEA are actually antagonized by SR144528, a selective CB2 receptor blocker [16-18]. Furthermore, N-palmitoylethanolamine was demonstrated to protect neurons in primary culture against excitotoxic death by an unknown cannabinoid receptor-independent mechanism that may be related to one of the mechanisms mentioned above. Nowadays PEA is more correctly referred to as a cannabinoid receptor-inactive eCB-related molecule [4, 19]. Indeed several mechanisms have been proposed to explain the anti-inflammatory and anti-hyperalgesic effects of PEA, including: (i) the activation of a cell surface receptor (i.e. the ‘‘CBn’’ (or CB2-like) or, alternatively, the orphan GPR55 receptor) or otherwise a nuclear receptor of the peroxisome proliferator-activated receptors (PPARs) family [20-22]; (ii) the down-modulation of mast cell hyper-activity (ALIA mechanism) [23]; (iii) an action as ‘‘entourage’’ compound, i.e. the augmentation of eCBs activities at their receptors and/or the inhibition of eCBs degradation [2]. The most recently proposed mechanisms will be discussed further in this review along with evidence on the protective role and the beneficial effects of PEA. In this review we will focus on the new insights indicating an involvement of PEA in several type of trauma as well as brain trauma. These findings highlight the potential of PEA as therapeutic targets for the treatment of trauma and related disorders.

TOLERABILITY AND SAFETY

PEA has been extensively researched in several different cell types *in vitro*, without exhibiting any toxic effects [24-26]. Furthermore, in the several preclinical studies published so far, the biopharmacologic effects of PEA were not associated with any changes in overt behaviour, neither in rodents [27] nor in dogs [28] indicating that all the tested doses were well tolerated. Moreover, the body weight gain of PEA-treated animals was reported to be similar to that of control animals [27].

Interestingly, it has been shown that the anti-inflammatory effect of PEA (unlike other NAEs) does not undergo tolerance following repeated administration of high doses, this being a critical finding with respect to the potential therapeutic utility of the compound [29].

Most importantly, PEA is devoid from the classic tetrad of behaviours, used to identify the cannabinergic psychoactive effect [18], i.e., depression of spontaneous locomotor activity, production of antinociception, hypothermia and catalepsy. Conversely, i.c.v. administration of PEA increased wakefulness and reduced both slow-wave and REM sleep [30]. Moreover, given i.c.v. in mice, PEA did not produce changes in locomotor activity, EEG parameters and permanence in the rotarod apparatus, suggesting the absence of any sedative and moto-neurological effect [31, 32]. Finally, PEA administered to rats at 10 and 30 mg/kg os did not change any of the physiological variables evaluated before and after an experimentally-induced acute stroke, i.e., pH, pO2 (mm Hg), pCO2 (mm Hg), base excess and body temperature [33]. These data stand for a non-psychoactive nature of PEA (i.e., free from psychotropic side effects).

Actually, the lack of any adverse effect has been repeatedly reported in published clinical studies, both in companion animals and humans. In a veterinary clinical study performed on 17 privately owned European shorthaired cats affected with allergic skin disease and treated with PEA (20 mg/kg/die, 30 days), no adverse effect was reported and tolerability was judged by the owners and investigators to be good or excellent [34]. In a recently published study on 6 hypersensitive dogs, the treatment with a single oral dose of PEA (3, 10, 30 mg/kg) did not produce any side effects [28].

In a pilot human study specifically aimed to preliminarily assess the tolerance of PEA, it was shown that oral administration of PEA to 5 children (50 mg/kg for 2 weeks) and 15 adults (100 mg/kg for 3 weeks) did not lead to any significant change in the value of any biochemical parameters which were analyzed before and after treatment. In a double-blind controlled randomized clinical study, recently performed on over 600 patients affected with lumbosciatic pain and treated with PEA (300 or 600 mg/die p.o., 21 days), no alterations in blood and urine biochemical studied parameters were observed; moreover, no adverse events were reported throughout the study [35]. A second similar study, performed on over 100 patients, resulted in similar findings, i.e. no changes in urine and hematological parameters and no adverse effect during the treatment period (PEA 300 or 600 mg/die p.o., 21 days) [36]. In a further randomized placebo-controlled trial on 26 patients suffering from carpal tunnel syndrome, the treatment with PEA (600 and 1200 mg/die p.o., 30 days) was considered to be safe and well tolerated: no adverse events were reported by PEA-treated patients or observed by physicians [37]. An open study on 30 patients suffering from neuropathic pain and treated with a combination of pregabalin and PEA (1200 mg/die p.o., 45 days) reported a 100% compliance rate (i.e., all patients completed the study) and no side effects [38].

Moreover a randomized doubleblind crossover clinical trial was performed to investigate the effect of oral PEA administration, on intraocular pressure (IOP) in primary open angle glaucoma (POAG) and ocular hypertension (OH). In a prospective, 42 patients with POAG or OH who were treated with timolol 0.5% and whose IOP was between 19 and 24 mm Hg received oral PEA (300-mg tablets twice a day) or placebo (PEA vehicle tablets twice a day) for 2 months (period 1), and, after a 2-month washout, received the other treatment for 1 month (period 2). After PEA treatment IOP was reduced by 3.2 ± 1.3 mm Hg at 1 month and by 3.5 ± 1.2 mm Hg (15.9% ± 5.1%) at 2 months; after placebo IOP was reduced by 0.4 ± 1.2 mm Hg at 1 month and by 0.3 ± 1.3 mm Hg at 2 months. In conclusion systemic administration of PEA reduces IOP in patients with glaucoma and ocular hypertension. PEA could be a valuable tool for the treatment of glaucoma.

Finally, it is noteworthy to mention that the topical use of PEA for long-term management of atopic eczema resulted to be safe in a multicentre non-controlled, prospective cohort study, performed on 2456 patients [39]. No serious adverse events were reported, and the tolerance was assessed by the physicians as very good or good in 66.3% and 25.7% of the patients, respectively, and as moderate in 4.6% of the patients. Only in 3.4% of all cases was the tolerance reported to be poor [39].

Altogether the aforementioned data on the safety and tolerability of PEA lay the foundations for a potential therapeutic use of the compound.

A HISTORICAL PERSPECTIVE

PEA was first discovered in the late 1950s, when it was shown that the anti-allergic and anti-inflammatory activity exerted by dietary supplementation with egg yolk, peanut oil or soybean lecithin [40, 41] was due to a specific lipid fraction corresponding to PEA [42, 43]. Particularly, PEA was found to be active at very low doses (0.3 μg/kg i.p., 3 to 6 hrs before inflammation) in the guinea pig joint anaphylaxis assay, and, notably, large doses of PEA produced no greater anti-inflammatory action than small doses [43]. Moreover, PEA was found to protect mice against fatal anaphylactic shock when given at a dose of 0.5 and 5 mg/kg i.p., and the number of survivors observed after the lower dose of PEA was about equal to that seen with 100 mg/kg hydrocortisone [44], corresponding to PEA being 200 hundred times more active than hydrocortisone in this model.

After a ten-year hiatus, there was a renewed interest on PEA, and its anti-inflammatory and protective activities were confirmed in several models of inflammation, i.e. carrageenin-induced paw oedema, adjuvant-induced arthritis and tuberculin hypersensitivity [45, 46]. In the mid '70s, based on the positive results of several large scale double-blind human trials investigating the effect of PEA on the upper respiratory tract disease [47, 48], a product containing PEA was marketed for a few years in the Czech Republic, under the trade name of Impulsin. The drug was recommended for the prevention of virus infections of the respiratory tract and shown to lower the incidence and to reduce morbidity from acute respiratory disease both in children and adults.

A dozen years passed since new data on PEA were released. It was from the cooperation between an Italian pharmaceutical Group (Innovet-Epitech group, formerly called Lifegroup) and the research group headed by the 1996 Nobel Prize Winner Rita Levi Montalcini that PEA received renewed attention. In particular, it was shown that pre-treatment with PEA (20mg/kg s.c.) significantly reduced mast cell degranulation induced by the local administration of substance P (SP) in the rat ear pinna [49]. It was thus speculated that endogenous local production of PEA might be an adaptive response for the regulation of mast cell activation and consequent expression of inflammatory processes. The acronym ALIA (Autacoid Local Inflammation Antagonism) was coined for this novel mast cell modulation mechanism [49]. The result is a milestone in the history of PEA, its strength being mainly dependent on the crucial role played by mast cells not only in SP-induced neurogenic inflammation [50], but in a much wider spectrum of physiological and pathological responses, e.g., allergy, infection, angiogenesis and pain [51-60]. In fact, mast cells and mast cell mediators are now considered to be deeply involved in a wide variety of human and animal disease processes, ranging from dermatologic, cardiopulmonary, joint, gastrointestinal and urinary disorders to central and peripheral nervous system diseases [61-72].

It was later discovered that PEA protects cultured cerebellar granule cells from glutamate toxicity in a delayed postagonist paradigm [26]. Based on this finding, it was speculated that the function of PEA is not limited to an autacoid reduction of inflammation, but a more broad local anti-injury function, thus the acronym ALIA was used to describe an Autacoid Local Injury Antagonism [26]. The hypothesis is outlined and discussed in an illuminating

review paper by Levi Montalcini and colleagues [23], where it is speculated that the local production of PEA might inhibit both inflammation and the nerve sensitization. Particularly, it is proposed that PEA accumulates in tissues following injury and exerts a local, autacoid, anti-injury function via down-modulating mast cells and protecting neurons against excitotoxic, thus reducing tissue inflammation, decreasing hyperalgesia and exerting a neuroprotective function [23].

ENDOGENOUS LEVELS IN PLANTS, INVERTEBRATES AND VERTEBRATES

N-acylphosphatidylethanolamines (NAPEs), i.e. the membrane phospholipid precursors of NAEs [73], were originally isolated from a multiplicity of living beings, i.e., pea seeds [74], soyabean [75], mammalian skin [76], anaerobic bacteria [77], amoeba Dictyostelium discoideum [78], leech [79], infarcted canine myocardium [80], fish brain and spinal cord [81], dog brain [82], ischemic rat brain [83], neuroblastoma cells [84], mouse macrophages [85], and in rat testis homogenate [15]. Thus, the NAPE-forming transacylase does not appear to be confined to neurons, but is present in several animal cell types as well as plants [3]. For a comprehensive review on NAPEs isolation, characterization, occurrence, and metabolism, see Schmid and coll. [86]. The tissue amount of NAPEs is generally found to be tens-fold higher than the level of total NAEs [73, 79, 87, 88].

The first identification of the NAE molecular species in plant cells was accomplished by Chapman and coll. in tobacco and the total NAE content was measured thereafter in several plant species by gas chromatography-mass spectroscopy and found to range from around 500 ng/g to 1600 ng/g depending on the specific variety [89]. Out of the several NAE types identified in desiccated seeds of a variety of plant species, PEA is one of the most abundant [3]. Originally isolated from egg yolk, peanut oil and soybean lecithin [42, 90], PEA unlike other NAEs, is highly conserved during evolution and it is found even in microscopic, single-cell organism, like the yeast Saccharomyces cerevisiae [91]. Together with its synthetic and degradative machinery, PEA may thus represent a widespread tuning system of various different physiological responses. In mammals, PEA was first detected in the mid '60s, the occurrence being reported in the brain, liver, and skeletal muscle of fasted rats and guinea pigs [92]. The authors of this pioneering study also highlighted that the level of PEA was most constant and higher in the brain than in other tissues, representing almost 0.1% of the total brain lipid, while in the liver and skeletal muscle PEA accounted respectively for 0.015% and 0.002% of the total lipid [92]. Recently, this finding was confirmed by the use of an advanced target lipidomics approach [93], and PEA was confirmed to represent over 65% of rat brain NAEs, a very high content compared to N-stearoylethanolamide, N-oleoylethanolamide and anandamide, whose content was respectively 20% and 8% [94]. Furthermore, PEA resulted to be the most abundant NAE in all vertebrate and invertebrate specimens evaluated so far [79, 95-98]. In the mouse stomach, for instance, PEA is five-fold higher than the levels of OEA which, in turn, are six-fold higher than those of AEA [95]. In plasma of rats on the first week of pregnancy, PEA levels are 10 and 100 times higher than OEA and AEA, respectively [98]. In human reproductive fluids (i.e., seminal plasma, oviductal fluid, and follicular fluids), the levels of PEA were significantly higher than AEA [99]. Finally, in human subcutaneous fat, PEA was found to be the most abundantly produced among all the NAEs quantified [100].

It is now becoming apparent that PEA exists in virtually all cells, tissues and body fluids, with a widespread occurrence both in plants and animals. The main findings about the occurrence of PEA in living organisms are reported in Table **1** (A, B, C). In mammals, its levels vary among different tissues and body fluid, being 4-20 pmol/ml and 45 pmol/ml in human and mice blood respectively. The highest value reported so far is that in the human synovial fluid, where PEA level was found to be 1500 pmol/ml [101]. In the brain (both as a whole and particular areas), PEA levels have been reported to range from around 0.13 to 6.84 pmol/mg. It is noteworthy to point out that circadian variation occurs in the CNS concentration of PEA, levels being higher in the CSF, pons, hippocampus and hypothalamus during the dark period (8:00 p.m. - 4 a.m.) compared to the light period [102]. Although the biological significance of this variation is still unknown, it has been speculated that the nocturnal increase in the CNS levels of PEA might be linked to the anti-inflammatory and /or antinociceptive effect of the compound [102].

The levels of endogenous PEA in muscle and adipose tissues (0.04 - 6.00 pmol/mg) are similar to those observed in the CNS and something higher compared to the gastrointestinal tract (0.05-1.50 pmol/mg), skin (0.3- 0.69 pmol/mg) and eye (0.10 - 0.64 pmol/mg). As for the latter, it is noteworthy to mention that among all the human eye tissues analyzed so far, iris seems to contain the highest levels of PEA [103] and more particularly three-fold higher than elsewhere in the eye [104].

CHANGES IN THE LOCAL LEVEL OF PEA IN RESPONSE TO ACTUAL OR POTENTIAL DAMAGE

PEA is produced by several different cell types, ranging from immune and fat cells to CNS cells (i.e., neurons, microglia, astrocytes). The main findings about cells producing PEA are summarized in Table **2**. Interestingly, activated mast cells which are thought to be one of the possible cellular targets of PEA, do synthesize PEA [8]. PEA has also been found in peritoneal macrophages [88], adipocytes, pancreatic beta-cells [105], astrocytes from different species under basal conditions [106] and microglial cell line [107]. More particularly, the latter were found to produce 1.5-fold more PEA than 2-AG and 1.3-fold less than AEA [107]. Primary cultures of rat neurons also produce PEA. Di Marzo and coll. [108] have demonstrated the formation of NAEs, including PEA, in primary cultures of rat striatal and cortical neurons stimulated with kainate or ionomycin (i.e. a calcium ionophore). Moreover, glutamate-induced formation of NAEs in neocortical neurons was shown, and receptor-dependent formation of PEA by coactivation of NMDA and acetylcholine receptors was recently demonstrated in neurons [109]. PEA has thus been suggested to represent an ideal mediator for neuro-immune interactions during neurogenic and neuropathic pain [110].

Table 1. A– Levels of PEA in Plants, Yeast and Invertebrates.

Plant seeds and plant derived products	Barrel clover *(Medicago truncatula)*	13,0 µg/g fw	Kilaru *et al.,* 2007 [149]
	Soybean	6,7 µg/g fw	Kilaru *et al.,* 2007 [149]
	Peanut	3,73 µg/g fw	Kilaru *et al.,* 2007 [149]
	Alfalfa (erba medica)	1,1 µg/g fw	Kilaru *et al.,* 2007 [149]
	Texas bluebonnet *(Lupinus texensis)*	0,37 µg/g fw	Kilaru *et al.,* 2007 [149]
	Cotton	0,35 µg/g fw	Kilaru *et al.,* 2007 [149]
	Corn	0,2 µg/g fw	Kilaru *et al.,* 2007 [149]
	Garden pea	0,1 µg/g fw	Kilaru *et al.,* 2007 [149]
	Tomato	0,1 µg/g fw	Kilaru *et al.,* 2007 [149]
	Soy lecithin	950 µg/g fw	Kilaru *et al.,* 2007 [149]
Yeast	Saccharomyces cerevisiae	650 pmol/g of protein	Muccioli *et al.,* 2009 [91]
Invertebrates	Leech *Hirudo officinalis* (CNS)	32.3±1.5 pmol/g ww	Matias *et al.,* 2001 [79]
	Edible bivalve molluscs (five species)	Range ~ 20-60 ng/g ww	Sepe *et al.,* 1998 [150]

fw= fresh weight; ww = wet weight.

Table 1. B– Levels of PEA in Mammals.

Body Tissue	Species	Area	Original value	Normalized value (pmol/mg)	Reference
Brain and brain regions	Pig	Whole brain	2.05 ± 0.49 µ/g (ww)	6.84 ± 1.64	Schmid *et al.,* 1995 [151]
	Sheep	Whole brain	0.75 µ/g (ww)	2.50	Schmid *et al.,* 1995 [151]
	Cow	Whole brain	1.10 µ/g (ww)	3.67	Schmid *et al.,* 1995 [151]
	Guinea pig	Whole brain	45 µ/g	150.25	Bachur *et al.,* 1965 [151]
	Rat	Whole brain	35.7 µ/g	119.20	Bachur *et al.,* 1965 [151]
	Rat	Whole brain	~ 330 pmol/g	0.33	Artmann *et al.,* 2008 [152]
	Mouse	Whole brain	430 ± 24 pmol/gm	0.43 ± 0.02	Franklin *et al.,* 2003 [152]
	Mouse	Brain and spinal chord	~ 220–240 pmol/g	0.22-0.24	Baker *et al.,* 2001 [153]
	Mouse	Whole brain	~ 2 nmol/g (fw)	2.00	Kilaru *et al.,* 2010 [153]
	Mouse	Brain	0,45 nmol/g	0.45	Muccioli and Stella, 2008
	Muse	Striatum	5.7 ± 0.1 pmol/mg e.l.	5.7 ± 0.10 e.l.	Bisogno *et al.,* 2008
	Mouse *(Hungthington model)*	Striatum	3.4 ± 0.1 pmol/mg e.l.	3.40 ± 0.1 e.l.	Bisogno *et al.,* 2008
	Rat	Striatum	3.98 ± 0.39 nmol/g	3.98 ± 0.39	Richardson *et al.,* 2007 [154]
	Mouse	Cortex and hippocampus	~ 3 pmol/mg e.l.	3.00 e.l.	Bisogno *et al.,* 2008
	Rat	Hippocampus	0.716 ± 0.09 nmol/g	0.72 ± 0.09	Richardson *et al.,* 2007 [154]
	Rat	Frontal cortex	1.94 ± 0.36 nmol/g	1.94 ± 0.36	Richardson *et al.,* 2007 [154]
	Rat	Prefrontal cortex	144.6 ± 2.8 pmol/g	0.14 ± 0.003	Rubio *et al.,* 2007 [155]
	Rat	Rest of cortex	0.91 ± 0.08 nmol/g	0.91 ± 0.08	Richardson *et al.,* 2007 [154]

(Table 1) contd….

Body Tissue	Species	Area	Original value	Normalized value (pmol/mg)	Reference
	Rat	Thalamus	1.13 ± 0.14 nmol/g	1.13 ± 0.14	Richardson *et al.,* 2007
	Rat	Hypothalamus	5.11 ± 1.4 nmol/g	5.11 ± 1.40	Richardson *et al.,* 2007
	Rat	Hypothalamus	290.6 ± 14.2 pmol/g	0.29 ± 0.01	Rubio *et al.,* 2007
	Rat	Midbrain	1.53 ± 0.34 nmol/g	1.53 ± 0.34	Richardson *et al.,* 2007
	Rat	Cerebellum	1.78 ± 0.23 nmol/g	1.78 ± 0.23	Richardson *et al.,* 2007
	Rat	Pons–medulla	0.814 ± 0.04 nmol/g	0.81 ± 0.04	Richardson *et al.,* 2007
	Rat	Amygdala	132.9 ± 12.5 pmol/g	0.13 ± 0.013	Rubio *et al.,* 2007
	Rat	Caudate-putamen	337.3 ± 10.9 pmol/g	0.34 ± 0.011	Rubio *et al.,* 2007
Muscle	Mouse	Heart	~ 0.6 nmol/g (fw)	0.6	Kilaru *et al.,* 2010
	Guinea pig	Muscle	0.88 μ/g	2.94	Bachur *et al.,* 1965
	Rat	Muscle	1.2 μ/g	4.01	Bachur *et al.,* 1965
	Human	Trapezius muscle	0.53 nM	0.53	Ghafouri *et al.,* 2010
Fat tissue	Mouse	Subcutaneous fat	4.3±1.7 nmol/g (ww)	4.30 ± 1.70	Matias *et al.,* 2007
	Human (obese)	Subcutaneous fat	~ 1100 pmol/g	~ 1.10	Matias *et al.,* 2007
	Human (normoweight)	Subcutaneous adipose tissue	~ 140 pmol/g (ww)	~0.14	Annuzzi *et al.,* 2010 [156]
	Human (DM2+obesity)	Subcutaneous adipose tissue	~ 390 pmol/g (ww)	~0.39	Annuzzi *et al.,* 2010
	Mouse	Visceral fat	0.4±0.05 nmol/g (ww)	0.40 ± 0.05	Matias *et al.,* 2007
	Human (normoweight)	Visceral fat	~ 1800 pmol/g	~ 1.80	Matias *et al.,* 2007
	Human (obese)	Visceral fat	~ 1900 pmol/g fat tissue	~ 1.90	Matias *et al.,* 2007
Reproductive system	Mouse	Uterus	~ 6 pmol/mg protein	6.00	Maccarrone *et al.,* 2005 [157]
	Rat	Testis	42.8 pmol/g	0.04	Kondo *et al.,* 1998
Joint	Human	Knee synovial membrane	1000-2300 pmol/g	1.00-2.30	Richardson *et al.,* 2008
GI tract	Rat	Jejunum mucosa	~ 40 pmol/mg e.l.	40,00 e.l.	D'Argenio *et al.,* 2007
	Mouse	Small intestine	77.5±6.3 pmol/g	0,08 ± 0,006	Capasso *et al.,* 2001
	Mouse	Colon	3.1±0.5 pmol/mg e.l.	3,10 ± 0,50 e.l.	Izzo *et al.,* 2008
	Rat	Jejunum	~ 230 pmol/g	0,23	Artmann *et al.,* 2008
	Mouse	Stomach	1.5 nmol/g	1,50	Borrelli e Izzo, 2009
	Human	Duodenum	~2.2 pmol/mg e.l.	2,20 e.l.	D'Argenio *et al.,* 2007
	Human	Colon	2.6±0.3 pmol/mg e.l.	2,60 ± 0,30 e.l.	Darmani *et al.,* 2005
	Human (Crohn's disease, inflamed mucosa)	Colon	123 pmol/g (range 57-1127)	0,12 (range 0,06-1,13)	Di Sabatino *et al.,* 2011
	Human (Ulcerative colitis, inflamed mucosa)	colon	151 pmol/g (range 55-380)	0,15 (range 0,06-0,06)	Di Sabatino *et al.,* 2011

(Table 1) contd….

Body Tissue	Species	Area	Original value	Normalized value (pmol/mg)	Reference
	Human (Crohon's disease, uninflamed mucosa)	colon	163 pmol/g (range 54-677)	0,16 (range 0,05-0,68)	Di Sabatino *et al.,* 2011
	Human (Ulcerative colitis, uninflamed mucosa)	colon	310 pmol/g (range 55-1394)	0,31 (range 0,06-1,39)	Di Sabatino *et al.,* 2011
	Human (normal)	Colon	109 pmol/g (range 28-401)	0,11 (range 0,03-0,40)	Di Sabatino *et al.,* 2011
	Guinea pig	Liver	17 μ/g	56,76	Bachur *et al.,* 1965
	Rat	Liver	5.75 μ/g	19,20	Bachur *et al.,* 1965
	Rat	Liver	~ 50 pmol/g	0,05	Artmann *et al.,* 2008
	Mouse	Pancreas	0.7±0.05 nmol/g (ww)	0,70±0,05	Matias *et al.,* 2007
Eye	Human	Meibomian Gland Secretions	Qualitative analysis	-	Nichols *et al.,* 2007 [159]
	Human	Eye tissues	Range 112-465 pmol/g	0.11-0.47	Matias *et al.,* 2006
	Human	Eye tissues	Range 95-637 pmol/g	0.10-0.64	Chen *et al.,* 2005
	Human	Iris	466±60 pmol/g (higher than in the other analyzed eye tissues)	0.47±0.06	Matias *et al.,* 2006
	Human	Iris	637±148 pmol/g (3-fold higher than in the other analyzed eye tissues)	0.64±0.15	Chen *et al.,* 2005
	Human	Ciliary body	158±19 pmol/g	0.16±0.02	Chen *et al.,* 2005
	Human	Choroid	200±30 pmol/g	0.20±0.03	Chen *et al.,* 2005
	Human	Retina	~ 130 pmol/g	0.13	Chen *et al.,* 2005
	Human	Cornea	~ 150 pmol/g	0.15	Chen *et al.,* 2005
Skin	Rat	Paw	692±119 pmol/g	0,69±0,12	Calignano *et al.,* 2008
	Rat	Paw	300 pmol/g	0.30	Guindon *et al.,* 2006 [160]
	Rat	Paw	5.60±1.0 pmol/mg e.l.	5,60±1,00 e.l.	Beaulieu *et al.,* 2000
	Rat	Paw	0.53±0.04 nmol/g	0,53±0,04	Darmani *et al.,* 2005
	Mice	Ear	29.9±1.5 pmol/mg e.l.	29,90±1,50 e.l.	Petrosino *et al.,* 2010

(ww) = wet weight; (fw) = fresh weight; e.l. = extracted lipids.

Table 1. C- Physiological Levels of PEA in Normal Mammalian Body Fluids.

Biological Fluid	FLUID TYPE	Original value	Normalized Value (pmol/ml)	Reference
	Human blood	14 pmol/ml	14	Darmani *et al.,* 2005 [161]
Plasma	Human blood (basal value)	9.4 - 16.7 pmol/ml	9.4 - 16.7	Zolese *et al.,* 2005 [161]
	Human serum (healthy volunteers)	28.44 ± 5.91 pmol/ml	28.44 ± 5.91	Schreiber *et al.,* 2007 [162]
	Human serum with cells (healthy volunteers)	29.07 ± 1.60 pmol/ml	29.07 ± 1.60	Schreiber *et al.,* 2007 [162]
	Women serum	12.6 pmol/ml	12.6	Hill *et al.,* 2009 [163]
	EDTA plasma (healthy human volunteers)*	1.556±0.163 ng/ml	5.20 ± 0.54	Ozalp and Barroso, 2009 [163]

(Table 1) contd….

Biological Fluid	FLUID TYPE	Original value	Normalized Value (pmol/ml)	Reference
	lithium-heparin plasma (healthy human volunteers)*	1.717±0.027 ng/ml	5.73 ± 0.09	Ozalp and Barroso, 2009 [164]
	Sodium fluoride plasma (healthy human volunteers)*	1.436±0.047 ng/ml	4.79 ± 0.16	Ozalp and Barroso, 2009 [164]
	Citrate plasma (healthy human volunteers)*	1.217±0.091 ng/ml	4.06 ± 0.30	Ozalp and Barroso, 2009 [164]
	Human plasma	16.91 ± 4.23 nmol/l	16.91 ± 4.23	Lam *et al.,* 2010
	Human serum	16.25 ± 3.67 nmol/l	16.25 ± 3.67	Lam *et al.,* 2010
	Human plasma	1.39±0.36 ng/mL	4.64 ± 1.20	Balvers *et al.,* 2009 [165]
	Human plasma	7.63±1.91 pmol/ml	7.63 ±1.91	Jumpertz *et al.,* 2011 [166]
	Mice serum	45 pmol/ml of blood	45.00	Hoareau *et al.,* 2009
	Fasted rats plasma	2.54 ± 0.08 ng/ml	8.48 ± 0.27	Sharma *et al.,* 2011 [167]
	deproteinated rat blood plasma	16.7 pmol/ml	16.7	Giuffrida and Piomelli, 1998 [168]
Other Biofluids	Human umbilical artery	22.34 ± 9.37 nmol/l	22.34 ± 9.37	Lam *et al.,* 2010
	Human umbilical vein	25.47 ± 10.75 nmol/l	25.47 ± 10.75	Lam *et al.,* 2010
	Human urine	0.42 ± 0.62 nmol/l	0.42 ± 0.62	Lam *et al.,* 2010
	Human saliva	7.29 ± 6.79 nmol/l	7.29 ± 6.79	Lam *et al.,* 2010
	Human peritoneal fluid	24.28 ± 18.64 nmol/l	24.28 ± 18.64	Lam *et al.,* 2010
	Human breast milk	8.98 ± 3.35 nmol/l	8.98 ± 3.35	Lam *et al.,* 2010
	Human breast milk (110±32.3 lactat.days)	23.4±7.2 nM	23.4±7.2	Schuel *et al.,* 2002
	Human seminal plasma	31.5±7.3 nM	31.5±7.3	Schuel *et al.,* 2002
	Human mid-cycle oviductal fluid	30.4±6.9 nM	30.4±6.9	Schuel *et al.,* 2002
	Human follicular fluid	11.3±1.3 nM	11.3±1.3	Schuel *et al.,* 2002
	Human amniotic fluid	8.8±2.9 nM	8.8±2.9	Schuel *et al.,* 2002
	Human amniotic fluid	9.27 ± 6.54 nmol/l	9.27 ± 6.54	Lam *et al.,* 2010
	Rat amniotic fluid (d14 gestation)	30.72 ± 8.03 nM	30.72 ± 8.03	Fonseca *et al.,* 2010
	Rat amniotic fluid (d16 gestation)	25.04 ± 8.00 nM	25.04 ± 8.00	Fonseca *et al.,* 2010
	Human knee synovial fluid	~ 1500 pmol/ml	~ 1500	Richardson *et al.,* 2008
	Cerebrospinal Fluid (CSF) healthy human subjects	2.12 ± 0.72 pmol/ml	2.12 ± 0.72	Jumpertz *et al.,* 2011
	Cerebrospinal Fluid (CSF) healthy human subjects	4.91±0.47 pmol/ml	4.91±0.47	Sarchielli *et al.,* 2007
	Cerebrospinal Fluid (CSF) of chronic migraine human patients	6.21±0.59 pmol/ml	6.21±0.59	Sarchielli *et al.,* 2007 [169]
	Cerebrospinal Fluid (CSF) of probable CM and probable analgesic-overuse headache (PCM+ PAOH) human patients	6.23±0.52 pmol/ml	6.23±0.52	Sarchielli *et al.,* 2007 [169]

Moreover, the formation of PEA has repeatedly been suggested to play a pro-homeostatic role, being part of a protective response to cellular injury [73, 86]. One of the first evidence in favor of the hypothesis came from the 40 fold increase and over in the NAE level in the infracted areas of canine myocardium 24 hrs after coronary artery ligation [111]. The mainly represented NAEs were PEA and 18:0, being respectively 37% and 39% of the total detected NAEs [111]. Preliminary data have also shown an increase of PEA levels in response to neurodegeneration and denervation-induced gliosis in organotypic hippocampal slice cultures subjected to perforant pathway transaction [112]. Interestingly, a microdialysis *in vivo* study, performed in a human patient with left-sided hemispheric infarction, has also revealed a

Table 2. Cellular producers of PEA.

Cell Type	Amount	Reference
Primary human astrocyte culture (under basal conditions)	6.11 ± 3.39 pmol/mg of protein	Walter *et al.,* 2002
Primary rat astrocyte culture (under basal conditions)	5.28 ± 2.89 pmol/ mg of protein	Walter *et al.,* 2002
Primary mouse astrocyte culture (under basal conditions)	2.57 ± 0.58 pmol/dish (3 x 10^5 cells/dish); 2.17± 0.49 pmol/mg of protein	Walter *et al.,* 2002
C6 rat glioma cell culture (under basal conditions)	2.65 ± 0.50 mg of protein	Walter *et al.,* 2002
Ionomycin-stimulated mast cells (RBL-2H3 cells)	10.9 ± 3.0 pmol/10^7 cells	Bisogno *et al.,* 1997
Ionomycin-stimulated monocytes/ macrophages (J774 cells)	12.9 ± 1.3 pmol/10^7 cells	Bisogno *et al.,* 1997
Mouse microglial cell line BV-2 (3 x10^6 cells/100 mm dish)	1.83 pmol / 10^6 cells	Muccioli and Stella, 2008
Cultured mouse epidermal JB6 p+ cells	18.32 ± 0.91 pmol/μ of lipid phosphorus	Berdyshev *et al.,* 2000
Primary cultures of mouse neocortical neurons	~ 3.5 pmol/mg protein	Stella and Piomelli, 2001
Human white subcutaneous adipocytes in primary culture	0.531 ± 0.048 pmol/mg lipid extract	Gonthier *et al.,* 2007
Human fat cells + culture medium	89.16 ± 8.08 pmol/I	Gonthier *et al.,* 2007
Culture medium of human fat cell culture	3.62 ± 0.54 pmol/I	Gonthier *et al.,* 2007
Human white subcutaneous adipocytes in primary culture	~ 1.7-2.7 pmol/mg of lipids	Hoareau *et al.,*, 2009
Mouse adipocytes (cell line 3T3F442A)	~ 90 pmol/mg lipid extract	Matias *et al.,* 2007
rat beta-pancreatic cells (RIN m5F insulonoma cell line)	~ 100 pmol/mg lipid extract	Matias *et al.,* 2007

massive release of PEA in the penumbral tissue surrounding the primary ischemic lesion, the increase being significantly associated with elevations in extracellular lactate, an early marker of the hypoxic insult [113]. Furthermore, a dramatic increase of PEA levels were found both in the epicentre and in the rostral segment of the spinal cord, one day after spinal cord injury in rats (i.e., the contusion/compression model) [114]. The level of PEA in the cerebral cortex was also dramatically increased in a model of focal cerebral ischemia (i.e. left carotid artery occlusion for 20 minutes), whether levels of AEA and 2-AG had only minor increase or remain unchanged, respectively [115]. All these findings greatly substantiate the hypothesis that PEA formation may serve a cytoprotective role in relation to neuronal injury [83].

Not only during nervous system disease states, but also during inflammatory/degenerative conditions involving different organs and tissues PEA levels are varied, i.e., they are usually increased during acute conditions [87, 110] and decreased in chronic inflammation models [116, 117]. Examples of diseases were changes in PEA levels have been demonstrated are osteoarthritis [101], atopic dermatitis [118], gut inflammation [110, 116, 119], eye degenerative diseases [103, 104], muscle pain (myalgia) [120]. The main findings are reported in Table **3**. It has been repeatedly suggested that, in view of the general anti-inflammatory and neuroprotective properties of PEA, the changes in its levels may contribute to cell damage (i.e., decreased levels) or alternatively to cell protection (i.e., increased levels) [103, 104, 121]. Accordingly, enhancement of colon endocannabinoid levels, through the blockade of enzymatic degradation, protects against preneoplastic lesions in the mouse colon [121]. It is also noteworthy that increased local levels of PEA might exert cytoprotective activity by facilitating the induction of apoptosis in injured PEA-producing cells or neighboring cells, thus inhibiting spreading of a necrotic process [73]. Supporting evidence also comes from *in vitro* experiments performed on cultured mouse epidermal JB6 P+ cells, where marked increased levels of PEA were reported 14 hours after UVB light irradiation [122].

Last but not least, the release of PEA and congeners has been suggested to play a role in plant defense signaling, representing an intriguing parallel to 'endocannabinoid signaling' in mammals [89].

ANTI-INFLAMMATORY EFFECTS OF PEA

The anti-inflammatory effects of PEA have been repeatedly demonstrated both *in vitro* and *in vivo*. At a cellular level, PEA was originally found to down-modulate antigen-evoked serotonin release from mast cells, with a reported EC_{50} of 0.27±0.19μM [11]. The finding confirmed the mast cell modulation mechanism (ALIA, Autacoid Local Inflammation Antagonism) previously hypothesized for PEA by Aloe and coll [49] and opened the way to the modern anti-inflammatory research on PEA, by virtue of the well-recognized involvement of mast cells both in neurogenic and immunogenic inflammation at peripheral tissues [52, 72, 123-125] and CNS level [126-128]. Actually, the ability of PEA to down-modulate MC degranulation has been confirmed by densitometric analysis on toluidine blue-stained sections from skin of allergic cats, before and after oral treatment with PEA [34]. Comparison of data obtained

at baseline and after 30 day-treatment revealed a significant increase in granular density in cutaneous MCs, thus suggesting that PEA is effectively able to decrease MC degranulation [34]. The downmodulating effect of PEA on MC degranulation is further sustained by a recent study showing that PEA inhibits the immunologically induced histamine release from freshly isolated MCs, the maximum inhibitory effect (about 54% inhibition) being observed at $3x10^{-6}$M PEA concentration [24]. Moreover, 10^{-5} M PEA significantly inhibited PGD2 and TNF-α release with about 25% and 29% of inhibition respectively [24]. Human PBM cells were also found to be a cellular target for PEA, since the compound was shown to downregulate LPS-induced synthesis of TNF- α, IL-6 and IL-8 at nanomolar concentrations [129]. Moreover, macrophages and adipocytes stimulated with LPS were both negatively-controlled by PEA, in that a significant inhibition of NO production and TNF- α secretion was respectively found [25, 130]. Finally, keratinocytes have been shown to represent a further cellular target for the anti-inflammatory effect of PEA, since the protein levels of the pro-inflammatory chemochine MCP-2 were strongly and significantly reduced by PEA (10μM) in immunologically-challenged HaCaT cells [1].

Table 3. Changes in PEA Levels in Response to Clinical and Experimental Disease.

Disease; Tissue or Body Fluid	Change	Main Finding	Reference
Mice with chronic relapsing experimental allergic encephalomyelitis (CREAE); spinal cord	↑	~200% increase	(Baker *et al.*, 2001)
Transgenic model of Huntington's disease (R6/2 mice); striatum	↓	Significantly decreased compared to wild type animals.	(Bisogno *et al.*, 2008)
Rat acute stroke; striatal and cortical infarcted hemisphere	↑	~ 25-fold increase compared to controlateral (non-infarcted) areas	(Berger et al, 2004)
Mice with focal cerebral ischemia (FCI) (left carotid artery occlusion for 20 min); ischemic cerebral cortex	↑	25-fold increase compared with sham-operated animals, at 24 hrs post FCI	(Franklin *et al.*, 2003)
Human cerebral ischemia: penumbral tissue surrounding the primary ischemic lesion in a human patient with left-sided hemispheric infarction (microdialysis study)	↑	Significantly increased levels within the first day after ischemia	(Shabitz *et al.*, 2002)
Human patients with chronic migraine or probable chronic migraine and probable analgesic-overuse headache; CSF	↑	Significantly higher levels in the two patient groups (without significant difference between them) compared to control subjects	(Sarchielli *et al.*, 2007)
Obese human patients; subcutaneous fat	↓	Significantly lower levels as compared to normoweight controls	(Matias *et al.*, 2007)
Streptozotocin- treated mice (with hyperalgesia on a hot plate); paw skin	↑	Significant enhancement of levels compared to untreated animals	(Darmani *et al.*, 2005)
Diabetic human patients; blood	↑	Significantly higher levels compared to blood of healthy controls.	(Matias *et al.*, 2007)
Cadmium chloride i.p.-injected rats (acute inflammation); testes	↑	Marked increase (39-fold) at 9 hrs post-injection	(Kondo *et al.*, 1998)
Dogs with spontaneous atopic dermatitis; skin	↑	~ 30-fold increase in AD lesional skin compared to control skin	(Petrosino *et al.*, 2008)
Rats with chronic inflammation (implant of λ-carrageenin-soaked sponges); granulomatous tissues	↓	Significantly decreased levels compared to saline-soaked sponges	(De Filippis *et al.*, 2010)
Croton oil-treated mice (gut chronic inflammation); small intestine	↓	Significantly decreased levels compared to control mice	(Capasso *et al.*, 2001)
Rats with metotrexate-induced transient intestinal mucositis (histologically resembling the gut appearance commonly evidenced in celiac patients); muscle/serosa and mucosa of the jejunum	↑	Significantly elevated levels in both muscle/serosa and mucosa at day 3, corresponding to the maximal histologic damage	(D'Argenio *et al.*, 2007)
Celiac disease patients; biopsy specimens from the distal duodenum	↑	Significantly elevated levels in patients with untreated celiac disease at the highest degree of atrophy as compared to those in non-celiac	(D'Argenio *et al.*, 2007)

(Table 3) contd….

Disease; Tissue or Body Fluid	Change	Main Finding	Reference
Human patients with ulcerative colitis; colonic mucosa biopsy specimens	↑	Significantly higher levels compared to control patients	(Darmani *et al.*, 2005)
Chronic low back pain patients, following osteopathic manipulation (OTM); blood	↑	Significantly increased levels, both vs baseline pre-OMT and vs no low back pain control group following OMT	(Darmani *et al.*, 2005)
Human patients with end-stageOA and RA; knee synovial fluid	↓	Markedly decreased levels compared to normal volunteers	(Richardson *et al.*, 2008)
Human myalgic trapezius muscle; dialysate samples	↑	Significantly higher compared to healthy controls	(Ghafouri *et al.*, 2010)
Patients with diabetic retinopathy; ciliary body	↑	1.3-fold increase compared to control values	(Nichols *et al.*, 2007)
Patients with glaucoma; ciliary body	↓	40% decrease compared to normal patients	(Chen *et al.*, 2005)
Patients with glaucoma; choroid	↓	54% decrease compared to normal patients	(Chen *et al.*, 2005)

Several preclinical studies have largely demonstrated the *in vivo* anti-inflammatory effects of PEA in the more different disease models and administration routes. Inhalated PEA, for example, has proven to inhibit pulmonary inflammation in LPS treated mice, significantly reducing TNF-α levels in the bronchoalveolar lavage fluid [131]. The effective dose of 750 nmol/kg was surprisingly low, corresponding to 0.225 mg/kg [131].

Furthermore PEA has been shown to reduce neurogenic inflammation in guinea pig airways [132]. The intravenous administration of PEA (0.32-3.2 mg/kg) dose-dependently reduced capsaicin-induced bronchoconstriction *in vivo*, without altering baseline airway resistance. Moreover, PEA (0.0033-3.3 μM) dose-dependently inhibited electrical field stimulation-induced bronchial smooth muscle contraction and significantly inhibited the capsaicin-induced release of substance P from airways tissues at 0.05 and 0.5 μM PEA concentration [132]. Substance P – induced neurogenic inflammation in the ear pinna of rats was reported to be significantly reduced by subcutaneous and oral treatment with PEA, at 0.1-20 mg/kg dosage range [49, 133].

Carrageenan-induced rat paw oedema, a model of acute inflammation, was significantly and dose-dependently inhibited by PEA at doses of 1-10 mg/kg p.o. [18, 27, 133], 10 mg/kg i.p. [21], and 0.01-1μg i.c.v. [134]. It is noteworthy that PEA behaved differently from other NAEs (i.e., anandamide and oleamide) in this model, being the only NAE that reliably and efficaciously reduced the magnitude of paw edema when administered before the carrageenan. An additional finding from the study of Wise and coll. [29] concerns the similarity among the extent of edema reduction achieved by PEA (12.5 mg/kg), the non-selective cyclooxygenase (COX) inhibitor diclofenac (5mg/kg) and the synthetic glucocorticoid dexametasone (10 mg/kg). Interestingly, PEA has proven to be active even if administrated after inflammation was established, thus showing a curative efficacy in this model [27].

Beyond carrageenan-induced edema, several different models of acute inflammation were demonstrated to benefit from PEA treatment. Formalin-induced rat paw edema was significantly reduced by PEA (10 mg/Kg p.o.) and lower doses (0.3; 1; 3 mg/Kg p.o.) were sufficient to dose-dependently reduce the dextran-induced edema in the rat [133]. Moreover, the topical application of PEA (15 and 150 nmol/cm^2) resulted in a significant decrease of TPA-induced ear edema [21]. Likewise, allergic skin reactions in mice and dogs were negatively controlled by PEA, that was able to reduce skin inflammation in DNFB-induced contact dermatitis in mice [1] and to decrease the area of immunologically-induced skin wheals in hypersensitive Beagle dogs [28]. Finally, a very strong anti-inflammatory effect was observed in LPS-injected mice treated with PEA (3, 30 and 200 mg/kg i.p.), as measured by the reduction of circulating TNF-α [25].

Interestingly, PEA was found to inhibit chronic inflammation as well the anti-inflammatory effect being demonstrated both in carrageenan-induce granuloma in rodents [117, 135] and a viral model of multiple scelorosis, i.e., the TMEV-IDD model [136]. In the former, the effect was shown to be mediated by the down-modulation of MC degranulation [137].

The main pre-clinical anti-inflammatory effects of PEA are reported in Table **4**.

EFFECTS OF PEA ON PAIN AND TRAUMA

In addition to its known anti-inflammatory activity, PEA elicited analgesia in acute and inflammatory pain [18, 138], inhibition of food intake [139], and neuroprotection [12, 115, 140]. N-acylethanolamides, like PEA, and N-acylphosphatidylethanolamides accumulate in conditions involving degenerative changes to tissues [86], including brain [141] and cardiac [80] ischemia. PEA, but not the unsaturated N-acylamide anandamide, down-modulated the toxic consequences of excitatory amino acids receptor (EAA) activation in cultured cerebellar granule cells, without

affecting EAA receptor function. PEA achieved maximal efficacy when added 15 min postglutamate [26].

Pain hypersensitivity that follows sciatic nerve constriction (CCI) in rats is associated with a significant decrease in the level of endogenous PEA in spinal cord and in brain areas directly or indirectly involved in nociception [142], so suggesting that this lipid might be involved in pain response. PEA, in fact, exerted inhibitory effects on mechanonociceptive hyperalgesia and sensory neuropeptide release *in vivo* suggesting its potential therapeutical use to treat chronic neuropathic pain. In fact PEA inhibited release of calcitonin gene-related peptide and somatostatin *in vivo*, two sensory neuropeptides playing important role in the development of neuropathic hyperalgesia. PEA significantly decreased neuropathic mechanical hyperalgesia 7 days after unilateral sciatic nerve ligation, and this effect was antagonized by the CB_2 receptor antagonist SR144528.

The efficacy of PEA in attenuating neuropathic pain is consistent with another report by Costa *et al.* [143] showing that the treatment of CCI mice with a single dose of PEA (10 mg/kg, i.p.) resulted in a significant relief of both thermal hyperalgesia and mechanical allodynia in mice. The anti-allodynic effect was maximum 90 min after the acute administration of PEA and disappeared at 2 h. Thus, PEA evoked a significant but short-lasting relief of pain in CCI mice after single systemic administration [143]. However, repeated treatment with PEA (10 mg/kg i.p., once daily) led to a more potent and long-lasting relief of neuropathic pain. The administration of PEA could lead to a further increase in AEA in the spinal and supraspinal areas of CCI animals. The cellular/receptor mechanism responsible for the actions of PEA is still under investigation and excited debate. It is now discussed whether PEA can interact with the so-called CB_2-like receptor or whether it can activate CB_2 receptor indirectly, augmenting the level of AEA that binds to CB_2 receptors causing anti-inflammation and analgesia (entourage hypothesis) [13, 144] neither CB_2 nor PPARα antagonists affected the PEA-elicited anti-hyperalgesia, suggesting that such receptors are not involved in its antinociceptive effect. On the contrary, CB_1 involvement in PEA-induced antinociception was expected.

Table 4. The Main Pre-Clinical Anti-Inflammatory Effects of PEA.

	Model	**Dose**	**Main Effect**	**Ref.**
Inflammation (*in vitro*)	LPS-stimulated hPBM cells	30-300 nM	Inhibition of IL-4, IL-6 and, IL- 8 production; decreased release of p75 TNF-α soluble receptor	[Berdyshev *et al.,*]
	Antigen-stimulated RBL-2H3 cells and RPMCs	EC_{50} of 0.3μM	Profound reduction of antigen-evoked serotonin release	[Facci *et al.,*]
	LPS-stimulated macrophage cell line RAW264.7	10μM	Significant inhibition of NO production	[Ross *et al.,*]
	LPS-stimulated adipocytes	100 μmol/l	Significant inhibition of TNF-α secretion	[Hoareau *et al.,*]
	Immunologically-challenged canine skin mast cells	$10^{-5} – 10^{-6}$ M	Inhibition of histamine, PGD2, and TNF-α release	[Cerrato *et al.,*]
	Cultured keratinocytes challenged with poly-(I:C)	0.1, 1, 10 μM	Strong reduction of the chemokyne MCP-2	[Petrosino *et al.,*]
Acute inflammation (*in vivo*)	Neurogenic inflammation (SP in the ear pinna of rats)	0.1, 1, 5, 20 mg/kg s.c.	Significant inhibition of skin mast cell degranulation	[Aloe *et al.,*]
	Neurogenic inflammation (SP in the ear pinna of rats)	0.1, 1, 10 mg/kg p.o.	Significant and dose-dependent inhibition of skin mast cell degranulation and plasma extravasation	[Mazzari *et al.,*]
	Carrageenan-induced rat paw oedema	3 - 10 mg/kg p.o.	Reduction of carrageenan-induced oedema n a time- and dose- dependent manner	[Mazzari *et al.,*]
	Passive cutaneous anaphylaxis in mice	1 mg/kg p.o.	Significant inhibition of PCA-induced extravasation	[Mazzari *et al.,*]
	Formalin-induced rat hind paw oedema	10 mg/kg p.o.	Significant reduction of paw oedema	[Mazzari *et al.,*]
	Dextran-induced oedema in the rat	0.3, 1, 3 mg/kg p.o.	Significant and dose-dependent reduction of hind paw oedema formation	[Mazzari *et al.,*]
	Carrageenan-induced rat paw oedema	10 mg/kg p.o.	Inhibition of paw oedema	[Conti *et al.,*]
	Carrageenan-induced rat paw oedema	1, 3, 5, 10 mg/kg p.o.	Inhibition of paw oedema when PEA is given after inflammation is established (curative efficacy), and inhibition of NO production	[Costa *et al.,*]

(Table 4) contd….

	Model	Dose	Main Effect	Ref.
	TPA-induced ear oedema in mice	Topical, 15 and 150 nmol/cm^2	Significant reduction of oedema	[Lo Verme *et al.,*]
	Carrageenan-induced paw oedema in mice	10 mg/kg i.p.	Significant decrease of oedema	[Lo Verme *et al.,*]
	Capsaicin-induced neurogenic inflammation in guinea pig airways	0.32-3.2 mg/kg i.v.	Significant and dose-dependent reduction of bronchoconstriction	Yoshihara *et al.,* 2005
	Carrageenan-induced paw oedema in mice	0.01-1μg i.c.v.	Significant decrease of oedema, and of COX-2 and iNOS expression	[D'Agostino *et al.,*]
	Carrageenan-induced paw oedema in mice	12, 25, 50 mg/kg i.p.	Significant decrease of oedema	Wise *et al.,* 2008
	LPS-injected mice	3, 30, 200 mg/kg i.p.	Very strong anti-inflammatory effect (reduced levels of circulating TNF-α)	[Hoareau *et al.,*]
	Allergic dermatitis in Beagle dogs	3, 10, 30 mg/kg p.o.	Significant reduction of the wheal area induced by both antigen and anti-canine IgE challenge	[Cerrato *et al.,*]
	DNFB-induced contact dermatitis in mice	5-10 mg/kg i.p.	Significant reduction of inflammation (ear thickness)	[Petrosino *et al.,*]
Chronic Inflammation (*in vivo*)	TMEV-IDD model of multiple sclerosis in mice	5 mg/kg i.p.	Improvement in the motor function	[Loria *et al.,* Eur J]
	s.c. implant of -carrageenin-instilled sponge in mice	50 μg/sponge	Significant reduction of leukocyte infiltration	[Solorzano *et al.,*]
	s.c. implant of -carrageenin-instilled sponge in rats	200, 400, 800 μg/ml	Significant and concentration-dependent decrease in granuloma formation and local angiogenesis	[De Filippis *et al.,*]

However, using the carrageenan-induced paw model of hyperalgesia in mice, it has been report that intracerebroventricular administration of PEA (0.1-1 microg) 30 min before carrageenan injection markedly reduced mechanical hyperalgesia up to 24 h following inflammatory insult. PEA significantly reduced the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in sciatic nerves and restored carrageenan-induced reductions of PPAR-α in the L4-L6 dorsal root ganglia (DRG). The role of PPAR- α in mediating PEA's actions was confirmed by the lack of anti-hyperalgesic effects in mutant mice lacking PPAR- α. In addition, PEA prevented IkB- α degradation and p65 NF-kappaB nuclear translocation, confirming the involvement of this transcriptional factor in the control of peripheral hyperalgesia [31].

New data about carpal tunnel syndrome in which the median nerve is compressed support the hypothesis of protection against inflammatory and neuropathic pain by PEA (Table **5**).

Endocannabinoid system is activated in a clinically relevant model of traumatic spinal cord injury (SCI) in rats [114]. In particular, an acute overproduction of PEA was observed in lesioned animals 1 day after the contusion both in the epicentre and in the adjacent rostral region.

PEA modulated tissue injury events associated with spinal cord trauma in mice in secondary damage induced by experimental SCI in mice. Repeated PEA administration (10 mg/kg i.p.; 30 min before and 1 and 6 h after SCI) significantly reduced the degree of spinal cord inflammation and tissue injury, neutrophil infiltration, nitrotyrosine formation, proinflammatory cytokine expression, nuclear transcription factor activation-kappaB activation, and apoptosis. Moreover, PEA treatment significantly ameliorated the recovery of motor function [145]. In a very recent paper by Esposito et al [146] significant less mast cells density and degranulation were observed after experimental SCI in the spinal cord tissues collected from mice which have been treated with PEA. Moreover, a local and sustained increase in neurotrophins' expression (NGF, NT-3 and GDNF) in the perilesioned tissue following intraperitoneally administration of PEA was evidenced. The effect of PEA against experimental compression model of spinal cord may be mediated by the inhibition of neutrophil accumulation, as well as by the ability of PEA of negatively modulating the secretion of mediators from MCs, the activation of microglia and astrocytes all expressing CB2 receptors, independently from CB receptor activation, via a so called 'entourage effect'.

PEA ability to mitigate beta-amyloid (Aβ)-induced astrogliosis was investigated in a recent study [147]. PEA (10^{-7}M) blunted the expression of pro-inflammatory molecules in rat primary astrocytes activated by soluble Aβ (1-42) (1μg/ml). PEA This effect was reduced by PPAR-α antagonist. These results indicate that PEA exhibits anti-inflammatory properties able to counteract Aβ-induced astrogliosis, and suggest novel treatment for neuroinflammatory/ neurodegenerative processes.

Table 5. The Main Pre-Clinical Effects of PEA on Inflammatory and Neuropathic Pain.

Inflammatory pain	somatic	Carrageenan-induced hyperalgesia in rats	10 mg/kg p.o.	Significant reduction of mechanical hyperalgesia	[Mazzari *et al.,*]
		Formalin-evoked nociception in mice	5, 10 mg/kg i.a.	Significant reduction of the second phase behavioural response	[Jaggar *et al.,*]
		Carrageenan-induced hyperalgesia in rats	10 mg/kg, p.o.	Abolishment of hyperalgesic response	[Conti *et al.,*]
		Formalin-evoked nociception in mice	5, 50 µg/animal (i.pl. injection)	Marked inhibition of pain behaviour	[Calignano *et al.,*]
		i.pl. NGF - induced hyperalgesia in rats	10, 25 mg/kg i.p.	Significant reduction of hyperalgesia and neutrophil accumulation	[Farquhar-Smith,]
		tail flick test in mice	5, 10, 50, 100 mg/kg p.o.	Remarkable decrease in antinociception behaviours	[Karimi *et al.,*]
		Formalin-evoked nociception in mice	5, 10, 50, 100 mg/kg p.o.	Significant antinociceptive activity in both early and late phase	[Karimi *et al.,*]
		i.pl. carrageenan-induced hyperalgesia in mice	0.01, 0.1, and 1 µg i.c.v.	Marked reduction of mechanical hyperalgesia in a time-dependent manner	[D'Agostino *et al.,*]
		Hyperalgesia evoked by s.c. implant of -carrageenin-instilled sponge in the rat	800 µg/ml	Significant reduction of new nerve formation and strongly reduction of granuloma-associated hyperalgesia	[De Filippis *et al.,*]
	visceral	Turpentine inflammation of the rat urinary bladder	10, 20, 30 mg/kg i.a.	Significant attenuation of the vesical hyper-reflexic response	[Jaggar *et al.,*]
		Acetic acid-evoked writhing in mice	1, 20 mg/kg i.p.	Dose-dependent attenuation of the writhing response	[Calignano *et al.,*]
		Turpentine inflammation of the rat urinary bladder	10, 25 mg/kg i.p.	Attenuation of referred hyperalgesia in a dose-dependent fashion	[Farquhar-Smith,]
		Kaolin-evoked writhing in mice	0.1 - 10 mg/kg i.p.	Potent inhibition of the nociceptive response	[Calignano *et al.,*]
		Magnesium sulphate-evoked writhing in mice	1-10 mg/kg i.p.	Dose-dependent inhibition of the nocifensive response	[Calignano *et al.,*]
		NGF-induced inflammation of the rat urinary bladder	2.5 mg/kg i.a.	Significant increase of micturition threshold and significant reduction of spinal cord Fos production	[Farquhar-Smith *et al.,*]
		Acetic acid-evoked writhing in mice	50, 100 mg/kg p.o.	Significant reduction of the number of writhes	[Karimi *et al.,*]
Neuropathic pain		Spinal cord injury in mice	10 mg/kg i.p. before or after surgery	Significant reduction of the severity of spinal cord trauma and of the spinal cord levels of TNF-α, IL-1, iNOS	[Genovese *et al.,*]
		Chronic constriction injury of sciatic nerve in mice	10 mg/kg i.p. once daily for one week	Significant relief of thermal hyperalgesia and mechanical allodynia	[Costa *et al.,*]

Moreover, in a model of transient middle cerebral artery occlusion PEA significantly reduced infarct volumes achieving a maximum protection of 35%. Treatment with PEA in doses of 30 mg/kg body weight showed a significant reduction of infarct size in cortical and total infarct areas [33].

In mouse cerebral cortex, focal cerebral ischemia greatly increases PEA [115]. Franklin and collaborators showed that PEA potentiates AEA-induced microglial cell migration, without affecting other steps of microglial activation, such as proliferation, particle engulfment, and nitric oxide production. This potentiation of microglial cell migration by PEA involves reduction in cAMP levels.

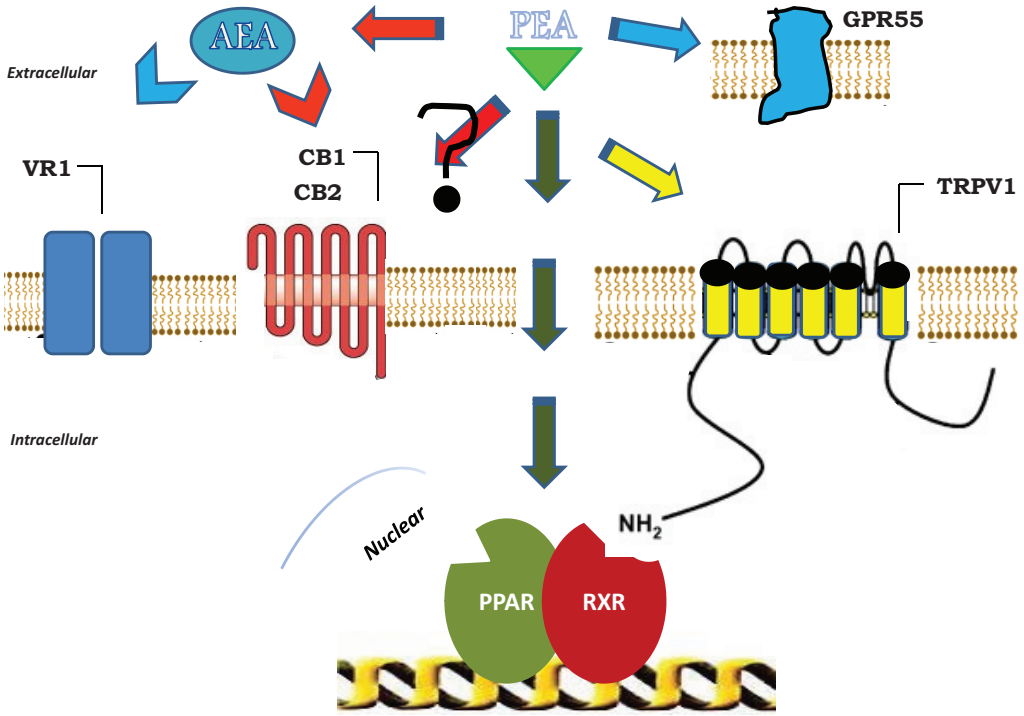

Fig. (1). Involvement of different receptors in the central and peripheral effects of PEA.

Interestingly, PEA regulated neurosteroidogenesis in astrocytes acting as a ligand of PPAR-α [148]. In particular, allopregnanolone levels were increased in PEA-treated astrocytes. Recent paper evidenced that PEA, activating PPAR-alpha receptor and involving neurosteroids de novo synthesis, modulates pentobarbital-evoked hypnotic effect [32].

These results add further support to the broad-spectrum of biological and pharmacological effects induced by PEA, but a definitive and clear action mechanism and involvement of specific receptors are needed to explain the discrepancy between binding and some pharmacological studies (Fig. **1**).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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