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Palmitoylethanolamide inhibits rMCP-5 expression by regulating MITF activation in rat chronic granulomatous inflammation

Daniele De Filippis [a,1,2], Annapina Russo [a,1], Daniela De Stefano [b], Mariateresa Cipriano [a,3], Davide Esposito [c], Gianluca Grassia [a], Rosa Carnuccio [a], Giulia Russo [a], Teresa Iuvone [a,*,2]

[a] Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy
[b] European Institute for Research in Cystic Fibrosis, San Raffaele Scientific Institute, Via Olgettina, 58, 20132 Milan, Italy
[c] Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Via S. Pansini, 5, 80131 Naples, Italy

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ABSTRACT

Chronic inflammation, a condition frequently associated with several pathologies, is characterized by angiogenic and fibrogenic responses that may account for the development of granulomatous tissue. We previously demonstrated that the chymase, rat mast cell protease-5 (rMCP-5), exhibits pro-inflammatory and pro-angiogenic properties in a model of chronic inflammation sustained by mast cells (MCs), granuloma induced by the subcutaneous carrageenan-soaked sponge implant in rat. In this study, we investigated the effects of palmitoylethanolamide (PEA), an anti-inflammatory and analgesic endogenous compound, on rMCP-5 mRNA expression and Microphtalmia-associated Transcription Factor (MITF) activation in the same model of chronic inflammation. The levels of rMCP-5 mRNA were detected using semi-quantitative RT-PCR; the protein expression of chymase and extracellular signal-regulated kinases (ERK) were analyzed by western blot; MITF/DNA binding activity and MITF phosphorylation were assessed by electrophoretic mobility shift assay (EMSA) and immunoprecipitation, respectively. The administration of PEA (200, 400 and 800 μg/ml) significantly decreased rMCP-5 mRNA and chymase protein expression induced by λ-carrageenan. These effects were associated with a significant decrease of MITF/DNA binding activity and phosphorylated MITF as well as phosphorylated ERK levels.

In conclusion, our results, showing the ability of PEA to inhibit MITF activation and chymase expression in granulomatous tissue, may yield new insights into the understanding of the signaling pathways leading to MITF activation controlled by PEA.

1. Introduction

Rat mast cell protease-5 (rMCP-5) belongs to a family of serine proteases classified as chymases (Sanker et al., 1997). Among rodent chymases, rMCP-5 is predominantly expressed in connective tissue type mast cells (MCs) and also in early phase of MC development (Sanker et al., 1997). Like other MC proteases (MCPs), rMCP-5 is packed in the MC secretory granules intimately bound to proteoglycans and is released, together with several other stored mediators, following degranulation (Forsberg et al., 1999). Several evidences show that MCPs play a crucial role in the inflammatory/immune process in mammals (Badertscher et al., 2005). It is known that MCPs play an important role in the allergen-induced biphasic skin reaction (Tomimori et al., 2002) and in eliciting or maintaining cutaneous inflammation in atopic dermatitis (Badertscher et al., 2005). Moreover, chymases have been proposed to increase vascular permeability both in skin disease (He and Walls, 1998) and brain edema during intracerebral hemorrhage (Strbian, et al., 2009). Chymases have been shown to induce the release of neutrophil chemoattractants by eosinophils (Terakawa et al., 2006) and to mediate interaction between MCs and eosinophils in allergic diseases (Wong et al., 2009).

We have previously demonstrated that rMCP-5 chymase exhibits pro-inflammatory and pro-angiogenic effects in rat λ-carrageenan-induced granuloma, i.e., a model of chronic inflammation actively sustained by MC activation (Russo et al., 2005). It has been demonstrated that Microphthalmia-associated Transcription Factor (MITF) controls the transcription of a spectrum of genes in MCs, including several MCPs, adhesion molecules, metabolic enzyme and growth factor receptors (Kitamura et al., 2006; Razin et al., 1999; Morii et al., 2001). MITF belongs to Myc supergene family of basic helix–loop–helix leucine zipper (bHLH-Zip) DNA-binding protein which is predominantly expressed in MCs, melanocytes, heart and skeletal muscles (Hershey and Fish, 2004).

* Correspondence to: Dipartimento di Farmacia, Università degli Studi di "Napoli "Federico II, Via D. Montesano, 49, Napoli 80131, Italy Tel.: +39 081 678 429; fax: +39 081 678 403.
E-mail address: iuvone@unina.it (T. Iuvone).
[1] These authors contributed equally to the work.
[2] Endocannabinoid Research Group.
[3] Present address: Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-90187 Umeå, Sweden.

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Palmitoylethanolamide (PEA) is an endogenous lipid signaling molecule produced locally "on demand" and exhibits potent anti-inflammatory properties, which define PEA as ALIAmide (autacoid local inflammation antagonist amide) (Aloe et al., 1993). The ALIA mechanism is based on the important role played by PEA on MCs during inflammation; PEA is able to naturally control MC hyperactivity, which occurs not only in inflammation, but also in hyperalgesia and allodynia (Skaper et al., 2013; De Filippis et al., 2013). Although it has been suggested that PEA can activate different receptors, a specific receptor responsible for PEA effects is still under debate. In fact, PEA exhibits low affinity for cannabinoid CB_1 (Ki $>5\,\mu M$) and CB_2 (Ki $>5\,\mu M$) receptors, and the activation of a CB_2-like receptor has only been hypothesized (Calignano et al., 1998). It has also been described that PEA is able to activate and desensitize the transient receptor potential cation channel V type1 (TRPV1) and K(+) channels (Kv4.3 and Kv1.5) (Ambrosino et al., 2013; De Novellis et al., 2012). Moreover, some anti-inflammatory and anti-nociceptive effects of PEA have been ascribed to a PPAR-α (peroxisome proliferator-activated receptor) direct mechanism (Lo Verme et al., 2005). In addition, the ability of PEA to potentiate anandamide tone (Lambert and Di Marzo, 1999), known as "entourage effect", has been used to explain its pleiotropic effects (Scuderi et al., 2011; D'Agostino et al., 2012). Finally, a so-called orphan receptor GPR-55 has been evoked as responsible for some other PEA-mediated actions (Cantarella et al., 2011).

We have previously demonstrated that PEA was able to reduce granuloma formation in a model of chronic inflammation, the subcutaneous implant of carrageenan-soaked sponge in rat (De Filippis et al., 2010). On the basis of these observations, the aim of the present study was to investigate the effect of PEA on MITF activation and rMCP-5 expression in the same model of chronic inflammation.

2. Material and methods

2.1. Sponge implantation

Male Wistar rats (Harlan, Italy), weighing 200–220 g, were used in all experiments. Animals were provided with food and water ad libitum. Sponge implant in the rat was performed as previously described (De Filippis et al., 2010). λ-Carrageenan (1% w/v) (Sigma) was dissolved in pyrogen-free saline (0.5 ml/sponge), in the presence or absence of 100 μl of micronized synthetic PEA (kindly provided by Epitech Group; purity > 98%) at different concentrations (200, 400, and 800 μg/ml) in final volume of 0.5 ml/sponge; saline (0.5 ml/sponge) was used as control. 96 h after sponge implant rats were sacrificed in an atmosphere of CO_2. The granulomatous tissue around the sponge was dissected by using a surgical blade, weighed, quickly frozen in liquid nitrogen, and stored at −80 °C. Animal care as well as all experiments was in accordance with European Community Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Preparation of cytosolic and nuclear extracts

Cytosolic and nuclear extracts from granulomatous tissues were performed as previously described (De Filippis et al., 2010). Protein concentration was determined by Bio-Rad protein assay kit.

2.3. mRNA analysis

The mRNA level of rMCP-5 in granulomatous tissue was determined using the semi-quantitative RT-PCR method as previously described (Russo et al., 2006, 2008). The PCR-primers were selected according to the rat rMCP-5 cDNA sequence (forward primer 5′-TCCTGCAAACACTTCACCAG-3′, and reverse primer 5′-CGAGATCCAGAGTTAATTCT-3′); and rat β-actin cDNA (forward

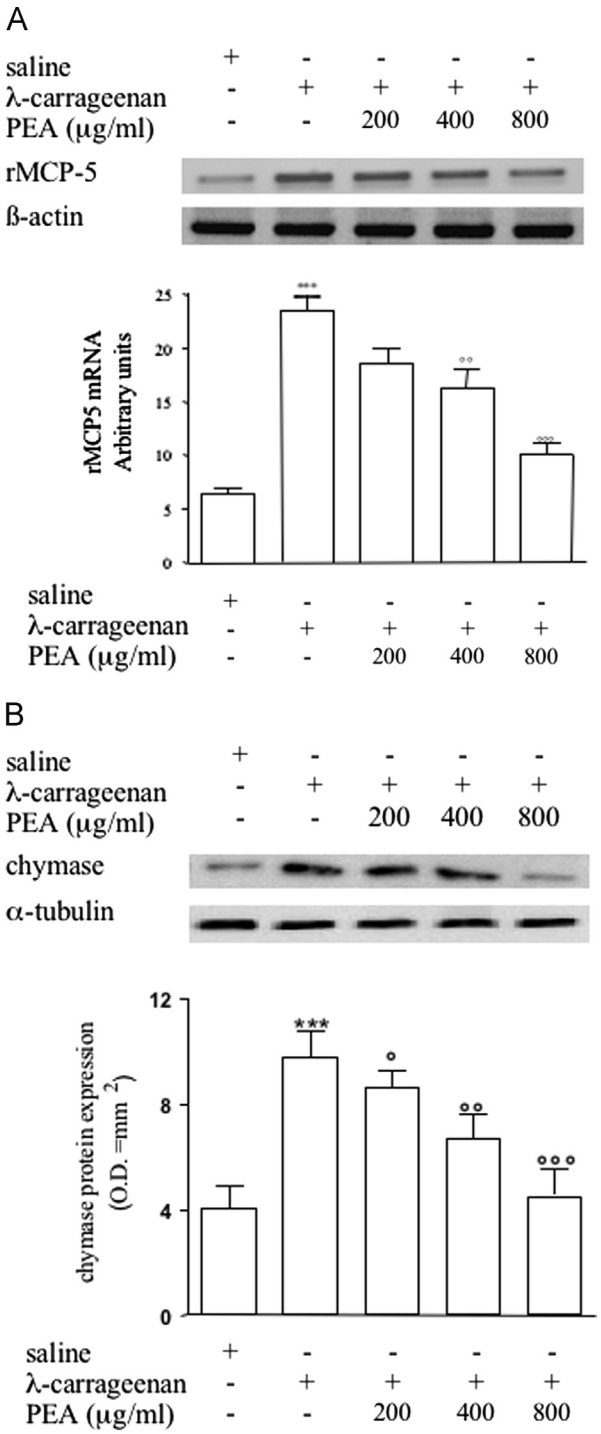

Fig. 1. (A) Effect of PEA on rMCP-5 transcription induced by λ-carrageenan in granulomatous tissue. Representative Vistra green-stained agarose gel of RT-PCR products, corresponding to rMCP-5mRNA in sponges injected with saline, λ-carrageenan (1% w-v), or λ-carrageenan in the presence of increasing amount of PEA (200, 400, and 800 μg/ml). β-actin, a housekeeping gene, was used as control. (B) Effect of PEA on λ-carrageenan-induced chymase expression in granulomatous tissue. Representative western blot analysis and relative densitometric analysis of chymase levels in sponges injected with saline, λ-carrageenan (1% w-v), or λ-carrageenan in the presence of increasing amount of PEA (200, 400, and 800 μg/ml). Tubulin expression is shown as control. Quantification of results is expressed as mean ± S.E.M. of three experiments. ***$P<0.001$ vs. saline.; °$P<0.05$, °°$P<0.01$, and °°°$P<0.001$ vs. λ-carrageenan alone.