PALMITOYLETHANOLAMIDE COUNTERACTS NEUROGENIC INFLAMMATION *IN VITRO* BY STIMULATING 2-ARACHIDONOYL-GLYCEROL BIOSYNTHESIS AND SYNERGISING WITH ENDOCANNABINOID SIGNALING AT THE CANNABINOID RECEPTOR TYPE-2

Stefania Petrosino[1], Aniello Schiano Moriello[1], Roberta Verde[2], Marco Allarà[1], Roberta Imperatore[2], Alessia Ligresti[2], Ali Mokhtar Mahmoud[2], Alessio Filippo Peritore[2], Fabio Arturo Iannotti[2], Vincenzo Di Marzo[3]

[1]Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA), ITA; Epitech Group SpA, Saccolongo (PD), ITA, Pozzuoli (NA) - Italy, [2]Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA), ITA, Pozzuoli (NA) - Italy, [3]Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA), ITA; Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health, CRIUCPQ and INAF, Faculties of Medicine and Agriculture and Food Sciences, Université Laval, Quebéc City, Canada, Pozzuoli (NA) - Italy

Introduction: Palmitoylethanolamide (PEA) is a pleiotropic endogenous lipid mediator used as a "dietary food for special medical purposes" against neuropathic pain and neuro-inflammatory conditions. Several mechanisms of actions underlie PEA effects, among which the "entourage" effect, consisting in PEA potentiation of endocannabinoid signaling at either cannabinoid receptors or transient receptor potential vanilloid type-1(TRPV1) channels. Here we report novel molecular mechanisms through which PEA controls mast cell degranulation and substance P (SP)-induced histamine release in a mast cell model.

Materials and methods: Rat basophilic leukemia (RBL-2H3) cells stimulated with SP were treated with PEA in the presence and absence of cannabinoid type-2(CB2) receptor antagonists (SR144528, AM630), or a diacylglycerol lipase (DAGL) enzyme inhibitor (OMDM188) to inhibit the biosynthesis of 2-arachidonoyl-glycerol (2-AG). Release of histamine was measured by ELISA and b-hexosaminidase release and toluidine blue staining were used as indices of degranulation. 2-AG levels were measured by LC-MS. The mRNA expression of proposed PEA targets, and of PEA and endocannabinoid biosynthetic and catabolic enzymes was also measured. Binding affinity and functional activity at the CB2receptors of 2-AG were assessed in the presence of PEA in in vitro cell systems over-expressing the human recombinant CB2receptor. PEA effects on the activity of DAGL-a or -b enzymes were assessed in COS-7cells over-expressing the human recombinant enzyme or in RBL-2H3cells, respectively.

Results: SP increased the number of degranulated RBL-2H3cells and triggered the subsequent release of b-hexosaminidase and histamine. PEA inhibited these effects in a manner antagonized by SR144528and AM630. PEA concomitantly increased the levels of 2-AG in SP-stimulated RBL-2H3cells, and this effect was reversed by OMDM188. PEA did not modulate the mRNA expression of any of the investigated target genes, slightly enhanced the functional activity at CB2receptors of nM concentrations of 2-AG, and significantly stimulated DAGL-a and -b activity and, consequently, 2-AG biosynthesis. Co-treatment with PEA and 2-AG at per se ineffective concentrations inhibited SP-induced release of histamine and degranulation, and these effects were reversed by OMDM188.

Discussion and conclusion: Activation of CB2underlies the inhibitory effects on SP-induced RBL-2H3cell degranulation by PEA alone or of subeffective concentrations of PEA and 2-AG. We demonstrate for the first time that these effects of PEA, which is per se inactive at CB2, are due to stimulation of 2-AG biosynthesis by DAGL and enhancement of endocannabinoid signaling at CB2receptors.