Brain, Behavior, and Immunity 25 (2011) 1099–1112

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Effects of palmitoylethanolamide on release of mast cell peptidases and neurotrophic factors after spinal cord injury

Emanuela Esposito [a], Irene Paterniti [a], Emanuela Mazzon [b], Tiziana Genovese [a], Rosanna Di Paola [b], Maria Galuppo [a], Salvatore Cuzzocrea [a,b,*]

[a] Department of Clinical and Experimental Medicine and Pharmacology, School of Medicine, University of Messina, Italy
[b] IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy

ARTICLE INFO

Article history:
Received 15 November 2010
Received in revised form 14 February 2011
Accepted 14 February 2011
Available online 25 February 2011

Keywords:
Mast cells
GDNF
NT-3
NGF
CB2
Spinal cord injury

ABSTRACT

Spinal cord injury (SCI) has a significant impact on quality of life, expectancy, and economic burden, with considerable costs associated with primary care and loss of income. The complex pathophysiology of SCI may explain the difficulty in finding a suitable therapy for limiting neuronal injury and promoting regeneration. Although innovative medical care, advances in pharmacotherapy have been limited. The aim of the present study was to carefully investigate molecular pathways and subtypes of glial cells involved in the protective effect of PEA on inflammatory reaction associated with an experimental model of SCI.

The compression model induced by applying an aneurysm clip to the spinal cord in mice is closer to the human situation, since it replicates the persistence of cord compression. Spinal cord trauma was induced in mice by the application of vascular clips to the dura via a four-level T5–T8 laminectomy.

Repeated PEA administration (10 mg/kg i.p., 6 and 12 h after SCI) significantly reduced the degree of the severity of spinal cord trauma through the reduction of mast cell infiltration and activation. Moreover, PEA treatment significantly reduced the activation of microglia and astrocytes expressing cannabinoid CB_2 receptor after SCI. Importantly, the protective effect of PEA involved changes in the expression of neurotrophic factors, and in spinal cord dopaminergic function.

Our results enhance our understanding about mechanisms related to the anti-inflammatory property of the PEA suggesting that this *N*-acylethanolamine may represent a crucial therapeutic intervention both diminishing the immune/inflammatory response and promoting the initiation of neurotrophic substance after SCI.

1. Introduction

Spinal cord injury (SCI) has a significant impact on quality and expectancy of life, and economic burden, with considerable costs associated with primary care and loss of income. During the first 24 h post-injury, the synthesis and release of the potent pro-inflammatory mediators are markedly elevated at the lesion site where they play a major role in the development of secondary tissue degeneration after SCI in animals and in humans. Neurons continue to die for hours after SCI due to several mechanisms including exitotoxicity, vascular abnormalities and inflammatory response that can contribute to evolution of SCI.

Historically, administration of high-dose methylprednisolone (MP), acutely after SCI has been considered the standard of care in the United States. Although clinical results were initially promising, there have been growing concerns that the modest neurological improvements seen with high-dose MP treatment in injured patients are not worth the associated risks. Therefore, there is a critical need to develop new pharmacologic therapies for treatment of SCI. Regeneration and compensatory sprouting of axons are very limited after injury to the adult SC. This deficiency has been attributed to a lack of growth promoting factors and the presence of inhibitory molecules and physical barriers to axonal regeneration (Schwab, 2002).

Resident microglia and macrophages originating from blood are two key cell types related to the occurrence of neuronal degeneration in CNS after traumatic injury. In particular, when SCI occurs, microglia in parenchyma is activated and macrophages in circulation cross the blood–brain barrier to act as intrinsic spinal phagocytes. Therefore, these cells can release various neurotrophic peptides, which are excellent substrates for neurite outgrowth.

In addition, various study have clearly demonstrated that mast cells (MCs) have long been known to participate in the inflammatory process, in fact MCs are present and recruited to

* Corresponding author. Address: Department of Clinical and Experimental Medicine and Pharmacology, School of Medicine, University of Messina, Torre Biologica, Policlinico Universitario Via C. Valeria, Gazzi, 98100 Messina, Italy. Fax: +39 090 2213300.
E-mail address: salvator@unime.it (S. Cuzzocrea).

0889-1591/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2011.02.006

all inflammatory sites (Andoh et al., 2006; Mican and Metcalfe, 1990). However, it has only recently become clear that MCs play an important role in orchestrating the whole inflammatory process from initiation events to chronic activation (Pejler et al., 2007). MCs are the first immune cells to considerably stimulate the inflammatory process due to a rapid release of pro-inflammatory and vasoactive mediators. MCs make and secrete an abundance of peptidases, which are stored in such large amounts in granules. The main peptidases (tryptases, chymases, carboxypeptidase A3, and dipeptidylpeptidase I) were best known as markers of degranulation, they are released locally in response to MC stimulation and can be distributed systemically and detected in blood (Trivedi and Caughey, 2010).

During the last years, the endocannabinoid system has attracted the attention of researchers working in neural damage and repair and is being considered a promising target for the development of new therapies (Di Marzo, 2008; Pacher et al., 2006). Moreover, some components of the endocannabinoid system, that are constitutively expressed in the normal spinal cord such as diacylglycerol (DAG) lipases, fatty acid amide hydrolase and CB1 receptor (Bisogno et al., 2003; Cravatt et al., 2004; Romero et al., 2002; Tsou et al., 1998a,b) are modulated after neurodegenerative diseases or after peripheral nerve lesions (Bilsland et al., 2006; Lim et al., 2003; Petrosino et al., 2007; Shoemaker et al., 2007; Witting et al., 2004; Wotherspoon et al., 2005; Zhang et al., 2003). Furthermore, recently have been reported that the endocannabinoid system is activated in a clinically relevant model of traumatic SCI in rats (Garcia-Ovejero et al., 2009).

The endogenous fatty acid palmitoylethanolamide (PEA) is one of the members of *N*-acyl-ethanolamines family. In addition to the hypothesis that PEA has potent immunoregulatory properties (Aloe et al., 1993; Berdyshev, 2000; Berdyshev et al., 1997; Facci et al., 1995; Mazzari et al., 1996; Ross et al., 2000; Scarampella et al., 2001), 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 (Di Marzo et al., 2000).

Recently, we have also clearly demonstrated that the treatment with PEA significantly reduced the inflammation associated with experimental SCI in mice (Genovese et al., 2008). In particular, PEA protected against locomotor dysfunction following SCI. However, the basic molecular mechanisms as well as cellular types accounting for the anti-inflammatory properties of PEA in SCI have not yet been carefully investigated.

Thus, the aim of the present study is to carefully investigate molecular pathways and subtypes of glial cells involved in the

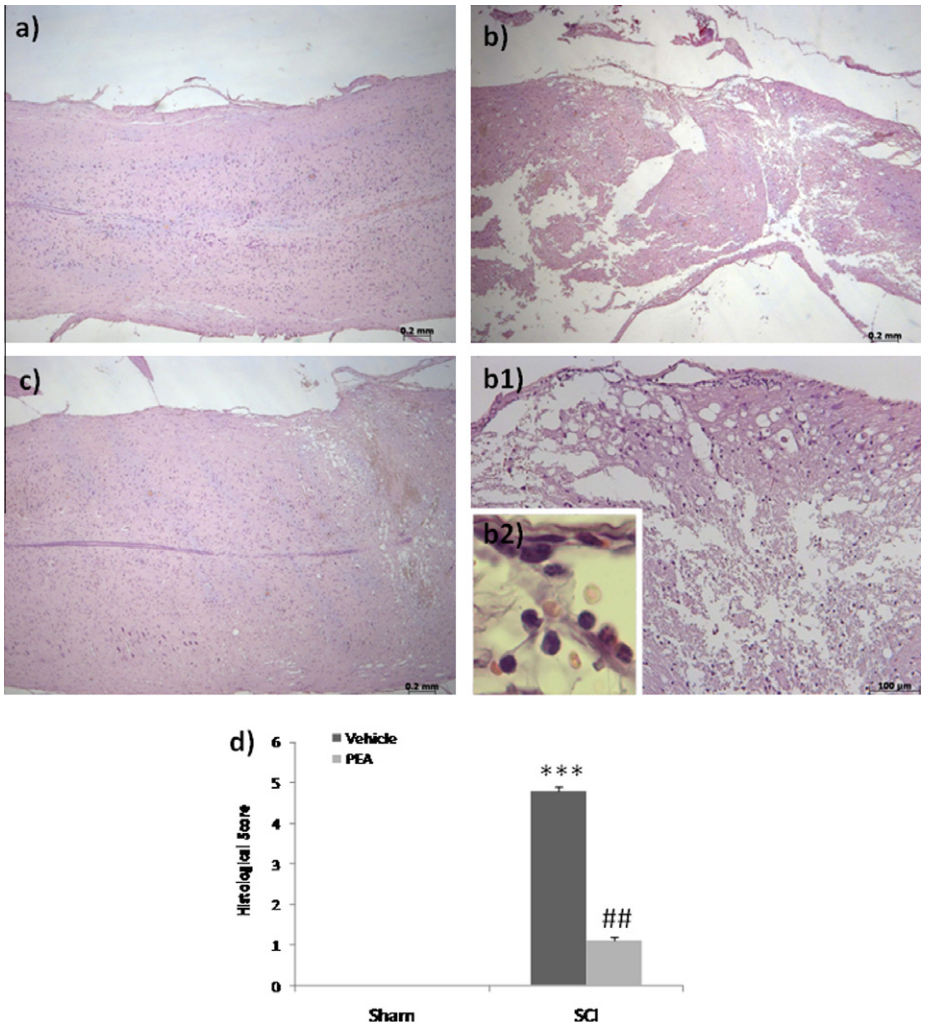

Fig. 1. PEA reduces the severity of spinal cord trauma. The low-magnification images of spinal cord after compression injury show tissue disorganization, white matter alteration, and inflammation in the perilesional area at 24 h after injury (panels b, b1). A significant protection from the SCI was evident in the tissue collected from PEA-treated mice (c). The histological score was made by an independent observer (d). $^{***}p<0.001$ vs. Sham and $^{\#\#}p<0.01$ vs. SCI. Values shown are means ± SE mean of 10 mice for each group.