PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2012) **367**, 3312–3325
doi:10.1098/rstb.2011.0391

Review

Mast cell–glia axis in neuroinflammation and therapeutic potential of the anandamide congener palmitoylethanolamide

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Communication between the immune and nervous systems depends a great deal on pro-inflammatory cytokines. Both astroglia and microglia, in particular, constitute an important source of inflammatory mediators and may have fundamental roles in central nervous system (CNS) disorders from neuropathic pain and epilepsy to neurodegenerative diseases. Glial cells respond also to pro-inflammatory signals released from cells of immune origin. In this context, mast cells are of particular relevance. These immune-related cells, while resident in the CNS, are able to cross a compromised blood-spinal cord and blood-brain barrier in cases of CNS pathology. Emerging evidence suggests the possibility of mast cell–glia communication, and opens exciting new perspectives for designing therapies to target neuroinflammation by differentially modulating the activation of non-neuronal cells normally controlling neuronal sensitization—both peripherally and centrally. This review aims to provide an overview of recent progress relating to the pathobiology of neuroinflammation, the role of glia, neuro-immune interactions involving mast cells and the possibility that glia–mast cell interactions contribute to exacerbation of acute symptoms of chronic neurodegenerative disease and accelerated disease progression, as well as promotion of pain transmission pathways. Using this background as a starting point for discussion, we will consider the therapeutic potential of naturally occurring fatty acid ethanolamides, such as palmitoylethanolamide in treating systemic inflammation or blockade of signalling pathways from the periphery to the brain in such settings.

Keywords: microglia; mast cells; neuro-immune; neuroinflammation; neurodegeneration; palmitoylethanolamide

1. INTRODUCTION

A fundamental advance in neuroscience research has been the understanding that an extensive communication exists between the immune system and the central nervous system (CNS). Pro-inflammatory cytokines occupy a key role in this communication, as they regulate host responses to infection, inflammation and reactions to stress or trauma. Astrocytes, and even more so microglia, constitute an important source of inflammatory mediators and may have cardinal roles in conditions ranging from chronic pain [1,2] and epilepsy [3] to neurodegenerative diseases, such as Alzheimer's (AD) [4–7], Parkinson's [8,9] and amyotrophic lateral sclerosis [10]—and may even contribute to schizophrenia, depression and other psychiatric disorders [11,12]. Microglia-mediated neuroinflammatory processes are thought to be implicated in brain ageing as well [13].

Heightened glial cell activity characterizes multiple pain-processing pathways in response to peripheral injury [14–16]. Systemic inflammation gives rise to signals that communicate with the brain and leads to changes in metabolism and behaviour. Our brain normally responds to stress and insults by transiently upregulating inflammatory processes, which are kept in check by endogenous protective elements. When upset, this homeostatic balance can result in disease or exacerbation of initiating factors that result in disease. Neuroinflammation may also raise the brain's sensitivity to stress [17–19].

Microglial activation cannot be viewed simply as a 'one size fit all' phenotypic manifestation. These resident myeloid-lineage cells in the brain and the spinal cord parenchyma participate in both innate and adaptive immune responses in the CNS. Microglial cells are suggested to exist in at least two functionally discernable states once 'activated': a phagocytic phenotype (innate activation); an antigen presenting phenotype (adaptive activation), as a function of their stimulatory environment [20]. When challenged with certain pathogen-associated molecular patterns (molecules associated with groups of

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One contribution of 15 to a Theme Issue 'Endocannabinoids in nervous system health and disease'.

pathogens that are recognized by cells of the innate immune system, lipopolysaccaride being the prototypical example), microglia seem to activate a 'mixed' response characterized by enhanced phagocytosis and pro-inflammatory cytokine production as well as adaptive activation of T cells. In an experimental autoimmune encephalomyelitis (EAE) model, microglia largely support an adaptive activation of encephalitogenic T cells in the presence of the CD40–CD40 ligand interaction. In the context of amyloid β-peptide (Aβ) challenge, CD40 ligation is able to shift activated microglia from innate to adaptive activation (reviewed in [20]).

Glia may respond to pro-inflammatory signals released from cells immune origin, such as mast cells. These effector cells of the innate immune system derive from a distinct precursor in the bone marrow [21] and mature under the influence of stem cell factor and various cytokines [22]. Mast cells are common at sites that are in close contact with the external environment (skin, gastrointestinal tract and airways) and are distributed in virtually all organs and vascularized tissues [23]. Mast cells are also normally resident in the peritoneum, synovium, hair follicles and many other organs. Like macrophages they reside in the brains of many species, where they enter during development via penetrating blood vessels with which they remain associated [24]. In the absence of inflammation, mast cells can move through normal brain via blood-brain barrier (BBB) passage [25], but may also cross the blood-spinal cord barrier and BBB when the barrier is compromised as a result of CNS pathology. Mast cells participate in innate host defence reactions and are found in peripheral tissues innervated by small calibre sensory nerve fibres and within the endoneurial compartment, where they orchestrate inflammatory processes. This last point is noteworthy, as recent findings demonstrate that systemic inflammation gives rise to signals that communicate with the brain and leads to changes in metabolism and behaviour, including the expression of a pro-inflammatory phenotype by microglia [26,27].

Mast cells produce an array of mediators, among which are biogenic amines, such as histamine and serotonin, cytokines (interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) in particular), enzymes, lipid metabolites, ATP, neuropeptides, growth factors (nerve growth factor (NGF) being a key example) and heparin [28]. Mast cells pack a one-two punch: in addition to a rapid mediator release via degranulation, longer-lasting activation results in the release of de novo-formed mediators [22]. Their immune regulatory role includes the release of chemoattractants that recruit eosinophils [29] and monocytes [30]. There is evidence that nervous system mast cells may play a role in the pathogenesis of the experimental autoimmune demyelinating diseases, experimental allergic neuritis and EAE [31], are degranulated in the brain of rats with EAE [32] and are associated with the multiple sclerosis lesions [33]. Mast cell trypase is elevated in the cerebrospinal fluid of patients with multiple sclerosis [34]. Moreover, mast cells can be activated by myelin [35], and activated mast cells cause demyelination [36], and induce apoptotic oligodendrocyte death *in vitro* [37]. Interestingly, brain mast cells have been considered as a bridge between the immune system and anxiety-like behaviour [38].

2. MICROGLIA, MAST CELLS AND NERVOUS SYSTEM PATHOLOGY

(a) *Neuropathic pain*

Clinical pain, for example, after nerve injury (neuropathic pain) is characterized by pain in the absence of a stimulus and reduced nociceptive thresholds so that normally innocuous stimuli produce pain. Not only neuronal pathways, but also Schwann cells, elements of the peripheral immune system, spinal microglia and astrocytes are involved in the creation and maintenance of neuropathic pain states [39,40]. Inflammation or nerve injury can result, e.g. in the synthesis and release of IL-1β that modulates neuronal cell activity [41]. In addition, microglia express several subtypes of purinergic P2X and P2Y receptors that play a key role in pain signalling in the spinal cord under pathological conditions, such as following peripheral nerve injury [42–45]. In such settings, dorsal horn microglia become activated and show upregulated expression of purinergic receptors, and interference with receptor function or expression suppresses neuropathic pain [46,47]. After nerve injury, mitogen-activated protein kinases are differentially activated in spinal microglia and astrocytes, leading to the synthesis of pro-inflammatory/pro-nociceptive mediators, thereby enhancing and prolonging pain. Inhibition of these kinase signalling pathways may attenuate inflammatory and neuropathic pain in different animal models [48,49].

Activated mast cells contribute directly to neuropathic pain by releasing algogenic mediators after degranulation [50]. Resident peripheral nerve mast cells are the first cells activated at the site of nerve damage and contribute to the recruitment of neutrophils and macrophages [51]. Their degranulation distinctly activates trigeminocervical and lumbosacral pain pathways and elicits widespread tactile pain hypersensitivity [52]. Histamine, a key mast cell mediator has sensitizing effects on nociceptors [53]. Another important mediator is NGF, which produces sensitization of nociceptors, directly via trkA receptors on nociceptors, and indirectly via other peripheral cell types [53]. Mast cell degranulation is a principal source of rapidly released NGF, and mast cells respond in a paracrine/autocrine fashion to NGF [54,55]. These initial events promote the recruitment of T cells, which reinforce and maintain inflammatory reactions. These mediators/factors may either induce activity in axons or are transported retrogradely to cell bodies in the dorsal root ganglia, where they may alter gene expression of the neurons. Mast cells may also contribute indirectly by enhancing the recruitment of other key immune cell types which, in turn, release pro-nociceptive mediators, such as IL-6 [56,57]. Moreover, a role for mast cells in chronic pain states is strengthened by recent data showing that systemic glucocorticoid therapy reduces pain and the number of TNF-α-positive mast cells in rats with chronic constrictive injury [58].

(b) *Acute CNS injury*

Acute CNS injuries, such as stroke or trauma result in a prolonged inflammatory response involving microglial activation and infiltration of macrophages and neutrophils, which has the potential to cause secondary injury [59]. Attenuation of microglial activation

has protective value, and there are examples making the case for damage-limiting action [60,61].

Much effort has been directed to inhibiting the inflammatory cascade of blood-borne neutrophil and phagocyte infiltration in ischaemia. Surprisingly, few studies have focused on resident brain cell types that are able to mount an immediate host response in the brain and meninges—the mast cell. The latter are normally resident in the CNS [62], in close association with cerebral blood vessels during development and adulthood [63,64]. In contrast with what had been long assumed [65], Jin *et al.* [66] showed that mast cell activation is the 'first responder' in this injury—not microglia. Although TNF-α is produced by many cells in response to stimuli, mast cells arrive 'armed' to initiate acute inflammation with their store of preformed TNF-α [67]. Microglia/macrophages [68,69] and endothelial cells [70] in the CNS also produce TNF-α; however, the presence and the release of TNF-α from mast cells preceded its detection in other cells. Inhibition of immediate mast cell activation limits hypoxic-ischaemic brain damage [66,71–74]. Mast cells are as early responders in the regulation of acute BBB changes after cerebral ischaemia and haemorrhage [75], via their complement of vasoactive and matrix-degrading components, such as histamine, and proteases capable of activating matrix metalloproteinases. Furthermore, cerebral mast cells can regulate acute microvascular gelatinase (matrix metalloproteinases-2 and -9) activation and consequent BBB disruption following transient cerebral ischaemia [76].

(c) *Stress*

Prior exposure to a stressor can potentiate CNS pro-inflammatory immune responses to a peripheral immune challenge [77]. Stressors such as inescapable shock and restraint enhance the inflammatory profile of microglia [78,79], perhaps by activating β-adrenergic receptors which increase the expression of IL-1β in the CNS [80]. Intriguingly, an increased peripheral inflammatory profile is detected in humans after prolonged social stress [81]. Indeed, β-adrenergic receptor antagonism prevents anxiety-like behaviour and microglial reactivity induced by repeated social defeat [82]. Furthermore, acute (immobilization) stress may increase BBB permeability via brain mast cell activation [83].

3. MICROGLIA AND MAST CELLS: LEADING A DOUBLE LIFE

As discussed earlier, activated microglia produce a potentially lethal mix of compounds capable of damaging neurons, oligodendrocytes or extracellular matrix molecules. In demyelinating disorders at both the clinical and the preclinical levels, depletion or blockade of microglia and macrophages prevents disease progression [84,85]. Yet, microglial paralysis inhibits the development and maintenance of inflammatory CNS lesions in toxin-induced models of de- and remyelination [86]. Microglia/macrophages may deliver trophic factors [87], and support myelin regeneration by phagocytic removal of obstructive myelin debris [88,89] or through activation and recruitment of endogenous oligodendrocyte precursor cells to the lesion site [90]. As for mast cells, mice engineered to lack mast cells are resistant to myelin oligodendrocyte glycoprotein-induced EAE [91]. Reconstitution of these animals with normal bone marrow-derived mast cells restores susceptibility to EAE induction [92]. Using mast cell transplantation and genetic mutations, Bennett *et al.* [93] showed that while bone marrow-derived mast cells are actively recruited to the CNS during EAE, the disease developed unabated in the complete absence of mast cells or bone marrow-derived mast cell reconstitution.

The subject of microglia and cerebral amyloidosis/AD pathogenesis remains a contentious one [94]. Microglia can be found adjacent to amyloid deposits [95], and anti-inflammatory drugs that suppress the inflammatory response in microglia attenuate symptoms in a mouse model of AD [96]. In one study, deletion of inducible nitric oxide synthase in a transgenic AD mouse model protected from plaque formation and premature mortality [97], yet others had observed that a marked reduction or a virtually complete ablation of resident microglia (including bone marrow-derived microglia) failed to alter amyloid plaque load in two distinct transgenic AD mouse models [98]. Furthermore, deleting the microglial chemokine receptor Ccr2 (which mediates the accumulation of mononuclear phagocytes at sites of inflammation) accelerated early disease progression and impaired microglial accumulation in an AD mouse model [99].

The case for brain ischaemia is also complex, as microglia produce cytotoxic molecules, as well as growth and repair factors [61]. After an ischaemic lesion, microglia accumulate at the lesion site and in the penumbra, suggesting a neuroprotective role. In transgenic mice in which microglial cells have been ablated, transient middle cerebral artery occlusion produces a larger infarct, associated with an increase in apoptotic neurons, compared with normal mice [100], while injection of microglia into the bloodstream of Mongolian gerbils (which is home to an ischaemic hippocampal lesion) resulted in greater neuron survival [101]. Furthermore, microglia may protect hippocampal neurons from excitotoxicity [102]. Microglia are probably also key players in developmental synaptic pruning, and disruptions in their number and/or function during the early postnatal period can impair synapse development and plasticity [103]. At the other end of the developmental curve, early activation of microglia can trigger long-lasting impairment of adult neurogenesis in the olfactory bulb [104].

Human mast cell granules contain angiogenin [105]. Angiogenin is reported to be neuroprotective and to promote the survival and neuritogenesis of motor neurons [106], suggesting a link between recent studies associating angiogenin gene mutations with amyotrophic lateral sclerosis [107].

4. MAST CELLS AND GLIA: ARE YOU TALKING TO ME?

> Oh, east is east, and west is west, and never the twain shall meet
>
> Rudyard Kipling, The Ballad of East and West (1889)

Table 1. Possible paths of microglia–mast cell interaction. Modified from Skaper *et al.* [108]. BDNF, brain-derived neurotrophic factor; C5aR, C5a receptor; MAPK, mitogen-activated protein kinase; PAMPs, pathogen-associate molecular patterns; PAR2, proteinase-activated receptor 2; TLR, Toll-like receptor.

	biological actions		
effector	microglia	mast cells	references
TLR2, TLR4	release of IL-6 and CCL5 affects surface expression of mast cell TLR2/TLR4	upregulation of cytokine/chemokine release; CCL5/RANTES induces pro-inflammatory profile in microglia; recruitment of immune cells to site of injury	[109–114]
ATP/P2 receptors	ATP stimulates IL-33 release from microglia pre-activated with PAMPs via TLRs	IL-33 binds to mast cell receptor leading to secretion of IL-6, IL-13 and monocyte chemoattractant protein 1 → modulate microglia activity	[42,115–117]
mast cell tryptase and PAR2	mast cell tryptase cleaves/activates PAR2 on microglia, resulting in: $P2X_4$ upregulation and BDNF release; pro-inflammatory mediator release via the MAPK-nuclear factor-κB pathway	IL-6 and TNF-α from microglia can upregulate mast cell expression of PAR2 → mast cell activation and TNF-α release	[118–120]
CXCR4/CXCL12	promotes migration and activation; CXCR4/CXCL12 upregulated in hypoxia/ischaemia	CXCR4 is a mast cell chemotaxin	[121–123]
C5aR	C5aR upregulated upon activation; C5a peptide released in neuroinflammtion; crosstalk between C5a and TLR4	C5aR upregulated upon activation; provides a strong mast cell chemoattractant signal towards C5a peptide; crosstalk between C5a and TLR4	[124,125]

Mast cells and microglia would appear to be an exception to this. Indeed, a number of potential contact points exist between these cell types, and include: Toll-like receptors (TLRs), especially isoforms-2 and -4 (upregulation of cytokine/chemokine release and recruitment of immune cells to site of injury); purinergic (ATP) P2 receptors (e.g. IL-33 from microglia binds to its receptor on mast cells and induces secretion of IL-6, IL-13 and monocyte chemoattractant protein 1 which, in turn may modulate microglia activity); proteinase-activated receptor 2 (PAR2) (e.g. mast cell tryptase cleaves/activates PAR2 on microglia, resulting in $P2X_4$ receptor upregulation and brain-derived neurotrophic factor release, while IL-6 and TNF-α from microglia can upregulate mast cell expression of PAR2, resulting in mast cell activation and TNF-α release); CXCR4/CXCL12 (promotes microglia migration and activation, and in microglial cells CXCR4/CXCL12 are both upregulated in hypoxia/ischaemia; CXCR4 acts as a mast cell chemotaxin); C5a receptor (C5aR; in microglia C5aR is upregulated upon activation, C5a peptide is released in neuroinflammtion, and there is crosstalk between C5a and TLR4; for mast cells C5aR is upregulated upon activation, and C5aR is a strong mast cell chemoattractant signal towards C5a peptide; there is also crosstalk between C5a and TLR4; table 1). The above points are discussed in greater detail elsewhere [108].

Although beyond the scope of this review, it is worth noting that there is evidence suggesting an interaction between mast cells and astrocytes. Astrocytes share perivascular localization with mast cells [126] are able to maintain the viability of rat serosal mast cells in culture [126] and have receptors for histamine [127]. Astrocyte-derived cytokines/chemokines trigger mast cell degranulation [128]. Co-culture of mouse bone marrow mast cells with cortical astrocytes evidenced autocrine/paracrine actions, with release of histamine and leukotrienes [129]; mast cells and astrocytes displayed enhanced surface expression of CD40L and CD40, respectively, whose crosstalk led to the production of inflammatory cytokines [130].

5. MICROGLIA AND MAST CELLS AS THERAPEUTIC TARGETS

(a) *'Classical' pharmacology*

Pharmacological attenuation of microglial and mast cell activation is emerging as promising targets for neuropathic pain (for reviews, see [131,132]). For example, propentofylline, pentoxifylline, minocycline and AV411 (ibudilast) inhibit cytokines and lower microglia activation, thereby suppressing the development of neuropathic pain [132]. These agents appear to be safe and clinically well tolerated. Chemical genetics of neuroinflammation has been used to identify natural and synthetic compounds as microglial inhibitors *in vivo*, e.g. obovatol [133]. Regarding mast cells, the established degranulation stabilizer sodium cromoglycate suppresses hyperalgesia induced by nerve injury and post-operative pain [50,51,134]. Apart from neuropathic pain, detrimental effects of neuroinflammation have been noted in association with psychiatric and neurodegenerative diseases. Within this context, much attention has been directed to therapeutic strategies aimed at inhibiting neurotoxic glial cell activation [135].

(b) N-*Palmitoylethanolamine: a wide-acting anti-inflammatory and neuroprotective* N-*acylethanolamine*

In addition to synthetic chemistry efforts aimed at controlling neuroinflammation, we now recognize the existence of endogenous molecules involved in endogenous protective mechanisms that are activated in the body as a result of different types of tissue damage or stimulation of inflammatory responses and nociceptive fibres. One interesting group of such molecules are the *N*-acylethanolamines, a class of naturally occurring lipidic mediator molecules composed of a fatty acid and ethanolamine, namely the fatty acid ethanolamines (FAEs). The principal FAE family members are the endocannabinoid *N*-arachidonoylethanolamine (anandamide, or 5Z,8Z,11Z,14Z)-*N*-(2-hydroxyethyl)icosa-5,8,11,14-tetraenamide) and its congeners *N*-stearoylethanolamine (*N*-(2-hydroxyethyl)-stearamide), *N*-oleoylethanolamine (*N*-2-hydroxyethyl-9(Z)-octadecenamide) and *N*-palmitoylethanolamine (PEA, or palmitoylethanolamide) (*N*-(2-hydroxyethyl)-hexadecanamide) [136]. PEA (figure 1) is abundant in mammalian brain and, intriguingly, there is evidence for the presence of PEA as well as other FAEs in marine species, such as bivalve molluscs [137] and sea urchin ovaries [138]. PEA has even been detected in the CNS of the leech *Hirudo medicinalis* [139]. PEA is produced through an on-demand synthesis within the lipid bilayer where *N*-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) releases it from its membrane precursor, *N*-palmitoylphosphatidylethanolamine [140].

The potential benefit of FAEs first came to the light in 1943 when Coburn & Moore [141] reported on the antipyretic properties of dried chicken egg yolk in children with rheumatic fever. A decade later, this same group identified the lipid fraction from egg yolk as the component responsible for this effect [142], with PEA being the active component [143]. The therapeutic applications of this lipid amide remained largely overlooked, however, until the emerging characterization of its anti-inflammatory [144], analgesic [145] and anti-convulsant [146] properties. These past 15 years have seen a remarkable rise in studies on PEA anti-inflammatory actions [147], and a first-ever international workshop on PEA was held in February of this year [148].

In biological terms, PEA is produced and hydrolysed by microglia [149], inhibits mast cell activation [150,151] and increases in glutamate-treated neocortical neurons *ex vivo* [152] and in cortex after CNS injury [153–155], as well as in muscle dialysate from women with chronic neck-/shoulder pain [156]. The level of PEA is increased in the spinal cord of spastic (but not non-spastic) mice suffering from chronic relapsing experimental allergic encephalomyelitis (an animal model of multiple sclerosis, induced by repeated administration to mice of syngenic spinal cord homogenate emulsified in Freund's complete adjuvant [157]. Collectively, these observations suggest that a key role of PEA may be to maintain cellular homeostasis when faced with external stressors provoking, for example, inflammation. However, one may envision pathological settings where PEA endogenous production is insufficient to control the ensuing inflammatory cascade.

A number of studies have addressed the above issue by applying PEA exogenously. This fatty acid amide, given orally (as pre-treatment), was effective in mast cell-mediated experimental models of acute inflammation, both immunogenic (passive cutaneous anaphylaxis-induced extravasation of leucocytes) and neurogenic (subcutaneous injection of substance P), as well as carrageenan- or dextran and formalin-induced hindpaw oedema in rats [144,158]. PEA reduced the pain behaviour elicited by subcutaneous formalin injection [145,159,160] and was efficacious also when administered after induction of acute inflammation [161]. Using the carrageenan-induced paw model of hyperalgesia in mice, D'Agostino *et al.* [162] reported that intracerebroventricular administration of PEA 30 min before carrageenan injection markedly reduced mechanical hyperalgesia up to 24 h following inflammatory insult. In a rat model of chronic granulomatous inflammation sustained by mast cell activation, locally administered PEA significantly reduced mast cell degranulation and the expression and release of NGF, prevented nerve fibre formation and sprouting, reduced mechanical allodynia and inhibited dorsal root ganglia activation [163]. Importantly, PEA has anti-inflammatory activity and elicits analgesia in rodent neuropathic pain models [164,165].

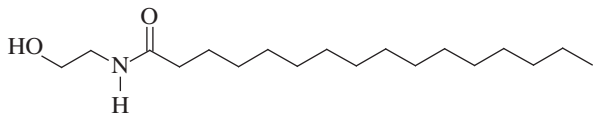

Figure 1. Chemical structure of *N*-palmitoylethanolamine.

The endocannabinoid system is modulated in response to spinal cord injury in rats. Lesion-induced increases of anandamide and PEA levels occur in the early stage with an upregulation of NAPE-PLD and a downregulation of the degradative enzyme fatty acid amide hydrolase (FAAH), while in delayed stages 2-arachidonoylglycerol increases [166]. In this context, PEA is endowed with neuroprotective effects as well. For example, in a compression model of spinal cord trauma in mice (induced by applying an aneurysm clip to the spinal cord, which replicates the persistence of cord compression as seen in human injury) PEA given systemically 6 and 12 h post-injury induction significantly reduced the severity of spinal cord trauma via reduction of mast cell infiltration and activation [167]. Furthermore, PEA limited the activation of microglia and astrocytes expressing cannabinoid CB2 receptors, and its protective effect appeared to involve changes in neurotrophic factor expression and in spinal cord dopaminergic function. In an earlier study using this experimental model of spinal cord injury, the authors showed that intraperitoneal administration of PEA reduced spinal cord inflammation and tissue injury, neutrophil infiltration, nitrotyrosine formation, pro-inflammatory cytokine expression, nuclear transcription factor κB activation, inducible nitric oxide synthase expression and apoptosis, and ameliorated recovery of motor limb function [168]. In a model of mixed neuron-glia cultures from hippocampus, the introduction of stimulated mast cells led to neuron loss as a result of mast cells releasing TNF-α which then triggered astrocyte production of nitric oxide [169]. PEA decreased neuron loss resulting from mast cell stimulation in the mixed cultures (figure 2), but

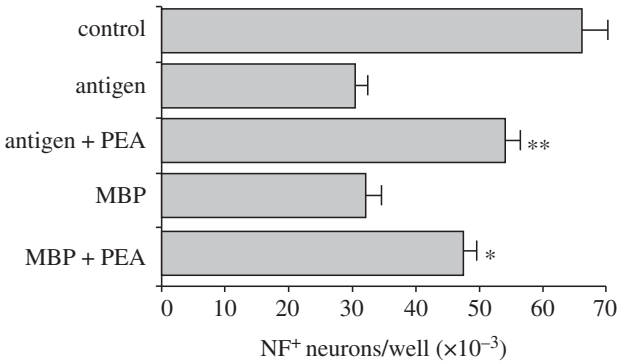

Figure 2. *N*-Palmitoylethanolamine (PEA) reduces hippocampal neuron death caused by antigen- or myelin basic protein (MBP)-treated mast cells. Mixed neuron-glia cultures were incubated for 12 h with transwell membrane inserts containing 5×10^4 mast cells treated with either anti-dinitrophenol lgE/dinitrophenol-human serum albumin ('antigen') or 20 μM MBP, alone or together with 30 μM PEA. Hippocampal cell cultures were fixed 60 h after insert removal, and neurofilament-immunopositive (NF^+) neurons quantified. Values are means ± s.d. (four to five experiments). $*p < 0.01$ or $**p < 0.001$ compared with the same condition but without PEA. (Modified from Skaper *et al.* [169] (figure 3). Copyright (1996), with permission from John Wiley & Sons.)

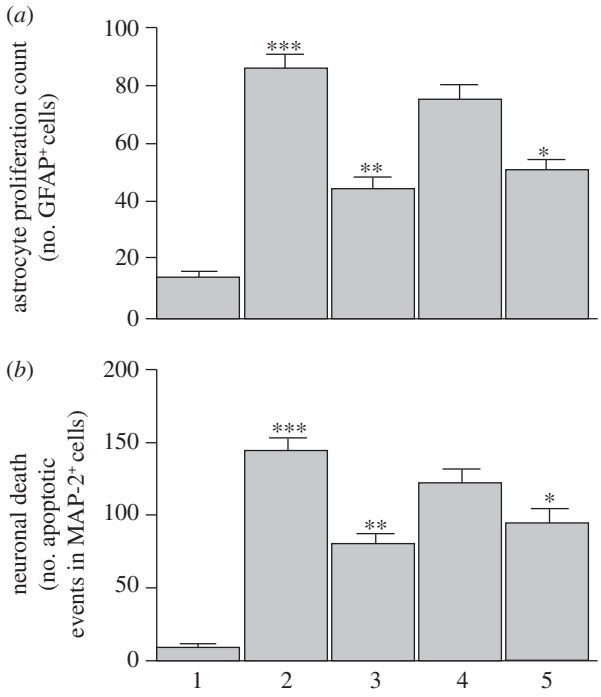

Figure 3. *N*-Palmitoylethanolamine (PEA) decreases astrocyte activation in organotypic cultures of rat hippocampus and rescues neuronal CA3 damage caused by Aβ challenge. Aβ(1-42)-challenged (1 μg ml^{-1}) slices of rat hippocampi were treated for 24 h with PEA (0.1 μM) in the presence of the selective PPARγ antagonist (GW9662, 9 nM) or the selective PPARα antagonist (MK886, 3 μM). (*a*) Relative quantification of glial fibrillary acidic protein (GFAP)-positive cell number as a count of astrocyte proliferation. (*b*) Apoptotic events detected on microtubule associated protein 2 (MAP2)-expressing cells as an indication of neuronal death. The average value was determined by counting cells in at least five microscopic fields for each treatment. Data are means ± s.e.m. of four separate experiments. Statistical analysis was performed using parametric one-way analysis of variance, and multiple comparisons were performed using the Bonferroni test. $***p < 0.001$ and $*p < 0.05$ versus control; $**p < 0.01$ and $*p < 0.05$ versus Aβ-challenge slices. 1, control; 2, Aβ; 3, Aβ + PEA; 4, Aβ + PEA + MK886; 5, Aβ + PEA + GW9662. (Modified from Scuderi *et al.* [172] (figure 2). Copyright (2012), with permission from Biomed Central.)

not that caused by direct cytokine induction of astrocytic nitric oxide synthase.

In another model, PEA was protective in a delayed post-glutamate paradigm of excitotoxic death [170]. Several new reports describe the neuroprotective action of PEA against Aβ(25-35)-induced learning and memory impairment in mice [171], or organotypic hippocampal slices challenged with Aβ(1-42) (figure 3) [172].

In mechanistic terms, there is gathering evidence that PEA may be an endogenous ligand for the peroxisome proliferator-activated receptor alpha (PPARα). PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. In particular, the α- and γ-isoforms are associated with pro-inflammatory events. Lo Verme *et al.* [173] were the first to show that PPARα mediates the anti-inflammatory effects of PEA and suggested that this fatty acid ethanolamine may serve, like its analogue oleoylethanolamine, as an endogenous ligand of PPARα. For example, PEA failed to rescue memory deficits induced by Aβ(25-35) injection in PPARα null mice, while a synthetic PPARα agonist mimicked the effect of PEA [171]. Furthermore, the neuroprotective action of PEA in organotypic hippocampal slices challenged with Aβ(1-42) was blocked by selective PPARα, but not PPARγ, antagonists [172]. PEA induces allopregnanolone synthesis in astrocytes in a PPARα-dependent fashion: its effects were blunted by a selective PPARα antagonist, or by PPARα silencing by RNA interference [174]. Moreover, PPARα antagonists reduced PEA's ability to counteract Aβ(1-42)-induced reactive gliosis [175]. All these effects were absent in PPARα null mice [38]. In yet other studies, acute intracerebroventricular administration of PEA modulated carrageenan-induced paw edema in mice in a PPARα-dependent manner [176]. Microinjection of PEA in the ventrolateral periaqueductal grey of male rats reduced the ongoing activity of ON and OFF cells in the rostral ventromedial medulla and produced an increase in the latency of the nociceptive reaction (the periaqueductal grey–rostral ventromedial medulla pathway is a key circuit in pain processing), effects that were prevented by a selective PPARα antagonist [177].

The so-called 'entourage effect hypothesis' has also been invoked to explain PEA's pharmacological actions. This hypothesis proposes that PEA may act to enhance the anti-inflammatory and anti-nociceptive activity of other endogenous compounds by raising their affinity for a receptor or by inhibiting their metabolic degradation [178]. One such compound whose activity may be potentiated by PEA is anandamide, which possesses anti-inflammatory and anti-nociceptive effects. A possible point of interaction between anandamide and its congeners (e.g. PEA) is the transient receptor potential vanilloid type 1 (TRPV1) receptor. The TRPV1

receptor, a non-selective cation channel expressed in small diameter sensory neurons, is activated by noxious heat, low pH and capsaicin. As it happens, anandamide is also an agonist for TRPV1 receptors, and PEA enhances anandamide stimulation of human TRPV1 receptors [179]. The finding that the cannabinoid CB2 receptor antagonist, SR144528 inhibits some of the analgesic responses to PEA *in vivo* (although PEA lacks affinity for either the CB1 or the CB2 receptors) has been attributed to the possibility of PEA acting indirectly by potentiating anandamide actions [145]. Mast cells [180] and cortical [181] and spinal cord [182] microglia have all been reported to express TRPV1 receptors. This, together with the close association of mast cells and microglia in nervous tissue further strengthens the existence of a line of communication between these two immune cell types.

FAAH is an intracellular integral membrane protein belonging to the amidase family of enzymes which catalyses the hydrolysis of FAEs into the corresponding fatty acid and ethanolamine [183]. Later, another enzyme which preferentially hydrolyses PEA was cloned [184]. Nominated *N*-acylethanolamine-hydrolysing acid amidase (NAAA), it is not related to FAAH but bears structural homology to ceramidase and belongs to the family of choloylglycine hydrolases. NAAA is localized to lysosomes. Inhibition of PEA breakdown presents a complementary and attractive therapeutic approach to treat inflammation. Indeed, this is an area of active investigation, and initial efforts have shown promise. Selective NAAA inhibitors have been reported [185–187], which blunt responses induced by inflammatory stimuli *in vivo* and *in vitro*, while elevating PEA levels *in vitro* [185].

6. CONCLUSIONS AND OUTLOOK

We now appreciate that inflammatory signalling molecules can profoundly affect a great many CNS functions. These effectors derive both from the innate and adaptive immune systems, as well as glia within the CNS. Microglia, in particular, serve as sensors for disturbed brain tissue homeostasis and accumulate locally in response to neuronal injury or entry of foreign material in the brain [188]. Yet, few studies have focused on resident brain cell types capable of mounting *immediate* host responses in the brain and meninges, namely mast cells. In spite of their recognized 'first responder' action in injury rather than microglia, one needs to bear in mind that *longer-lasting* activation of mast cells results in the release of de novo-formed mediators. Moreover, mast cells are multiple-use cells, capable of surviving and delivering repetitive hits [189].

In human chronic pain, unequivocal demonstration that glial and mast cell activation occurs in hypersensitized patients remains to be provided. Systematic studies are lacking in demonstrating a correlation between the magnitude of glial and/or mast cell markers in the cerebrospinal fluid or in spinal tissue and the intensity of pain in patients.

Currently available drugs for neuropathic pain were designed to hit neuronal targets and focus on blocking neurotransmission. Hence, they address pain symptoms but not the underlying pathology of neuropathic pain. Unfortunately, they only provide a transient relief of neuropathic pain in only a fraction of patients and produce marked CNS side effects. Mast cell stabilizers, while suppressing development of hyperalgesia do not touch microglia. On the other hand, current glial inhibitors for pain largely rely on their anti-inflammatory properties, and carry issues, such as non-selectivity in targeting one cell population, while risk of either acute or cumulative toxicity could hamper long-term use. Targeting regulators of neuroinflammation may prove to be a useful therapeutic strategy to affect a diverse array of nervous system disorders. Future studies should investigate the role of mast cells in inflammatory diseases as a network, which requires a critical examination of specific tissue localization, function and dynamic interaction with endogenous cells.

The capacity of PEA to modulate the protective responses of animals during inflammation and pain led to the hypothesis that endogenous PEA may be a component of the complex homeostatic system controlling the basal threshold of both inflammation and pain. The production of PEA during inflammatory conditions supports this role, and emerging data that selective inhibition of PEA degradation is anti-inflammatory provide more direct evidence for the involvement of PEA in the control of pain and inflammation. As an endogenous compound, PEA has basically no adverse effects, while possessing a double therapeutic effect (i.e. anti-inflammatory and anti-nociceptive).

Although clinical data are somewhat limited at present, PEA has been reported to improve myelinated-fibre function in patients with chemotherapy-induced painful neuropathy [190], and to reduce neuropathic pain in a patient with multiple sclerosis [191]. In addition, nearly 40 clinical trials have been conducted to date, with a total of more than 2000 patients having been entered in these trials. All these clinical trials have been reviewed recently [192].

Clearly, much remains to be learned about signalling mechanisms that regulate neuroinflammation. Targeting regulators of neuroinflammation may prove to be a legitimate therapeutic strategy capable to affect an array of nervous system disorders. PEA, its analogues and agents that specifically inhibit its degradation are likely to result in the development of new therapeutic strategies for the treatment of pathological conditions also different from pain and inflammation.

The authors thank Stefano Lovison for excellent graphic design assistance. L.F. was supported by Fondazione CARIPARO 'Progetto Dottorati di Ricerca' Anno 2009.

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