# INVESTIGATION OF ANTI-INFLAMMATORY EFFECTS OF PALMITOYLETHANOLAMIDE (PEA)

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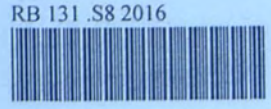





Lihat Sebelah

# Investigation of Anti-inflammatory Effects of Palmitoylethanolamide (PEA)

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## Abstract

Inflammation is a common feature of many pathological processes within the body. Although commonly perceived as being detrimental to the health of the organism, the inflammatory process is essential in the repair of damaged tissue. Following either tissue damage, infection or in some disease processes, the affected area, for example skin, swells, becomes hypersensitive to heat and pressure. These all reflect the infiltration of immune cells to the site of injury or infection from the blood stream. Activation of Toll-like receptor 4 (TLR4), which is expressed by both tissue resident and circulating immune cells, as well as neurons, initiates a well described intracellular signalling cascade that initiates inflammation. Ligands for this receptor include lipopolysaccharides (LPS-archetypally bacterial cell wall) as well experimental inflammogens such as carrageenan. Activation of TLR4 leads to increased expression of pro-inflammatory molecules (typically cytokines and chemokines as well as other molecules) that are secreted by TLR4 expressing cells to promote inflammation and which also sensitise primary afferent nociceptors leading to pain. Increased tissue levels of these pro-inflammatory molecules act to promote the infiltration of circulating neutrophils and monocytes to the site of injury that in turn release further pro-inflammatory mediators increasing plasma and cell recruitment (swelling) and hyperalgesia (pain). The fatty acid amide *N*-Palmitoylethanolamide (PEA) is an endogenous ligand of the peroxisome proliferator activated receptor alpha (PPARα). Activation of this receptor has analgesic and anti-inflammatory properties. The aim of this thesis was to investigate the downstream consequences of PPARα activation and how this lead to modulation of the inflammatory processes. Intra plantar subcutaneous (s.c) injection of 2% (v/v) λ-carrageenan (100 µl) or saline control into the rat hind paw (Sprague Dawley, male, 200-225 g) significantly altered hind-limb weight bearing and increased paw volume consistent with hyperalgesia and inflammation. Pre-treatment with PEA (50µg/50µl, s.c) 30 minutes pre-carrageenan significantly delayed the onset of hyperalgesia, but not

increases in paw volume, for 2 hours. Post mortem plantar skin samples were collected 2 hours after the carrageenan injection and mRNA from these was purified. mRNA for 83 genes associated with inflammation and pain were quantified using Taqman Low Density Arrays (TLDA). Carrageenan significantly up-regulated many pro-inflammatory genes. PEA was able to inhibit expression of some but not all of these.

Infiltration of immune cells to the site of damage is a characteristic of inflammation. In skin samples, I assessed the number and activation state of monocytes/macrophages and neutrophils using immunofluorescence based histology. PEA significantly inhibited the infiltration of monocytes into the skin but had no effect upon neutrophil numbers. The ability of PEA to modulate the migration of cultured neutrophil-like cells (HL60) and macrophage-like cells (U937) were also investigated. PEA significantly inhibited monocyte/macrophage-like cell migration, but had no effect upon neutrophil-like cells. In cultured primary cells, PEA and synthetic PPARα agonist GW7647 significantly inhibited the LPS-stimulated differentiation of monocytes into macrophages which subsequently led to the selective inhibition of the secretion of specific chemokine that are known to promote the recruitment of monocytes/macrophages but not neutrophils. These effects could be blocked by the PPARα antagonist GW6471, thus confirming the involvement of PPARα. Classically inflammation, leads to the activation of cells to enter an inflammatory phenotype. The effect of PEA on TLR4-mediated pro-inflammatory gene expression and NF-ƙB/Rel expression in monocytes/macrophages were determined using Taqman qRT-PCR. NF-ƙB/Rel isomer expressions were all significantly inhibited by PEA following TLR4 activation, whereas in vivo only some were reversed. This suggests that other signalling pathways must be involved in the differential regulation of pro-inflammatory gene expression mediated by PEA. This thesis provides a new understanding of the mechanism by which PEA differentially modulates TLR4-mediated signalling pathways and consequent inflammatory responses.