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2-pentadecyl-2-oxazoline: Identification in coffee, synthesis and activity in a rat model of carrageenan-induced hindpaw inflammation

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ABSTRACT

N-acylethanolamines (NAEs) comprise a family of bioactive lipid molecules present in animal and plant tissues, with *N*-palmitoylethanolamine (PEA) having received much attention owing to its anti-inflammatory, analgesic and neuroprotective activities.

2-Pentadecyl-2-oxazoline (PEA-OXA), the oxazoline of PEA, reportedly modulates activity of *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), which catabolizes PEA. Because PEA is produced on demand and exerts pleiotropic effects on non-neuronal cells implicated in neuroinflammation, modulating the specific amidases for NAEs (NAAA in particular) could be a way to preserve PEA role in maintaining cellular homeostasis through its rapid on-demand synthesis and equally rapid degradation. This study provides the first description of PEA-OXA in both green and roasted coffee beans and Moka infusions, and its synthesis. In an established model of carrageenan (CAR)-induced rat paw inflammation, PEA-OXA was orally active in limiting histological damage and thermal hyperalgesia 6 h after CAR intraplantar injection in the right hindpaw and the accumulation of infiltrating inflammatory cells. PEA-OXA appeared to be more potent compared to ultramicronized PEA given orally at the same dose (10 mg/kg). PEA-OXA markedly reduced also the increase in hindpaw myeloperoxidase activity, an index of polymorphonuclear cell accumulation in inflammatory tissues. NAAA modulators like PEA-OXA may serve to maximize availability of NAEs (e.g. PEA) while providing for recycling of the NAE components for further resynthesis.

1. Introduction

Inflammation is widely recognized as a key element across a spectrum of neuropathological conditions, including chronic pain and neuropathic pain, neurodegenerative diseases, stroke, spinal cord injury, and neuropsychiatric disorders [1–5]. Few would dispute that non-resolving inflammation is one of the principal contributors to the medical burden in industrialized societies. It is thus not surprising that a great deal of effort is being directed to identifying effective pharmacological strategies to deal with neuroinflammation [6,7].

Tissue damage, stress and their associated inflammatory response may trigger an endogenous program of resolution that encompasses the production of lipid mediators with the capacity to switch off inflammation and restore a homeostatic balance [8,9]. A number of molecules have been identified which take part in these protective mechanisms. Among these are the *N*-acylethanolamines (NAEs), a class of naturally occurring lipid signaling molecules composed of a fatty acid and ethanolamine—the so-called fatty acid ethanolamides (FAEs). The main family members are the endocannabinoid *N*-arachidonoylethanolamine (anandamide), together with its congeners *N*-stearoylethanolamine, *N*-oleoylethanolamine, and *N*-palmitoylethanolamine (PEA) (chemical name: *N*-(2-hydroxyethyl)hexadecanamide). That PEA plays a role in maintaining cellular homeostasis by acting as mediator of resolution of inflammatory processes draws support from a number of studies [10,11]. These past years have witnessed a continually growing number of studies confirming the anti-neuroinflammatory and neuroprotective actions of PEA [12–15]. Modulating responses induced by inflammatory stimuli can also be achieved by

Abbreviations: CAR, carrageenan; NAAAN, -acylethanolamine-hydrolysing acid amidase; NAE, N-acylethanolamines; PEA, *N*-palmitoylethanolamine; PEA-OXA, pentadecyl-2-oxazoline.

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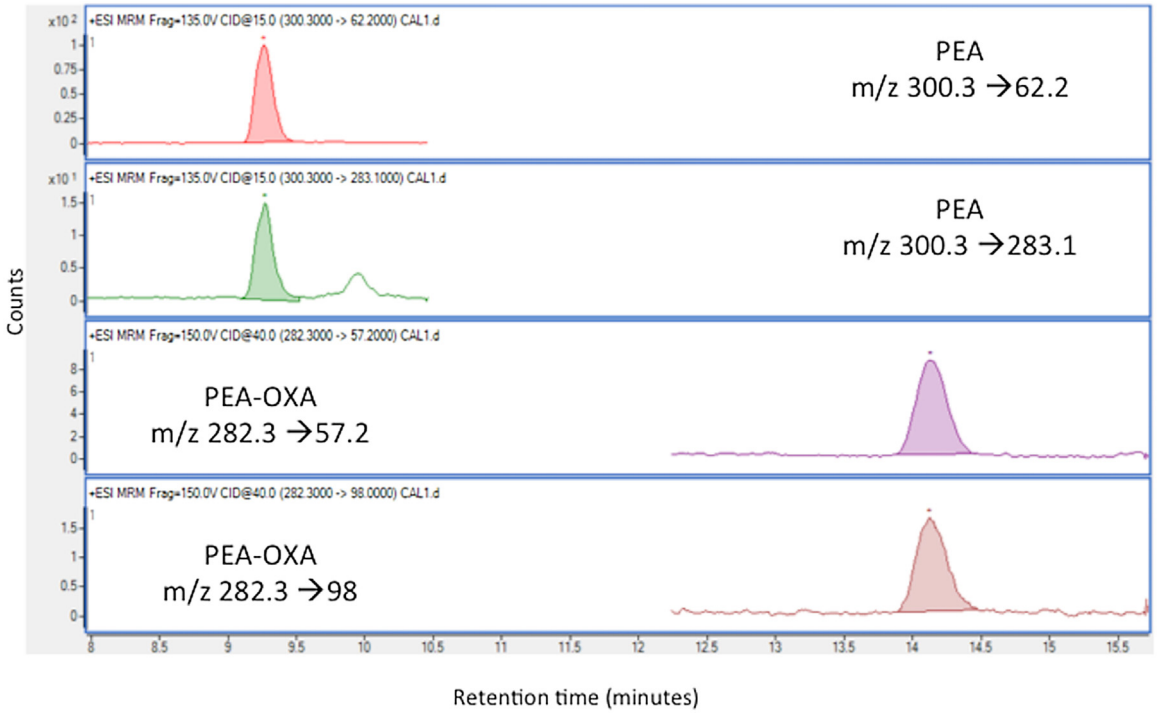

Fig. 1. Chromatographic separation of PEA and PEA-OXA (0.001 μg/ml each) and Multiple Reaction Monitoring. See Section 2 for further details.

increasing endogenous PEA levels through inhibition of its degradation, targeting either fatty acid amide hydrolase [16] or its principal catabolic enzyme, *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) [16–20]. Paradoxically, genetic or pharmacological manipulation of PEA catabolism may result in some instances lead to undesirable effects [21–24].

Because PEA is produced on demand rather than being constitutive, its ability to exert a regulatory effect on non-neuronal cells (e.g. glia and mast cells) implicated in neuroinflammation [25,26] would necessitate that PEA pleiotropic effects [11] be tightly controlled by a mechanism allowing for inactivation. One might thus suppose that NAE catabolic enzymes (NAAA in particular) are intended to modulate substrate availability. It has been proposed that pharmacologically modulating—and not blocking—the specific amidases for *N*-acylamides (in particular NAAA) could be a way to preserve PEA role in maintaining cellular homeostasis through its rapid on-demand synthesis and equally rapid degradation [27]. Recent studies described the pharmacological modulation of NAAA with the oxazoline of PEA (2-pentadecyl-2-oxazoline or PEA-OXA) [28]. The present study was designed to identify natural plant sources of PEA-OXA, describe its synthesis, and therapeutic application in a model of carrageenan (CAR)-induced inflammation in the rat paw, which represents a classical paradigm of edema formation and hyperalgesia [29] that has been extensively used in the development of anti-inflammatory drugs.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich Company Ltd (Poole, Dorset, U.K.). All other chemicals were of the highest commercial grade available. Ultramicronized PEA was kindly provided by Epitech SpA, Saccolongo, Italy. All stock solutions were prepared in non-pyrogenic saline (0.9% NaCl; Baxter, Biogenerica srl, Catania, Italy).

2.2. Identification of PEA and PEA-OXA in green and roasted coffee beans and moka infusions by LC/MS/MS

Coffee samples were obtained from DIEMME SpA (Albignasego, Italy). Roasted seeds were obtained by heating at 180 °C for 20 min. A common coffee grinder was employed to turn seeds in powder. Powders obtained from green and roasted coffee were extracted with dichloromethane while Moka infusion solutions were extracted with ethylacetate. Solvent extracts were then evaporated to dryness. The sample residues were re-suspended in 1 ml of acetonitrile and analysed without further purification. The present chromatographic method was adapted from Gouveia-Figueira and Nording [30], while maintaining the same mobile phases but modifying the gradient. Phase A, water; phase B, methanol added to 10 mM ammonium acetate. The gradient was: 0–5 min, 75% B; 5–6.5 min, 6.5–19 90% B; 19–19.1 min, 75% B, flow rate, 0.25 ml/min. The acquisition of MS/MS occurred prior optimization of collision energies for each analyte with infusion technique. The best source conditions [ESI+] model Jet Stream were: gas temp 140 °C, gas flow 8 l/min, nebulizer 20 psi, sheath gas temperature 375 °C, sheath gas flow 12 l/min.

The matrix effect was determined using the PEA-corresponding and ^{13}C-labeled compound EPT2110/1 (for dilutions ≥10, matrix effect 100–116%). A dilution of 1:10 in acetonitrile for all experiments was chosen as the best compromise so as not to lose information on the peak of PEA-OXA, which is present in lower concentrations than PEA. Extraction recovery was calculated using the structurally related compound EPT2110/19 (*N*-2-(hydroxyethyl)nonadecanamide) at 2.7 and 0.27 μg/ml. The final product recovery was 72%.

Intraday repeatability for PEA and PEA-OXA was calculated across the concentration range 0.01–0.1–1–10–50–100 μg/ml. The limits of detection were calculated using standard solutions of 0.12 and 0.72 ng/ml for PEA and PEA-OXA, respectively (defined statistically based on 7 repeats and Student's *t*-test, confidence interval 0.01). The extended calibration was tested in the range 0.005–100 μg/ml; this concentration range was reduced the case of