Palmitoylethanolamide in Homeostatic and Traumatic Central Nervous System Injuries

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Abstract: The role of palmitoylethanolamide (PEA) in the regulation of complex systems involved in the inflammatory response, pruritus, neurogenic and neuropathic pain is well understood. Growing evidence indicates that this N-acylethanolamine also exerts neuroprotective effects within the central nervous system (CNS), i.e. in spinal cord and traumatic brain injuries and in age-related pathological processes. PEA is abundant in the CNS, and is produced by glial cells. Several studies show that administering PEA during the first few hours after injury significantly limits CNS damage, reduces loss of neuronal tissue and improves functional recovery. PEA appears to exert its protective effect by decreasing the development of cerebral edema, down-regulating the inflammatory cascade, and limiting cellular necrosis and apoptosis. All these are plausible mechanisms of neuroprotection. This review provides an overview of current knowledge of PEA effect on glial functions in the brain and how targeting glial-specific pathways might ultimately impact the development of therapies for clinical management of neurodegenerative disorders. The diverse signaling mechanisms are also summarized.

Keywords: Glial cells, N-acylethanolamine, neuroprotection, spinal cord.

INTRODUCTION

Palmitoylethanolamide (PEA) is a saturated fatty acid derivative where the carboxylate function is amidated by the primary amine of ethanolamine [1]. PEA is a naturally occurring N-acylethanolamine (NAE) endorsed with pleiotropic effects, collectively considered to play protective and homeodynamic roles in the animal [2,3] and vegetable [4] kingdoms. PEA is produced "on demand", it is not stored in vescicles. The phosphatidylethanolamine, a cell membrane phospholipid characteristic of nervous tissue, forms N-acylphosphatidylethanolamines (NAPEs) when cells are subjected to potentially harmful stimuli. After being cleaved by phospholipases, NAPEs can be transformed into NAE. Like other NAEs, PEA acts locally, and its tissue levels are tightly regulated through a balance between anabolic and catabolic pathways, respectively catalyzed by the biosynthetic enzyme NAPE-selective phospholipase D and the degradative N-acylethanolamine-hydrolyzing acid amidase [2,5]. Since N-acylethanolamine-hydrolyzing acid amidase 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 take up PEA by a "carrier-mediated" transport process [6,7]. In fact, PEA, beside penetrating the cells by passive transfer, due to its high lipophilicity, is taken up by cells through a facilitated transport system that is apparently similar in neuronal and immune cells (i.e., mast cells) and pharmacologically distinct from that for the PEA analogues [7].

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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 [8,9] was due to a specific lipid fraction corresponding to PEA [10,11]. Its anti-inflammatory and protective activities were confirmed in several models of inflammation, i.e. carrageenan-induced paw oedema, adjuvant-induced arthritis and tuberculin hypersensitivity [12, 13]. In particular, it was shown that pre-treatment with PEA (20mg/kg, subcutaneously) significantly reduced mast cell degranulation induced by the local administration of substance P in the rat ear pinna [14]. It was 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 'Autacoid Local Inflammation Antagonism' was coined for this novel mast cell modulation mechanism [14]. This result is very important because mast cells play a crucial role not only in substance P-induced neurogenic inflammation [15], but in a much wider spectrum of physiological and pathological responses, e.g., allergy, infection, angiogenesis and pain [16-22]. In fact, mast cells and their mediators are 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 [23-28].

PEA was originally considered to be an endocannabinoid (eCB), since at first it was suggested to be a cannabinoid receptor 2 (CB2) agonist [29]. Although these results were not confirmed [7,30-33], some of the pharmacologic effects of PEA were antagonized by a selective CB2 receptor blocker SR144528 [34-36]. Actually, PEA is more correctly referred to as a cannabinoid receptor-inactive eCB-related molecule [37]. Currently, several mechanisms have been proposed to explain the anti-inflammatory and anti-

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hyperalgesic effects of PEA, including: (i) the activation of a cell surface receptor (i.e. CB2-like or, alternatively, the orphan GPR55 receptor) or otherwise a nuclear receptor of the peroxisome proliferator-activated receptor (PPAR) family [38-41]; (ii) the down-modulation of mast cell hyper-activity (Autocoid Local Inflammation Antagonism mechanism) [42]; (iii) an action as ''entourage'' compound, i.e. the augmentation of eCB activities at their receptors and/or the inhibition of eCB degradation [3].

PEA exists in all cells, tissues and body fluids, with a widespread occurrence both in plants and animals. The levels of endogenous PEA in normal muscle and adipose tissues (0.04 - 6.00 pmol/mg tissue) [43] are similar to those observed in the central nervous system (CNS) [44] and somewhat higher compared to the gastrointestinal tract (0.05-1.50 pmol/mg lipids) [45-47], skin (0.3- 0.69 pmol/mg of lipid extract) [48,49] and eye (0.10 - 0.64 pmol/mg) [50,51], where iris seems to contain the highest levels of PEA [50]. In blood, the physiological levels of PEA range from 4 to 45 pmol/lipid mg [52-56].

Examples of diseases where changes in PEA levels have been demonstrated are osteoarthritis [57], atopic dermatitis [58], gut inflammation [45,46], eye degenerative diseases [50,51] and myalgia [59]. 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 the spread of a necrotic process. The release of PEA and congeners has also been suggested to play a role in plant defense signaling, representing an intriguing parallel to 'endocannabinoid signalling' in mammals [60].

PEA has been extensively researched in several different cell types *in vitro*, without exhibiting any toxic effects [61-63]. In the several preclinical studies the biopharmacologic effects of PEA were not associated with any changes in behaviour in rodents [64] indicating that all the tested doses were well-tolerated. Interestingly, the anti-inflammatory effect of PEA is not accompanied by tolerance following repeated administration of high doses, which is very important for the potential therapeutic utility of the compound [65]. Actually, the lack of any side effect has been repeatedly reported in published clinical studies, both in animals and humans [66-68]. No serious adverse events were reported, and the tolerance was very good.

The role of PEA in the regulation of the inflammatory response, pruritus, neurogenic and neuropathic pain is well known, but growing evidence indicates that this lipid also exerts CNS neuroprotective effects, i.e. in spinal cord and traumatic brain injuries and in age-related pathological processes.

PEA AND THE CNS

In 1996 Skaper and collaborators showed that PEA protects cultured cerebellar granule cells from glutamate toxicity [63]. 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 (Autocoid Local Injury Antagonism). 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 excitotoxicity, thus reducing tissue inflammation, decreasing hyperalgesia and exerting a neuroprotective function [42].

In the brain, PEA levels have been reported to range from around 0.13 to 6.84 pmol/mg [44,69,70]. Circadian variation occurs in the CNS concentration of PEA, levels being higher in the cerebrospinal fluid, hippocampus and hypothalamus during the dark period (8:00 p.m. - 4 a.m.) compared to the light period [71].

PEA is produced by several different cell types: neurons, microglia, astrocytes [72-74] (Fig. **1**). Moreover, the formation of PEA has repeatedly been suggested to play a pro-homeostatic role, being part of a protective response to cellular injury. 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 [75]. 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 (contusion/compression model) [76].

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), while levels of N-arachidonoylethanolamine (AEA, or anandamide) and 2-arachidonoylglycerol had only minor increase or remain unchanged, respectively [77]. Interestingly, PEA levels behaved the same in rats with acute stroke [78] and in a human patient with left-side hemispheric infarction. All these findings greatly substantiate the hypothesis that PEA formation may serve a cytoprotective role in relation to neuronal injury.

EFFECTS OF PEA ON PAIN AND TRAUMA

In addition to its known anti-inflammatory activity, PEA elicited analgesia in acute and inflammatory pain [36] and neuroprotection [30,79-81]. PEA accumulates in conditions involving degenerative changes to tissues, including brain [82] and cardiac ischemia (data unpublished). Pain hypersensitivity that follows sciatic nerve constriction 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 [83], thus suggesting that this lipid compound might be involved in pain response. The administration of PEA could lead to a further increase in AEA in the spinal and supraspinal areas of sciatic nerve constriction animals. The cellular/receptor mechanism responsible for the actions of PEA is still under investigation. It is now discussed whether PEA can interact with the so-called CB_2-like receptor or whether it can activate CB_2 receptors indirectly, augmenting the level of AEA that binds to CB_2 receptors causing anti-inflammation and analgesia (entourage hypothesis) [31,84]. Neither CB_2 nor PPAR-α antagonists affected 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. However, the involvement of other nuclear receptors and their isoforms has not been investigated.

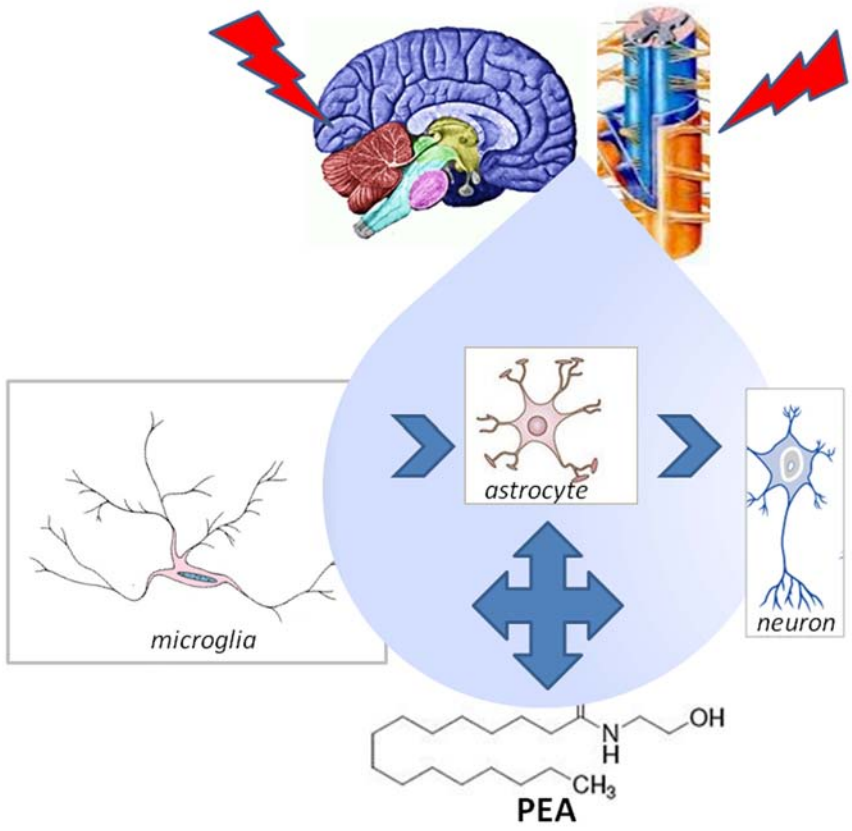

Fig. (1). Subtypes of glial cells involved in the protective effect of palmitoylethanolamide (PEA) on inflammatory reaction associated with experimental models of brain trauma (left) and spinal cord injury (right).

The eCB system is activated in a clinically relevant model of traumatic spinal cord injury (SCI) in rats [76]. 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. In the spinal cord, neurons and glial cells actively interact and contribute to neurofunction. Surprisingly, both cell types have similar receptors, transporters and ion channels and also produce similar neurotransmitters and cytokines. However, in trauma or disease states, spinal glia become activated and dorsal horn neurons become hyperexcitable, contributing to sensitized neuronal-glial circuits. In addition, thoracic SCI produces activation of astrocytes and microglia that contributes to dorsal horn neuronal hyperexcitability and central neuropathic pain in above-level, at-level and below-level segments remote from the lesion in the spinal cord. The cellular and molecular events of glial activation are not simple events; rather, they are the consequence of a combination of several neurochemical and neurophysiological changes following SCI. The ionic imbalances, neuroinflammation and alterations of cell cycle proteins after SCI are predominant components for changes that result in glial activation. More importantly, SCI-induced release of glutamate, pro-inflammatory cytokines, ATP, reactive oxygen species, and neurotrophic factors trigger activation of postsynaptic neuron and glial cells *via* their own receptors and channels that, in turn, contribute to neuron-neuron and neuron-glia interaction as well as microglia-astrocytic interactions. Glial cells play a critical role in brain homeostasis and synaptic plasticity. PEA modulated tissue injury events associated with spinal cord trauma in mice in secondary damage induced by experimental SCI in mice [85]. Repeated PEA administration (10 mg/kg intraperitoneally, 30 minutes before and 1 and 6 hours after SCI) significantly reduced the degree of spinal cord inflammation and tissue injury, neutrophil infiltration, nitrotyrosine formation, pro-inflammatory cytokine expression, nuclear transcription factor activation-kappaB activation, and apoptosis. Moreover, PEA treatment significantly ameliorated the recovery of motor function [85]. In a very recent paper by Esposito *et al.* [86] significantly less mast cell density and degranulation were observed after experimental SCI in the spinal cord tissues collected from mice which had been treated with PEA. Moreover, a local and sustained increase in neurotrophin expression (nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor) in the perilesional tissue following intraperitoneal administration of PEA was seen. The effect of PEA in the experimental compression model of spinal cord may be mediated by the inhibition of neutrophil accumulation, as well as by the ability of PEA to negatively modulate the secretion of mediators from mast cells, the activation of microglia and astrocytes all expressing CB2 receptors, independently from CB receptor activation ('entourage effect').

Specifically, gliotransmission (presence of glial neurotransmitters, and their receptors and active transporters), trophic support (release, maturation and degradation of neurotrophins) and metabolism (production of

lactate and glutathione components) are relevant aspects of astrocyte function in neuronal metabolism, synaptic plasticity and neuroprotection. Morpho-functional changes of astrocytes and microglial cells after traumatic or toxic insults to the CNS (namely, reactive gliosis) disrupt the complex neuro-glial networks underlying homeostasis and connectivity within brain circuits. Moreover, PEA is able to mitigate beta-amyloid (Aβ)-induced astrogliosis as reported recently [87]. PEA (10^{-7}M) blunted the expression of pro-inflammatory molecules in rat primary astrocytes activated by soluble Aβ (1-42) (1μg/ml). This effect was reduced by a PPAR-α antagonist. These results indicate that PEA is able to counteract Aβ-induced astrogliosis, and suggest a novel treatment for neuroinflammatory/neurodegenerative processes.

Moreover, in a model of transient middle cerebral artery occlusion PEA significantly reduced infarct volume, achieving a maximum protection of 35% (paper in press from our lab). Treatment with PEA at a dose of 30 mg/kg body weight significantly reduced infarct size in cortical and total infarct areas.

Microglial activation is one of the first steps in inflammatory processes within the CNS, and it is often followed by an infiltration of neutrophils, T lymphocytes and reactive astrocytosis. In parallel with their negative or neurotoxic effects microglia also play an important role in the maintenance of neuronal well-being. Based on their phagocytic function as the 'professional' phagocytes of the CNS, microglia can enter damaged brain regions and remove toxic products. Microglia seem to be much more integrated into neuronal function than was thought in the past, and recent findings indicate neuron-microglia crosstalk [88]. During activation microglia change from a ramified to a hyper-ramified phenotype and subsequently adopt an amoeboid morphology, a process which helps microglia invade lesions. Activated microglia not only change their phenotype, but also proliferate, migrate to the site of damage and secrete pro- and anti-inflammatory cytokines and chemokines and oxidative stress-inducing factors such as nitric oxide, as well as growth factors [89]. Microglial activation has often been the first – or at least a significant – cellular event detected in and around a lesion in several animal models of developing brain injuries such as those induced by mechanical trauma, infection/inflammation, excitotoxic insults and hypoxia/ischemia [90]. Moreover, microglia and astrocyte interaction seems important: pro-inflammatory cytokines secreted by activated microglia inhibit astrocyte gap junction communication, which influences the role of astrocytes in providing neuronal support. Franklin and collaborators [77] 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 cyclic AMP levels.

Interestingly, PEA regulated neurosteroidogenesis in astrocytes acting as a ligand of PPAR-α [91]. In particular, allopregnanolone levels were increased in PEA-treated astrocytes. The involvement of de novo neurosteroid synthesis in the modulation of pain behavior by PEA was also investigated by Sasso *et al*. [92] in two models of acute and persistent pain, the formalin test and carrageenan-induced paw edema. PEA antinociceptive activity was partially reduced when the animals were treated with aminoglutethimide (which blocks the conversion of cholesterol to pregnenolone) or finasteride (an inhibitor of the enzyme that converts testosterone to dihydrotestosterone), implying that de novo neurosteroid synthesis is involved in the effect of PEA. Accordingly, in the spinal cord, the allopregnanolone levels were increased by PEA treatment both in formalin- and carrageenan-exposed mice. In both pain models, PEA administration specifically restored the expression of two proteins involved in neurosteroidogenensis, the steroidogenic acute regulatory protein and cytochrome P450 side-chain cleavage in the ipsilateral horns of spinal cord, without affecting their expression in the contralateral side. These data provide new information about the involvement of de novo neurosteroid synthesis in the modulation of pain behavior by PEA.

In their effort to clarify the specific function of this lipid in the CNS, Sasso *et al.* [93] showed that PEA, by activating PPAR-α receptors and involving neurosteroid de novo synthesis, positively modulates γ-aminobutyric acid-alpha receptor and pentobarbital-evoked hypnotic effect [93]. Moreover, D'Agostino and collaborators [94] described the neuroprotective activities of PEA in mice injected intracerebroventricularly with Aβ(25-35) peptide. Spatial and non-spatial memory tasks to evaluate learning and memory dysfunctions showed that PEA, administered for 2 weeks once a day (10 mg/kg, subcutaneously) starting 3 hours after Aβ, reduced or prevented behavioral impairments induced by Aβ injection. Acute treatment with PEA was ineffective. According to the neuroprotective profile of PEA observed during behavioral studies, also lipid peroxidation, protein nitrosylation, inducible nitric oxide synthase expression, and caspase-3 activation, were reduced by PEA treatment [94]. These data disclose an unknown therapeutic possibility to treat memory deficits.

CONCLUSION

The attraction about the neuroprotective effect of PEA is that it coherently affects multiple defense mechanisms that can be invoked in CNS damage settings. In fact, several data from our and other laboratories suggest that PEA may be useful in the therapy of conditions associated with CNS injury.

ABBREVIATIONS

Aβ = Beta-amyloid

AEA = N-arachidonoylethanolamine

CB2 = Cannabinoid type 2 receptor

CNS = Central nervous system

eCB = Endocannabinoid

NAE = N-acylethanolamine

NAPE = N-acylphosphatidylethanolamine

PEA = Palmitoylethanolamide

PPAR = Peroxisome proliferator-activated receptor

SCI = Spinal cord injury

CONFLICT OF INTEREST

None of the other authors have financial or other conflict of interest to disclose.

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