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# Central administration of palmitoylethanolamide reduces hyperalgesia in mice via inhibition of NF-κB nuclear signalling in dorsal root ganglia

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ABSTRACT

Despite the clear roles played by peroxisome proliferators-activated receptor α (PPAR-α) in lipid metabolism, inflammation and feeding, the effects of its activation in the central nervous system (CNS) are largely unknown. Palmitoylethanolamide (PEA), a member of the fatty-acid ethanolamide family, acts peripherally as an endogenous PPAR-α agonist, exerting analgesic and anti-inflammatory effects. Both PPAR-α and PEA are present in the CNS, but the specific functions of this lipid and its receptor remain to be clarified. Using the carrageenan-induced paw model of hyperalgesia in mice, we report here that intracerebroventricular administration of PEA (0.1–1 μg) 30 min before carrageenan injection markedly reduced mechanical hyperalgesia up to 24 h following inflammatory insult. This effect was mimicked by GW7647 (1 μg), a synthetic PPAR-α agonist. The obligatory role of PPAR-α in mediating PEA's actions was confirmed by the lack of anti-hyperalgesic effects in mutant mice lacking PPAR-α. PEA significantly reduced the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in sciatic nerves and restored carrageenan–induced reductions of PPAR-α in the L4-L6 dorsal root ganglia (DRG). To investigate the mechanism by which PEA attenuated hyperalgesia, we evaluated inhibitory kB-α (IkB-α) degradation and p65 nuclear factor kB (NF-κB) activation in DRG. PEA prevented IkB-α degradation and p65 NF-κB nuclear translocation, confirming the involvement of this transcriptional factor in the control of peripheral hyperalgesia. These results add further support to the broad-spectrum of biological and pharmacological effects induced by PPAR-α agonists, suggesting a centrally mediated component for these drugs in controlling inflammatory pain.



## 1. Introduction

Peroxisome proliferators-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three different isotypes have been described, all of which display distinct physiological functions dependent on their differential ligand activation profiles and tissue distribution (Kliewer et al., 1994; Forman et al., 1996). Both PPAR-α and PPAR-gamma receptor subtypes regulate in *vivo* and in *vitro* inflammatory responses (Devchand et al., 1996; Delerive et al., 2001; Kostadinova et al., 2005). Multiple classes of lipids have been identified as ligands for PPARs (Forman et al., 1996). Among these are fatty acid ethanolamides, a class of lipid mediators that includes endogenous ligands for PPAR-α (Fu et al., 2003; LoVerme et al., 2005, 2006; O'Sullivan, 2007).

Palmitoylethanolamide (PEA), the amide of palmitic acid and ethanolamine, is one such compound that has been proposed as an endogenous agonist for PPAR-α. PEA has been widely studied for its analgesic (Calignano et al., 1998, 2001; Jaggar et al., 1998) and anti-inflammatory effects (Mazzari et al., 1996; Berdyshev et al., 1998; Costa et al., 2002; D'Agostino et al., 2007). Both of these properties have been shown to be dependent on PPAR-α expression, and PEA activates this nuclear receptor with a potency comparable to the synthetic agonist WY14,643 (LoVerme et al., 2005, 2006). Indeed, in both the carrageenan-induced paw edema and phorbol ester-induced ear edema models, PEA attenuates inflammation in wild-type mice, but has no effect in mice lacking PPAR-α (PPAR-α$^{-/-}$; LoVerme et al., 2005). Moreover PPAR-α agonists, including PEA, suppress pain behaviours induced in mice by chemical tissue injury, nerve damage, or inflammation (LoVerme et al., 2006; Russo et al., 2007).

Both PPAR-α (Benani et al., 2004; Moreno et al., 2004; Cimini et al., 2005) and PEA (Cadas et al., 1997) are found in the central nervous

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system (CNS) and may be modulated during pathological states, such as neuropathic pain (Petrosino et al., 2007). However, the endogenous roles of this lipid amide in the CNS remain unknown.

We have recently identified a peripheral anti-inflammatory action of PPAR-α agonists, which occurs following intracerebroventricular (i.c.v) administration of these agents (D'Agostino et al., 2007). The reduction of paw inflammation produced by i.c.v. PEA was likely mediated by a spinal mechanism, which involved the modulation of expression of PPAR-α and pro-inflammatory enzymes (D'Agostino et al., 2007). The aim of the present study was to further explore the role for central PPAR-α receptors in controlling peripheral hyperalgesia, and to evaluate the mechanism by which the peripheral anti-hyperalgesic effects of i.c.v. administered PEA might occur.

## 2. Materials and methods

### 2.1. Animals

Male Swiss mice weighing 20–25 g were purchased from Harlan (Italy) and housed in stainless steel cages in a room kept at 22 ± 1 °C on a 12:12 h light–dark cycle. The animals were acclimated to their environment for one week and had *ad libitum* access to tap water and standard rodent chow. Mice (4–5 weeks old, 20–22 g) with a targeted disruption of the *PPAR-α* gene and their wild-type littermate controls (PPAR-α WT) were purchased from Jackson Laboratories (Harlan Nossan, Italy). Mice homozygous for the Ppara$^{tnjGonz}$ targeted mutation mice are viable, fertile and appear normal in appearance and behaviour.

Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

### 2.2. Intracerebroventricular and spinal injections and carrageenan challenge

Groups of at least six mice were briefly anesthetized with enflurane. PEA, GW7647 (chemical name: 2-[[4-[2-[[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]-phenyl]thio]-2-methylpropanoic acid; Tocris, Bristol, UK), or vehicle (10% polyethylene glycol and 5% TWEEN 80 in sterile distilled water), were administered i.c.v. in a volume of 2 µl/mouse. Drug solutions were freshly prepared immediately before injection. We used a 25-µl glass Hamilton syringe (Reno, NV) with a stainless steel needle modified with a shaft to control the depth of injection to 2 mm. The injection was made into the lateral ventricle 2 mm caudal and 2 mm lateral from the bregma, 30 min before paw carrageenan challenge. Mice received subplantar injections of 50 µl of 1% λ-carrageenan in sterile saline (Sigma-Aldrich, Milano, Italy) or vehicle into the right hind paw.

Spinal administration were made as described by Fairbanks (2003). Briefly, a soft cloth was placed over the head and upper body of the animal to keep it calm. The skin above the ileac crest was tautly pulled to create a horizontal plane where the needle was inserted. After contact with the bone of the spinal column, the needle/syringe angle was reduced and the needle gently advanced between the vertebrae. Puncture of the *dura* was indicated by a marked tail flick.

The injection volume was reduced to 3 µl instead of the most common injection volume of 5 µl, to avoid a possible redistribution to the basal cistern of the brain. The injection was done over the course of 1–2 s.

### 2.3. Behavioral tests

Pain withdrawal thresholds to mechanical stimuli were measured on both the ipsilateral paw (treated with carrageenan) and contralateral paw (untreated) 30 min and 2, 4, 6, 24, 48, 72 h after intraplantar carrageenan injection. As previously described (LoVerme et al., 2006), mechanical hyperalgesia was assessed by measuring the latency in *s* to withdraw the paw away from a constant mechanical pressure exerted onto its dorsal surface. A 15 g calibrated glass cylindrical rod (diameter = 10 mm) chamfered to a conical point (diameter = 3 mm) was used to exert the mechanical force. The weight was suspended vertically between two rings attached to a stand and was free to move vertically. A cut-off time of 180 s was used.

#### 2.3.1. Rotarod test

The integrity of motor function was assessed in treated mice using an accelerating rotarod (Ugo Basile, Italy). The animals were acclimated to acceleration in 3 training runs. Mean performance time (*s*) determined on the fourth and fifth run served as control value. Performance time was measured the day before i.c.v. injection and at all the time point observed for hyperalgesia evaluation.

### 2.4. Preparation of nuclear and cytosolic protein extracts from dorsal root ganglia (DRG)

Lumbar (L)4-L6 DRG tissues were collected 5 h after carrageenan injection and suspended in extraction buffer (0.32 M sucrose, 10 mM TRIS–HCl pH 7.4, 1 mM ethyleneglycol-bis(β-aminoethyl)-N,N,N',N'-tetraacetic acid [EGTA], 2 mM ethylenediaminetetraacetic acid [EDTA], 5 mM NaN3, 10 mM 2-mercaptoethanol, 50 mM NaF, 0.2 mM phenylmethylsulphonylfluoride [PMSF], 0,15 µM pepstatin A, 20 µM leupeptin, and 1 mM sodium orthovanadate) and homogenized at the highest setting for 2 min in a Polytron PT 3000 tissue homogenizer (Kinematica, Littau-Lucerne, Switzerland). The homogenates were chilled on ice for 15 min and then centrifuged at 1000 ×*g* for 10 min at 4 °C. The supernatant, containing the cytosolic fraction, was processed for Ik-B-α measurement. The pellets were suspended in the supplied complete lysis buffer containing 1% Triton X-100, 150 mM NaCl, 10 mM Tris–HCl pH 7.4, 1 mM EGTA, 1 mM EDTA, 0,2 mM PMSF, 20 µM, 0,2 mM sodium orthovanadate and then centrifuged, 30 min at 15,000 ×*g* at 4 °C to yield the nuclear fraction for the NF-κB p65 level determination.

### 2.5. Preparation of total tissue protein extracts from DRG and sciatic nerve

L4-L6 DRG and sciatic nerve were obtained from each animal 6 h after carrageenan or saline injection, and were homogenized on ice in lysis buffer (10 mM Tris–HCl, 20 mM pH 7.5, 10 mM NaF, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulphonyl fluoride, 1 mM Na3VO4, leupeptin and trypsin inhibitor 10 µg/ml; 0.25 ml/50 mg tissue). After 1 h, tissue lysates were centrifuged at 100,000 ×*g* for 15 min at 4 °C, and the supernatant was stored at −80° until use.

### 2.6. Western blot analyses

Protein content was measured using bovine serum albumin (Sigma Aldrich, Milan, Italy) as a standard. Total extract was used for cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and PPAR-α detection, while cytosolic or nuclear extracts were used for inhibitory kB-α (IkB-α) and nuclear factor kB (NF-κB) respectively. Protein extracts containing equal amount of protein were separated on sodium dodecyl sulfate-polyacrylamide minigels and transferred onto nitrocellulose membranes (Protran Nitrocellulose Transfer Membrane Schleicher & Schuell Bioscience, Dassel, Germany), blocked with phosphate-buffered saline (PBS) containing 5% nonfat dried milk for 45 min at room temperature, and incubated at 4 °C, overnight in the presence of commercial antibodies in PBS containing 5% nonfat dried milk, and 0.1% Tween 20: iNOS (dilution 1:2000; BD Biosciences Transduction Laboratories, Lexington, KY); COX-2 (dilution 1:1500; Cayman Chemical, Ann Arbor, MI); PPAR-α (dilution 1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA); IκB-α (dilution 1:1000, Santa Cruz

Biotechnology); NF-κB p65 (dilution 1:500; Santa Cruz Biotechnology, Inc.).

Secondary antibodies (anti-mouse IgG, or anti-rabbit IgG, or anti-goat peroxidase conjugate) were incubated for 1 h at room temperature. Blots were washed with PBS, developed using enhanced chemiluminescence detection reagents (Amersham Pharmacia, Piscataway, NJ) following manufacturer's instructions, and exposed to X-Omat film (Eastman Kodak Co., Rochester, NY). Protein bands for iNOS (~130 kDa), COX-2 (~72 kDa), PPAR-α (~55 kDa), IκB-α (~37 kDa) and NF-κB p65 (~65 kDa), were quantified using a model GS-700 imaging densitometer (Bio-Rad Laboratories, Hercules, CA). α-tubulin or β-actin proteins (dilution 1:1000 and 1:20000 respectively; Sigma-Aldrich) were performed to ensure equal sample loading.

### 2.7. Statistical analyses

Results are expressed as the mean ± S.E.M. of *n* experimental replicates. All analyses were conducted using GraphPad Prism (GraphPad Software Inc., San Diego, CA). The significance of differences between groups was determined by one–way (for *ex vivo* experiments) and two-way (for *in vivo* experiments) analyses of variance (ANOVA) followed by Bonferroni post hoc tests for multiple comparisons.

## 3. Results

### 3.1. Effects of central PPAR-α activation on carrageenan-induced hyperalgesia

As expected, carrageenan injection into the mice paw elicited significant paw hyperalgesia as determined by a reduction in withdrawal latencies (expressed in *s*) following mechanical stimulation. This effect was evident up to 72 h after carrageenan challenge. PEA treatment (0.01, 0.1, and 1 μg i.c.v.), 30 min before carrageenan injection, markedly reduced mechanical hyperalgesia in a time-dependent manner, as shown by the increase in paw withdrawal latency (Fig. 1). During the first 4 h, all doses tested, but not vehicle alone, induced a significant increase in paw withdrawal latency ($P<0.05$ or $P<0.001$ vs carrageenan). At 6 h, the lowest dose of PEA did not produce an appreciable effect, while the doses of 0.1 and 1 μg were effective ($P<0.05$ and $P<0.001$ vs carrageenan, respectively). At the highest dose, PEA's anti-hyperalgesic effects continued for up to 24 h following carrageenan injection ($P<0.001$).

We next tested the effects of the synthetic PPAR-α agonist, GW7647 on mechanical hyperalgesia. Similarly to PEA, administration of GW7647 (1 μg/2 μl i.c.v. 30 min before carrageenan; Fig. 2B) produced a significant increase in paw withdrawal latency ($P<0.001$) up to 24 hs following carrageenan injection(Fig. 2A).

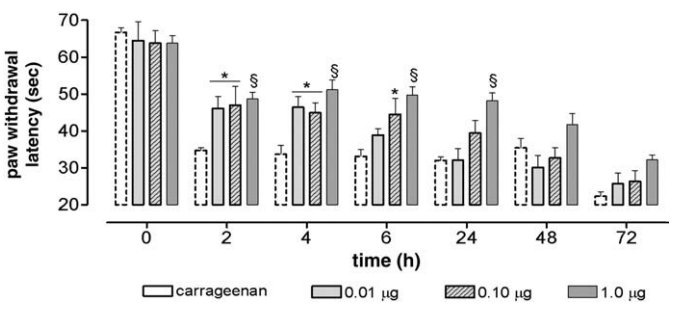


**Fig. 1.** Effect of a single 30-min pre-treatment of PEA (0.01, 0.1 and 1 μg/2 μl i.c.v.) on carrageenan-induced hyperalgesia in mice paws evaluated at 2, 4, 6, 24, 48, and 72 h after the carrageenan challenge. Paw withdrawal latencies to the force exerted on the injured paws is expressed as *s*. Data are expressed as mean ± S.E.M. of at least 6 animals for each group. *$P<0.05$ and §$P<0.001$, *vs* carrageenan group.

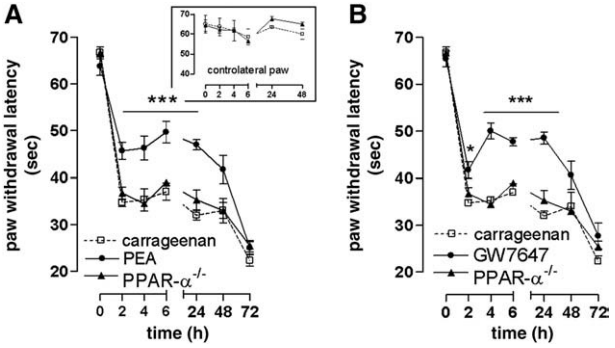


**Fig. 2.** Effect of a single treatment of PEA (panel A) or GW7647 (panel B) on carrageenan-induced hyperalgesia in mouse paws evaluated at 2, 4, 6, 24, 48, and 72 h after challenge. PEA, GW7647 (1 μg/2 μl i.c.v.), or vehicle were administered 30 min before carrageenan. Open square shows wild type animals receiving i.c.v. vehicle and i.pl. carrageenan; filled circle wild type animals receiving i.c.v. PEA (panel A) or GW7647 (panel B) and i.pl. carrageenan; filled triangle PPAR-$\alpha^{-/-}$ mice receiving i.c.v. PEA (panel A) or GW7647 (panel B) and i.pl. carrageenan. The insert in panel A shows no effect of PEA on withdrawal latency of uninflamed (contralateral) paw. Data are expressed as mean ± S.E.M. of at least 6 animals for each group. *$P<0.05$ and ***$P<0.001$ *vs* carrageenan group.

To demonstrate the obligatory role of PPAR-α in the central antihyperalgesic effect of PEA, in another set of experiments PEA or GW7647 (1 μg/2 μl i.c.v. 30 min before carrageenan) were administered (i.c.v.) to wild type or PPAR-$\alpha^{-/-}$ mice. As expected, both PEA and GW7647 did not modify hyperalgesia development in PPAR-$\alpha^{-/-}$ (▲, Fig. 2A and B), but retained their effects in wild-type animals.

To verify the specificity and selectivity of PEA's effects on mechanical hyperalgesia, we evaluated paw withdrawal latencies in the contralateral paws of ipsilaterally treated animals. As shown by the insert in Fig. 2A, i.c.v. administration of PEA at the highest dose used in this study (1 μg) did not significantly modify withdrawal latencies in contralateral paws.

Although favourable trend might be revealed at 1 and 2 h after carrageenan, the highest dose used for i.c.v. administration (1 ug) did not reduce carrageenan-induced hyperalgesia in a significant manner when spinally injected (data not shown). We used a 3 uL volume to avoid a possible redistribution of PEA at supraspinal level.

### 3.2. Effects of i.c.v administration of PEA or GW7647 on motor coordination

To evaluate possible sedative or muscle relaxant effects of i.c.v. administration of PEA (1 μg) or GW7647 (1 μg), we treated mice with either drug and tested the animals' performance on a rotarod apparatus. Neither PEA nor GW7647, given 30 min prior to testing, affected motor responses in mice (performance time are expressed in *s*, cut-off at 60 s: vehicle 60 ± 0; PEA 60 ± 2; GW7647 58 ± 3).

### 3.3. Effect of central administration of PEA on PPAR-α levels in DRG

Intraplantar injection of carrageenan significantly reduced levels of immunoreactive PPAR-α, as determined by Western blot and densitometric analysis, in the DRG of i.c.v. vehicle-treated animals 6 h after injection ($^{\#\#\#}P<0.001$ vs control group). A single i.c.v. administration of PEA (1 μg) 30 min before carrageenan injection significantly reverted PPAR-α down-regulation induced by carrageenan (*$P<0.05$ vs carrageenan) (Fig. 3).

PPAR-$\alpha^{-/-}$ mice did not express PPAR-α protein in DRG (data not shown), as previously reported (LoVerme et al., 2006).

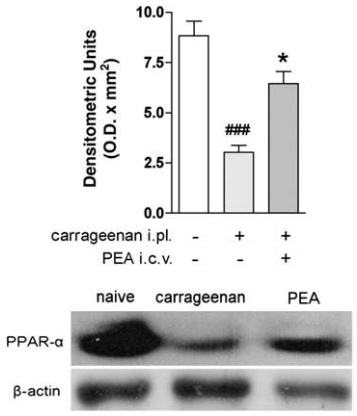


**Fig. 3.** PPAR-α expression in DRG. Western blot analysis of tissue lysates from mice treated with a single administration of PEA (1 μg/2 μl i.c.v.) or vehicle, 30 min before carragenan injection. DRG tissue lysate of mice treated with subplantar saline injections are also reported (control). Data are representative of blots obtained from 6 different animals. The modulation of PPAR-α expression was revealed by densitometric analysis of protein bands and normalized for β-actin. The modulation of PPAR-α expression was revealed by densitometric analysis of protein bands. The animals were sacrificed 6 h after subplantar carrageenan injection. *$P$<0.05 *vs* carrageenan group; ###$P$<0.001 *vs* control group.

### 3.4. PEA inhibits COX-2 and iNOS induction in sciatic nerves

To further characterize the anti-hyperalgesic effects of PEA, we investigated COX-2 and iNOS expression in sciatic nerves 6 h after carrageenan injection (Fig. 4A and B, respectively). This time point was chosen because the acute phase of inflammatory pain (0–6 h) is characterized by a central sensitization primarily mediated by COX-2 over-expression. COX-2 was induced in the sciatic nerve from carrageenan-injected paws of i.c.v. vehicle-treated mice ($^{\#\#\#}P$<0.001 *vs* control group; Fig. 4A). Similarly, sciatic nerve iNOS expression was up-regulated by carrageenan ($^{\#}P$<0.05 *vs* control group; Fig. 4B). A single i.c.v. administration of PEA (1 μg), 30 min before carrageenan, significantly reduced both COX-2 and iNOS expression in the sciatic nerve (*$P$<0.05 *vs* carrageenan group; Fig. 4A and B, respectively).

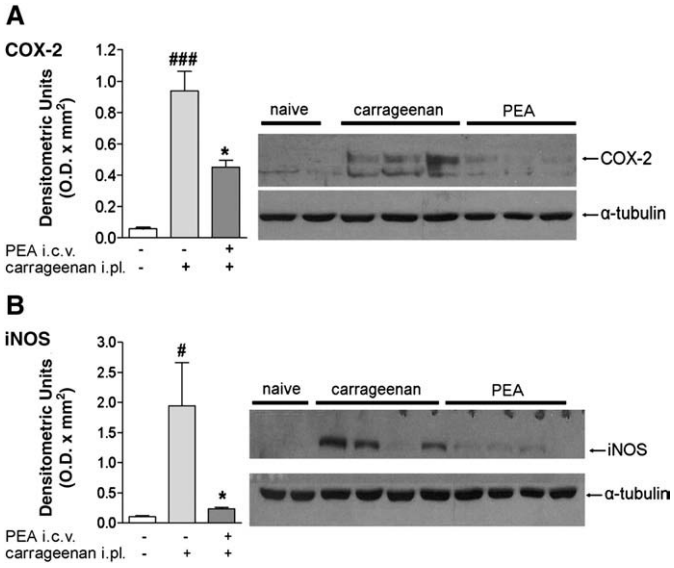


**Fig. 4.** COX-2 (A) and iNOS (B) expression on the sciatic nerve. Western blot analysis of tissue lysates from mice treated with a single administration of PEA (1 μg/2 μl i.c.v.) or vehicle, 30 min before carragenan injection. Sciatic nerve tissue lysate of mice treated with subplantar saline injection is also reported (control). Data are representative of blots obtained from 6 different animals. Equal loading was confirmed by α-tubulin staining. The modulation of COX-2 and iNOS expression was revealed by densitometric analysis of protein bands. The animals were sacrificed 6 h after subplantar carrageenan injection. *$P$<0.05 *vs* carrageenan group; #$P$<0.05 and ### $P$<0.001 *vs* control group.

### 3.5. NF-κB involvement in PEA's response in sensory DRG

To investigate the mechanism by which an acute i.c.v. administration of PEA might attenuate the development of carrageenan-induced hyperalgesia, we performed Western analyses to measure IkB-α degradation in DRG cytosolic cell fractions and NF-κB p65 nuclear translocation (Fig. 5A and B, respectively). Basal levels of IkB-α were detected in the cytosolic fraction of DRGs from control mice, whereas a carrageenan challenge significantly decreased IkB-α expression compared to the control group ($^{\#\#}P$<0.01 vs control group; Fig. 5A). A single i.c.v. administration of PEA (1 μg), 30 min before carrageenan, significantly prevented IkB-α degradation (*$P$<0.05 vs carrageenan group; Fig. 5A). The translocation of the p65 subunit of NF-κB into the nucleus was strongly increased 5 h after carrageenan injection ($^{\#\#}P$<0.01 vs control group; Fig. 5B), and this effect was significantly reduced by PEA treatment (*$P$<0.05 vs carrageenan group).

## 4. Discussion

Despite the clear physiological roles played by PPAR-α in lipid metabolism and inflammation, the effects of pharmacological activation of these nuclear receptors in the CNS remain unclear. All PPAR subtypes (alpha, beta/delta and gamma) have been found in the CNS, where they exhibit specific patterns of localization (Moreno et al., 2004). Recent evidence strongly suggests that PEA acts as an endogenous PPAR-α agonist (LoVerme et al., 2005, 2006; D'Agostino et al., 2007). On the other hand, nanomolar to micromolar levels of PEA have been found in the CNS (Cadas et al., 1997), but the physiological roles played by PEA in the CNS are still largely undetermined.

PEA activates the nuclear receptor PPAR-α with a potency comparable to the synthetic agonist, WY14,643, exerting its pharmacological effects in wild-type but not in mutant PPAR-$\alpha^{-/-}$ mice (LoVerme et al., 2005). More recently, we have reported a significant centrally-mediated antiinflammatory action of endogenous and synthetic PPAR-α agonists: in carrageenan-induced paw oedema in mice, central administration of PEA or GW7647 reduced paw inflammation and this effect was absent in mutant mice lacking PPAR-α (D'Agostino et al., 2007).Here, we report that a single

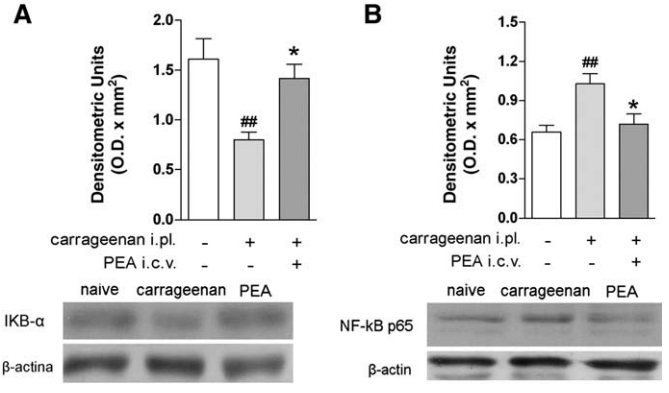


**Fig. 5.** IkB-α (A) and p65NF-κB (B) expression in DRG. Western blot analysis of tissue lysates from mice treated with a single administration of PEA (1 μg/2 μl i.c.v.) or vehicle, 30 min before carragenan injection. DRG tissue lysate of mice treated with subplantar saline injection is also reported (control). The animals were sacrificed 5 h after hyperalgesia induction. Data are representative of blots obtained from 6 different animals. The modulation of cytosolic IkB-α and nuclear p65 NF-κB expression was revealed by densitometric analysis of protein bands. Equal loading was confirmed by α-tubulin staining. #$P$<0.05 and ##$P$<0.01 *vs* control group, *$P$<0.05 and **$P$<0.01 *vs* carrageenan group.

centrally administered dose of PEA markedly reduced mechanical hyperalgesia after intraplantar carrageenan challenge. Moreover, PEA produced a significant reduction in hyperalgesia in the first h after the challenge and this effect lasted up to 24 h. The anti-hyperalgesic actions of central PPAR-α activation by PEA are highlighted by the similar effect of GW7647, a synthetic PPAR-α selective agonist. Moreover, the obligatory role of PPAR-α in mediating the anti-hyperalgesic effect of PEA and GW7647 was confirmed by the failure of these drugs to reduce hyperalgesia in PPAR-$\alpha^{-/-}$ mice.

Our results also show a significant reduction in sensory DRG PPAR-α levels induced by a peripheral noxious stimulus (i.e. carrageenan). In animals treated with centrally administered PEA, the reduced levels of PPAR-α in DRG following carrageenan treatment were restored to basal levels, suggesting that supraspinal administration of PEA likely modulates PPAR-α expression in DRG through a descending pathway. Supporting this possibility, previous experiments have identified the presence of PPAR-α in DRG neurons (LoVerme et al., 2006). The possibility that i.c.v. PEA, administered at the low doses tested in this study, could directly reach the DRG and induce PPAR-α expression is unlikely, since PEA inactivation primarily consists of its intracellular hydrolysis by lipid amidases such as fatty acid amide hydrolase and/or N-acylethanolamine-hydrolyzing acid amidase (Tsuboi et al., 2007). On the other hand, it has been also reported that when injected into the paw, PPAR-α agonists do not enter the CNS, yet strongly suppress nocifensive behaviour by acting at peripheral sites (LoVerme et al., 2006). Moreover, the highest dose used for i.c.v. administration (1ug) does not produce a significant hyperalgesia reduction when spinally administered, indicating a pivotal role for supraspinal regions in our experimental conditions. Taken together, these findings suggest that PPAR-α receptors may serve pivotal roles in regulating pain through ascending/descending pathway in the CNS to control noxious peripheral pain along the main somato-sensory structures (supraspinal< >spinal< >DRG< >nerve). Indeed, in the sciatic nerve we found that the carrageenan-induced over-expression of COX-2 and iNOS were strongly reduced in mice receiving i.c.v. administration of PEA.

During carrageenan-induced inflammation, several pro-inflammatory mediators stimulate the nuclear translocation and DNA-binding activity of NF-κB, causing this nuclear factor to regulate the expression of pro-inflammatory cytokines, factors, and enzymes, such as iNOS and COX-2 (Auphan et al., 1995; George et al., 2000). Thus, we focused our attention on IkB-α degradation (Grilli et al., 1996; Di Donato et al., 1997) and p65 NF-κB nuclear translocation, whose presence in DRG neurons can be reliably quantified (Wood, 1995). Indeed we found that PEA suppressed NF-κB signalling in DRG nuclei, which may explain the mechanisms by which PEA down-regulates COX-2 and iNOS expression in sciatic nerves. We assume that these enzymes are an outcome measure of NF-κB nuclear activity in DRG, because protein levels in nerve fibres are strictly dependent on DNA transcription in DRG cell bodies. It is reasonable to hypothesize a reduction of other pro-inflammatory factors downstream to NF-κB activation, as well as the modulation of enzymatic pathways leading to the phosphorylation of p65 subunit (Genovese et al., 2008). Consistent with our results, a role for NF-κB in hyperalgesia has been also suggested, in different models of inflammatory pain (Tegeder et al., 2004; Igwe, 2005; Ledeboer et al., 2005).

These observations agree with our previous report, in which we found that supraspinal injection of PEA reduced COX-2 and iNOS expression at the spinal level after peripheral inflammation (D'Agostino et al., 2007). Thus, the reduction of PPAR-α expression at several sites in the central (spinal) or peripheral nervous system (DRG) are proposed to be involved in peripheral damage and related hyperalgesia signalling and maintenance.

PPAR-α agonists acting only at CNS sites, are sufficient to prevent changes at spinal and peripheral sites, involving PPAR-α, which lead to central sensitization.

It is noteworthy that the antihyperalgesic effects of PPAR-α agonists, described here, are likely dependent on the modulation of neuronal activity for two additional reasons (i) COX-2 expression in carrageenan treated paw tissue was not modulated by i.c.v. administration of PEA; (ii) the same dose of PEA or GW7647 used for i.c.v. administrations did not modify hyperalgesia development when administered intravenously or intraplantarly (data not shown). In fact, to observe an appreciable antihyperalgesic effect of PEA intraplantarly, doses ten fold higher than those needed for i.c.v. administration, are required for direct injection into the paw (LoVerme et al., 2005, 2006).

In conclusion, our results indicate that intracerebroventricularly administered PEA, an endogenous PPAR-α agonist, or the synthetic PPAR-α agonist GW7647, can inhibit hyperalgesia development in a PPAR-α-dependent-manner. A single supraspinal injection of either drugs leads to a reduction of pro-inflammatory enzyme expression in the sciatic nerve. This effect may be due, at least in part, to a normalization of PPAR-α expression in L4-L6 DRG and an inhibition of NF-κB nuclear translocation. Our results add further support to the broad-spectrum of analgesic and anti-inflammatory effects induced by PPAR-α agonists, providing new evidence supporting a centrally mediated component for the actions of these drugs.

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