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Acute Intracerebroventricular Administration of Palmitoylethanolamide, an Endogenous Peroxisome Proliferator-Activated Receptor- α Agonist, Modulates Carrageenan-Induced Paw Edema in Mice

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ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)- α is a nuclear transcription factor. Although the presence of this receptor in different areas of central nervous system (CNS) has been reported, its role remains unclear. Palmitoylethanolamide (PEA), a member of the fatty-acid ethanolamide family, acts peripherally as an endogenous PPAR- α ligand, exerting analgesic and anti-inflammatory effects. High levels of PEA in the CNS have been found, but the specific function of this lipid remains to be clarified. Using carrageenan-induced paw edema in mice, we show that i.c.v. administration of PEA may control peripheral inflammation through central PPAR- α activation. A single i.c.v. administration of 0.01 to 1 μ g of PEA, 30 min before carrageenan injection, reduced edema formation in the mouse carrageenan test. This effect was mimicked by 0.01 to 1 μ g of GW7647 [2-[[4-[2-[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic

acid], a synthetic PPAR- α agonist. Moreover, central PEA administration significantly reduced the expression of the proinflammatory enzymes cyclooxygenase-2 and inducible nitric-oxide synthase, and it significantly restored carrageenan-induced PPAR- α reduction in the spinal cord. To investigate the mechanism by which i.c.v. PEA attenuated the development of carrageenan-induced paw edema, we evaluated inhibitor κ B- α (I κ B- α) degradation and nuclear factor- κ B (NF- κ B) p65 activation in the cytosolic or nuclear extracts from spinal cord tissue. PEA prevented I κ B- α degradation and NF- κ B nuclear translocation, confirming the involvement of this transcriptional factor in the control of peripheral inflammation. The obligatory role of PPAR- α in mediating the effects of PEA was confirmed by the lack of the compounds anti-inflammatory effects in mutant mice lacking PPAR- α . In conclusion, our data show for the first time that PPAR- α activation in the CNS can control peripheral inflammation.

PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three different isoforms (α , β/δ , and γ) have been described, all of which display

distinct physiological functions dependent on their differential ligand activation profiles and tissue distribution (Kliwer et al., 1994; Forman et al., 1997). Both PPAR- α and PPAR- γ receptor subtypes have been reported to regulate in vivo and in vitro inflammatory responses (Devchand et al., 1996; Dellerie et al., 2001; Kostadinova et al., 2005). The first indication for a role of PPAR- α in modulating inflammation was demonstrated by that the ability of leukotriene B₄, a potent chemotactic inflammatory eicosanoid, to bind PPAR- α and

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ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; NF- κ B, nuclear factor- κ B; CNS, central nervous system; PEA, palmitoylethanolamide; COX, cyclooxygenase; iNOS, inducible nitric-oxide synthase; PBS, phosphate-buffered saline; I κ B- α , inhibitor κ B- α ; Wy-14,643, pirinixic acid; 4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthioacetic acid; GW7647, 2-[[4-[2-[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic acid; GW0742, [4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]thio]-2-methylphenoxy]acetic acid.

induce gene transcription of enzymes involved in ω - and β -oxidation pathways that metabolize leukotriene B₄. Moreover, mutant mice lacking PPAR- α (PPAR- $\alpha^{-/-}$) showed a prolonged inflammatory response when challenged with leukotriene B₄ or arachidonic acid (Devchand et al., 1996). The inhibition of proinflammatory factors, such as interleukin-6 and prostaglandins, also seems to participate in the PPAR- α -mediated control of inflammation, probably through the suppression of NF- κ B activity (Poynter and Daynes, 1998).

It has also been postulated that activation of PPAR- α by nonsteroidal anti-inflammatory agents contributes to the anti-inflammatory, antipyretic, and analgesic properties of these drugs through stimulation of oxidative pathways involved in the catabolism of eicosanoids (Desvergne and Wahli 1999). In the CNS, the expression of PPAR- α has been described in brain (Moreno et al., 2004) and spinal cord (Benani et al., 2004; Moreno et al., 2004); however, the receptor-specific role in these areas is currently unknown.

Previous data suggest that palmitoylethanolamide (PEA) can be considered an endogenous activator of PPAR- α . PEA interacts with this receptor to inhibit inflammatory response with a potency comparable with that of the synthetic PPAR- α agonist GW7647, without activating PPAR- β/δ or PPAR- γ (Lo Verme et al., 2005). More recently, we have identified PPAR- α as the molecular target responsible for the analgesic effects of PEA (Lo Verme et al., 2006).

PEA, the endogenous amide of palmitic acid and ethanolamine, belongs to the superfamily of acylethanolamides, a class of lipid mediators. PEA exerts antinociceptive effects (Calignano et al., 1998, 2001), and it inhibits peripheral inflammation and mast cell degranulation in rats and mice (Mazzari et al., 1996; Berdyshev et al., 1998). The anti-inflammatory effects of PEA have been demonstrated in the carrageenan-induced rat paw edema model when intraperitoneally administered. In this model, PEA markedly reduces the expression of proinflammatory mediators such as cyclooxygenase (COX)-2, nitric-oxide synthase, and malondialdehyde (Costa et al., 2002). Carrageenan-induced paw edema is widely used as a model for inflammation, and it is well characterized in rats (Garcia Leme et al., 1973; Di Rosa, 1974) and mice (Posadas et al., 2004).

The aim of the present study was to investigate a possible role for PPAR- α receptor at the CNS level in mediating peripheral inflammation. Therefore, we have studied the effects of i.c.v. administration of PPAR- α agonists, including PEA at 0.01 to 1 μ g and GW7647 at 0.01 to 1 μ g, in mice subjected to carrageenan-induced paw edema. To further characterize the involvement of PPAR- α in this model by its agonists, we evaluated the modulation of PPAR- α , COX-2, and iNOS expression in the spinal cord, which represents the main relay station of the neural firing between the inflamed area and the CNS. The involvement of NF- κ B, a well known transcription factor involved in the inflammatory process, was also investigated.

Materials and Methods

Animals. Male Swiss mice weighing 20 to 25 g were purchased from Harlan (Udine, Italy). They were housed in stainless steel cages in a room kept at $22 \pm 1^\circ\text{C}$ on a 12/12-h light/dark cycle. The animals were acclimated to their environment for 1 week, and they had ad libitum access to tap water and standard rodent chow. For adrena-

lectomies, mice were adrenalectomized or sham-operated under ketamine and xilazine anesthesia (100 mg and 10 mg kg^{-1} i.p., respectively), and they were used 7 days after the surgical procedure. Adrenalectomized animals received isotonic saline as drinking water. Mice (4–5 weeks old; 20–22 g) with a targeted disruption of the PPAR- α gene (PPAR- α knockout) and their wild-type littermate controls (PPAR- α wild type) were purchased from Jackson Laboratories (Harlan Nossan, Italy). Mice homozygous for the Ppara^{tniJGonz}-targeted mutation are viable, fertile, and they seem normal in appearance and behavior (Genovese et al., 2005). 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 European Economic Community regulations (O.J. of E.C. L 358/1 12/18/1986).

Chemicals. PEA, GW7647, GW0742, and ciglitazone were purchased from Tocris Cookson Inc. (Bristol, UK). λ -Carrageenan, xylazine, and ketamine were purchased from Sigma-Aldrich (Milan, Italy).

Intracerebroventricular Injections. Animals were briefly anesthetized with enflurane. Drugs were dissolved in sterile distilled water with 10% polyethylene glycol and 5% Tween 80. They were administered in a volume of 2 μ l per mouse by using a 25- μ l glass Hamilton syringe Hamilton Co. (Reno, NV) with a stainless steel needle modified with a shaft to control the depth of injection at 2 mm. The injection was made into the lateral ventricle 2 mm caudal and 2 mm lateral from the bregma. Drug solutions were freshly prepared immediately before use.

Paw Edema. Paw edema was induced by a subplantar injection of 50 μ l of sterile saline containing 1% λ -carrageenan into the right hind paw. Paw volumes were measured by a plethysmometer (Ugo Basile, Milan, Italy) at different time intervals. The increase in paw volume was evaluated as the difference between the paw volume measured at each time point and the basal paw volume measured immediately before carrageenan injection.

Rotarod Test. In a pivotal experiment, integrity of motor function was assessed in treated mice using an accelerating rotarod (Ugo Basile). The animals were acclimated to acceleration in three training runs. Mean performance time (seconds) 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 points observed for paw edema evaluation.

Preparation of Nuclear and Cytosolic Protein Extracts from Spinal Cord. All the extraction procedures were performed on ice using ice-cold reagents. In brief, spinal cord tissues collected at 5 h after paw edema induction were suspended in extraction buffer (0.32 M sucrose, 10 mM Tris-HCl, pH 7.4, 1 mM EGTA, 2 mM EDTA, 5 mM Na₂S₂O₃, 10 mM 2-mercaptoethanol, 50 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 0.15 μ M pepstatin A, 20 μ M leupeptin, and 1 mM sodium orthovanadate), and then they were 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 1000g for 10 min at 4°C. 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 phenylmethylsulfonyl fluoride, 20 μ M leupeptin, and 0.2 mM sodium orthovanadate, and then they were centrifuged for 30 min at 15,000g at 4°C to yield the nuclear fraction for the NF- κ B p65 level determination.

Preparation of Total Tissue Protein Extracts from Spinal Cord. Spinal cords were obtained from each animal 6 h after carrageenan or saline injection, and they 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 phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 10 μ g/ml leupeptin, and 10 μ g/ml trypsin inhibitor; 0.25 ml/50-mg tissue). After 1 h, tissue lysates were centrifuged at 100,000g for 15 min at 4°C, and the supernatant was stored at -80°C until use.

Western Blot Analysis. Protein content was measured using bovine serum albumin (Sigma-Aldrich) as a standard. Total extract (for iNOS, COX-2, and PPAR- α) or cytosolic (for I κ B- α) or nuclear (NF- κ B) fractions containing equal amounts of protein were separated on sodium dodecyl sulfate-polyacrylamide minigels and transferred onto nitrocellulose membranes (Protran nitrocellulose transfer membrane; Whatman Schleicher and Schuell, 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 for iNOS (dilution 1:2000; BD Biosciences Transduction Laboratories, Lexington, KY); COX-2 (dilution 1:1500; Cayman Chemical, Ann Arbor, MI), PPAR- α (1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), I κ B- α (1:1000; Santa Cruz Biotechnology, Inc.), or NF- κ B p65 (1:500; Santa Cruz Biotechnology, Inc.) in PBS containing 5% nonfat dried milk and 0.1% Tween 20. The secondary antibody (anti-mouse IgG, anti-rabbit IgG, or anti-goat IgG peroxidase conjugate) was incubated for 1 h at room temperature. Blots were washed with PBS, developed using enhanced chemiluminescence detection reagents (GE Healthcare, Piscataway, NJ) following manufacturer's instructions, and exposed to X-Omat film (Eastman Kodak, Rochester, NY). Protein bands for iNOS (~130 kDa), COX-2 (~72 kDa), PPAR- α (~55 kDa), I κ B- α (~37 kDa), and NF- κ B p65 (~65 kDa) were quantified using a model GS-700 imaging densitometer (Bio-Rad, Hercules, CA). α -Tubulin protein (dilution 1:1000; Sigma-Aldrich) was performed to ensure equal sample loading.

Statistical Analyses. Results are expressed as the mean \pm S.E.M. of n experiments. 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.

Results

Effects of Central PPAR- α Activation on Carrageenan-Induced Paw Edema. Confirming a previous characterization of the model (Posadas et al., 2004), mouse paw edema was found to develop in two distinct phases: an acute first phase peaking at 6 h and a second phase peaking at 72 h. PEA treatment (0.01, 0.1, and 1 μ g i.c.v.), 30 min before carrageenan injection, markedly reduced paw edema in a time-dependent manner (Fig. 1). During the first phase (0–6 h), all doses tested significantly inhibited edema formation ($p < 0.001$). The effects of PEA were effective up to 24 h following carrageenan injection ($p < 0.01$ for 0.01 μ g and $p < 0.001$ for 0.1 and 1 μ g). In the second phase (24–96 h), only the highest dose of PEA, 1 μ g, was still able to reduce paw edema at 48 h ($p < 0.001$). Intracerebroventricular administration of the synthetic PPAR- α agonist GW7647 at 0.01 to 1 μ g, 30 min before carrageenan, resulted in a reduction of paw edema in a time-dependent manner ($p < 0.001$; Fig. 2). Twenty-four hours after carrageenan injection, the effect of GW7647 at a dose of 0.1 μ g was less evident ($p < 0.05$), whereas the effect of the highest dose, 1 μ g, was persistent and significant until 48 h ($p < 0.001$).

The obligatory role of PPAR- α in the anti-inflammatory effects of i.c.v. PEA was demonstrated by two different findings. First, the anti-inflammatory effects of 1 μ g of PEA or 1 μ g of GW7647, evaluated as area under the curve, were absent in mutant mice lacking the PPAR- α receptor (PPAR- $\alpha^{-/-}$; Fig. 3). Second, the selective PPAR- δ agonist GW0742 or the PPAR- γ agonist ciglitazone at the same dose of 1 μ g did not modify paw edema development

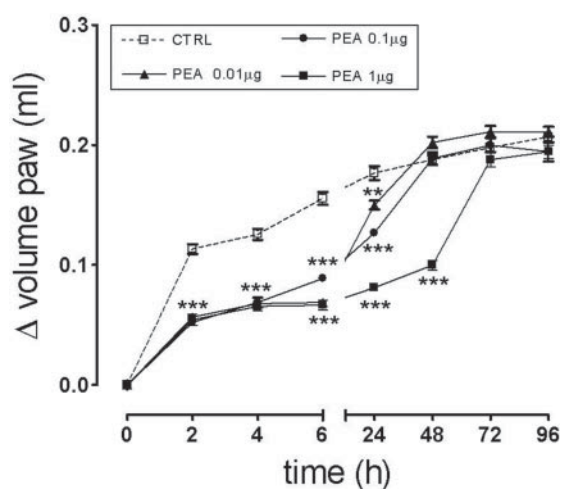


Fig. 1. Anti-inflammatory effect of i.c.v. administration of PEA on mouse carrageenan paw edema. PEA was administered i.c.v. in 2 μ l of vehicle. Control animals were treated with vehicle i.c.v. vehicle or PEA 30 min before subplantar injection of carrageenan. Data are expressed as means \pm S.E.M. of at least six animals for each group. ***, $p < 0.001$; **, $p < 0.01$ versus control group.

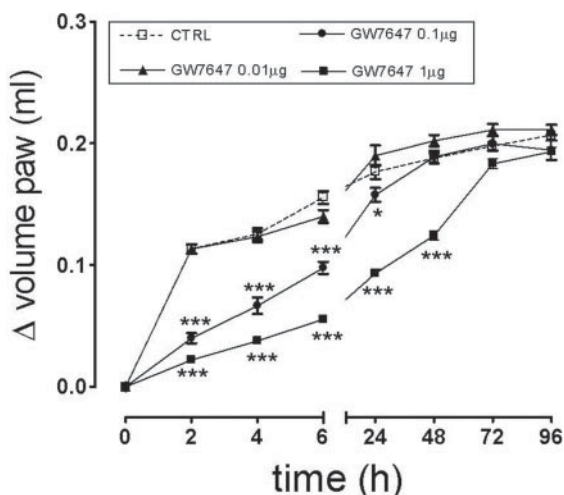


Fig. 2. Anti-inflammatory effect of i.c.v. administration of GW7647 on mouse carrageenan paw edema. GW7647 was administered i.c.v. in 2 μ l of vehicle. Control animals were treated with i.c.v. vehicle or GW7647 30 min before subplantar injection of carrageenan. Data are expressed as means \pm S.E.M. of at least six animals for each group. ***, $p < 0.001$; *, $p < 0.05$ versus control group.

(Fig. 4). Even when these compounds were administered at doses up to 10 times higher (10 μ g) than those of PEA or GW7647, they were still ineffective (data not shown). To exclude a possible glucocorticoid involvement in the anti-inflammatory effects of PPAR- α agonists, we also tested 0.01 to 1 μ g i.c.v. PEA in adrenalectomized mice, and our results demonstrate that PEA was still active (data not shown).

Effects of Intracerebroventricular Administration of PEA or GW7647 on Motor Coordination. To evaluate a possible sedative or muscle-relaxant effects after i.c.v. administration of 1 μ g of PEA or 1 μ g of GW7647, treated mice were tested on rotarod. Neither PEA nor GW7647, given 30 min before testing, affected motor responses in mice (performance times: vehicle, 60 \pm 0.0 s; PEA, 59.5 \pm 2.3 s; and GW7647, 57.8 \pm 3.3 s). Moreover, at the same doses, PPAR- α

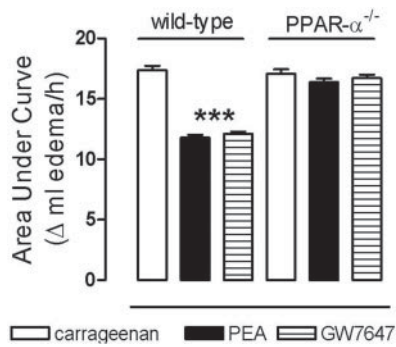


Fig. 3. Effect of i.c.v. administration of PEA or GW7647 on carrageenan paw edema in mutant mice lacking the PPAR- α receptor. PEA (1 μ g; black bar) or GW7647 (1 μ g; striped bar) was administered i.c.v. in 2 μ l of vehicle. Control animals (white bar) were treated with i.c.v. vehicle. Drugs or vehicle was administered 30 min before subplantar injection of carrageenan. Data are expressed as means \pm S.E.M. of at least six animals for each group. ***, $p < 0.001$ versus carrageenan group.

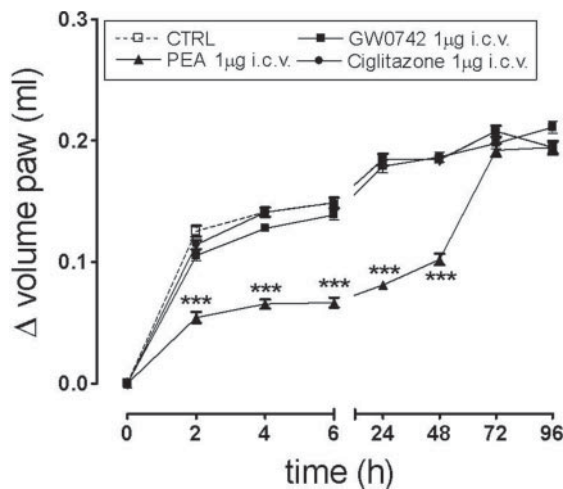


Fig. 4. Effect of i.c.v. PEA, GW0742, or ciglitazone on mouse carrageenan paw edema. PEA (1 μ g; \blacksquare), the synthetic PPAR- β/δ agonist GW0742 (1 μ g; \blacktriangle), or the synthetic PPAR- γ agonist ciglitazone (1 μ g; \bullet) was administered in 2 μ l of vehicle. Control animals (\square) were treated with vehicle i.c.v. PEA, GW0742, ciglitazone, or vehicle 30 min before subplantar injection of carrageenan. Data are expressed as means \pm S.E.M. of at least six animals for each group. ***, $p < 0.001$ versus control group.

agonists significantly (**, $p < 0.01$ versus carrageenan) enhanced the motor performances reduced in mice treated with carrageenan (carrageenan, 37.2 ± 2.2 s; PEA, 50.0 ± 2.8 s; and GW7647, 44.8 ± 1.6 s).

Effect of Intracerebroventricular Administration of PEA on Spinal PPAR- α , COX-2, and iNOS Expression.

Carrageenan significantly reduced the levels of PPAR- α in the spinal cords of vehicle (i.c.v.)-treated animals (##, $p < 0.01$ versus untreated group; Fig. 5). A single i.c.v. administration of 1 μ g of PEA, 30 min before edema induction, significantly reversed the spinal PPAR- α levels down-regulated by carrageenan (*, $p < 0.05$ versus carrageenan), as determined by densitometric analysis (Fig. 5).

To further characterize the anti-inflammatory effects of PEA, we investigated COX-2 and iNOS expression in spinal cord 6 h after paw edema induction. We chose this time point because the acute phase of paw edema (0–6 h) is characterized by a central sensitization primarily mediated by prostanoids. As expected, COX-2 expression was up-regulated in the spinal cord from carrageenan-injected and i.c.v. vehicle-

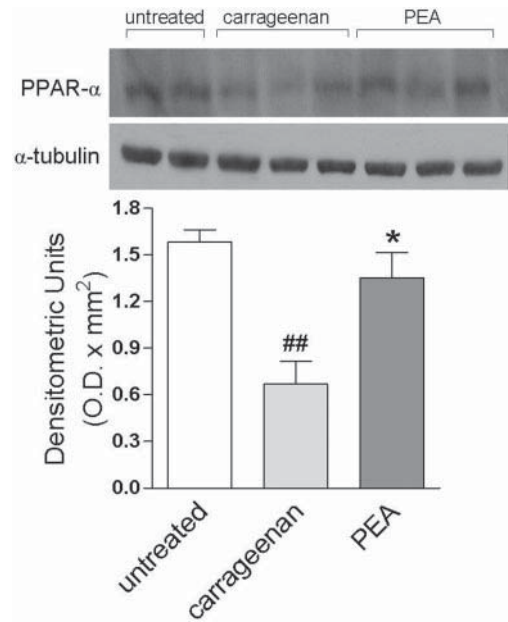


Fig. 5. PPAR- α expression in the spinal cord. Western blot analysis of PPAR- α receptor protein in spinal cord tissue lysates from mice treated with 1 μ g of PEA and vehicle i.c.v. (carrageenan) 30 min before carrageenan treatment. Content of spinal PPAR- α receptor from mice that received saline subplantar and vehicle i.c.v. injections is also reported (untreated). The animals were sacrificed 6 h after paw edema induction. A representative blot of lysates obtained is shown. Equal loading was confirmed by α -tubulin staining. ##, $p < 0.01$ versus untreated group; *, $p < 0.05$ versus carrageenan group.

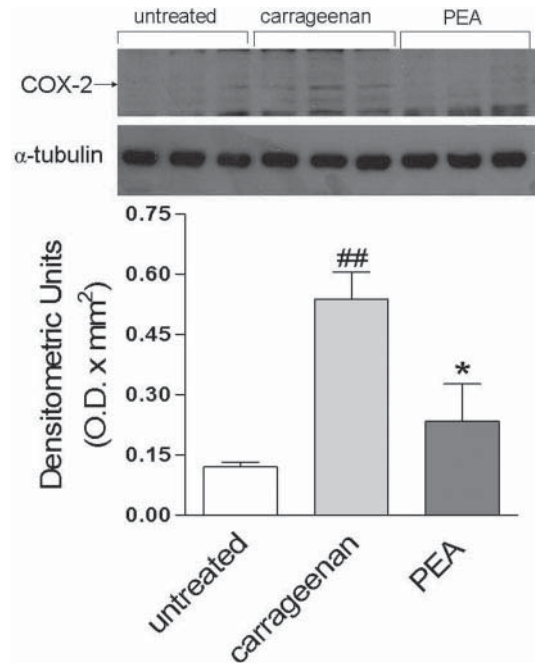


Fig. 6. COX-2 expression on the spinal cord. Western blot analysis of COX-2 expression in spinal cord tissue lysates from mice treated with 1 μ g of PEA or vehicle i.c.v. (carrageenan) 30 min before carrageenan subplantar injection. Spinal cord tissue lysates from mice treated with subplantar saline and vehicle i.c.v. injections is also reported (untreated). The animals were sacrificed 6 h after paw edema induction. A representative blot of lysates obtained is shown. Equal loading was confirmed by α -tubulin staining. ##, $p < 0.01$ versus untreated group; *, $p < 0.05$ versus carrageenan group.

treated mice (##, $p < 0.01$ versus untreated group; Fig. 6). Likewise, spinal iNOS expression was up-regulated by carrageenan (##, $p < 0.01$ versus untreated group; Fig. 7). A single i.c.v. administration of 1 μg of PEA, 30 min before carrageenan, significantly reduced spinal COX-2 (*, $p < 0.05$ versus carrageenan group; Fig. 6) and iNOS expression (**, $p < 0.01$ versus carrageenan group; Fig. 7).

Effect of Intracerebroventricular Administration of PEA on I κ B- α Degradation and NF- κ B p65 Nuclear Translocation. To investigate the mechanism by which i.c.v. administration of PEA may attenuate the development of carrageenan paw edema, we evaluated, using Western blot analysis, I κ B- α degradation, and NF- κ B p65 activation in the cytosolic or nuclear extracts from spinal cord, respectively. Basal level of I κ B- α was detected in the cytosolic fraction from untreated mice, whereas carrageenan induced a decrease of I κ B- α 5 h after paw edema induction, compared with untreated group (#, $p < 0.05$ versus untreated group; Fig. 8A). A single i.c.v. administration of 1 μg of PEA, 30 min before paw edema induction, significantly prevented I κ B- α degradation (*, $p < 0.05$ versus carrageenan group). Moreover, the translocation of the p65 subunit of NF- κ B into the nucleus (Fig. 8B) was dramatically increased 5 h after carrageenan injection (##, $p < 0.01$ versus untreated group). PEA significantly reduced the level of NF- κ B p65 in the nuclear fraction (**, $p < 0.01$ versus carrageenan group).

Discussion

Despite the clear roles played by PPAR- α in lipid metabolism, inflammation, and feeding, the effects of pharmacological activation of the nuclear receptors in the CNS have remained unclear (Benani et al., 2004; Moreno et al., 2004).

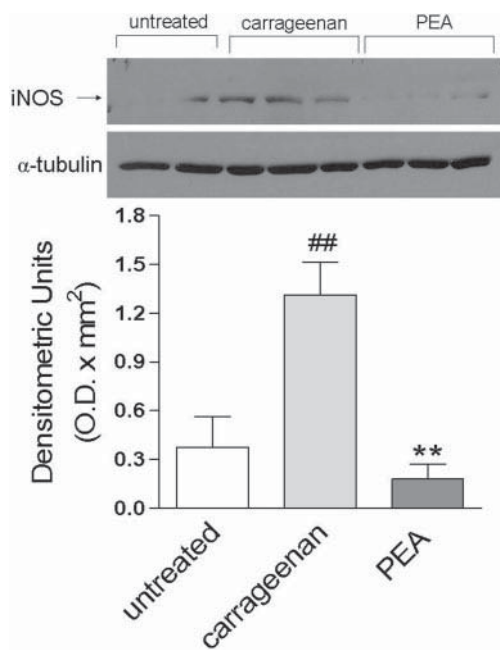


Fig. 7. iNOS expression on the spinal cord. Western blot analysis of iNOS expression in spinal cord tissue lysates from mice treated with 1 μg of PEA or vehicle i.c.v. (carrageenan) 30 min before carrageenan treatment. Spinal cord tissue lysates of mice treated with subplantar saline and vehicle i.c.v. injections are also reported (untreated). The animals were sacrificed 6 h after paw edema induction. A representative blot of lysates obtained is shown. Equal loading was confirmed by α -tubulin staining. ##, $p < 0.01$ versus untreated group; **, $p < 0.01$ versus carrageenan group.

Here, we report that i.c.v. injection of an endogenous PPAR- α agonist, PEA, or a synthetic PPAR- α agonist, GW7647, reduces carrageenan-induced paw edema in mice. In treated animals, central PEA administration restores the physiological expression of spinal PPAR- α content reduced by carrageenan. Recently, we have shown that PEA activates the nuclear receptor PPAR- α with a potency comparable with those of the synthetic agonists GW7647 and Wy-14,643 (Lo Verme et al., 2005). In two animal models, carrageenan-induced paw edema and phorbol ester-induced ear edema, PEA attenuates inflammation in wild-type mice, but it has no effect in PPAR- α ^{-/-} mice (Lo Verme et al., 2005).

Nanomolar-to-micromolar levels of PEA have been found in the CNS (Cadas et al., 1997), but the physiological roles for PEA in the brain remain an open question. Likewise, the physiological roles of PPAR- α , whose expression has been described in the adult rat CNS (Moreno et al., 2004), remain unknown.

Here, we found that a single i.c.v. administration of PEA, 30 min before carrageenan, markedly reduced paw edema formation. During the first phase, PEA showed a significant reduction in paw edema with a maximal inhibition of inflammation of ~50%. Likewise, the synthetic PPAR- α agonist GW7647 reduced mouse paw edema as a function of time. We used this synthetic agonist as a reference drug, to confirm that the central anti-inflammatory activity of PEA is mediated by PPAR- α , as we have found in peripheral tissue (Lo Verme et al., 2005). We demonstrated the obligatory role of PPAR- α in the anti-inflammatory effects of centrally administered PEA by two additional experiments. First, mutant PPAR- α ^{-/-} mice failed to respond either to GW7647 or PEA. Second, the selective PPAR- δ agonist GW0742 and PPAR- γ agonist ciglitazone did not modify carrageenan-induced paw edema development, even when administered at doses up to 10 times higher than those needed for PEA to exert paw edema reduction. Notably, the central administration of PEA or GW7647 does not cause sedation or other central effects, because it is not accompanied by an impairment of motor activity, as demonstrated in the rotarod test. Moreover, at the same doses, PPAR- α agonists significantly enhanced motor performances reduced in mice with paw inflammation.

The CNS usually reacts to peripheral stimuli in several ways. It is well known that peripheral damage can activate thalamic and cortical areas that in turn activate a descending inhibition, resulting in spinal opioid release that can in turn modulate afferent stimuli (Iversen et al., 1980; Hammond and Yaksh, 1981). Recent studies indicate that persistent pain after tissue or nerve injury is linked to an enhanced activation of descending modulatory circuits. A net increase in descending facilitatory drive leads to an amplification and spread of the pain (Urban and Gebhart, 1999; Vanegas and Schaible, 2004). Likewise, peripheral damage causes an increase in COX-2 and iNOS, which are involved in inflammatory signaling to the CNS (Ichitani et al., 1997; Samad et al., 2001). Indeed, experimental evidence indicates that COX-2-mediated (but not COX-1-mediated) increase in prostaglandin E₂ production in the CNS contributes to the severity of the inflammatory and pain peripheral responses in rat (Ichitani et al., 1997). COX-2 is rapidly induced in the spinal cord and other regions of the CNS following carrageenan injection in the paw (Ichitani et al., 1997), and it plays a pivotal role in sustaining pain and peripheral inflammation (Dolan et al.,

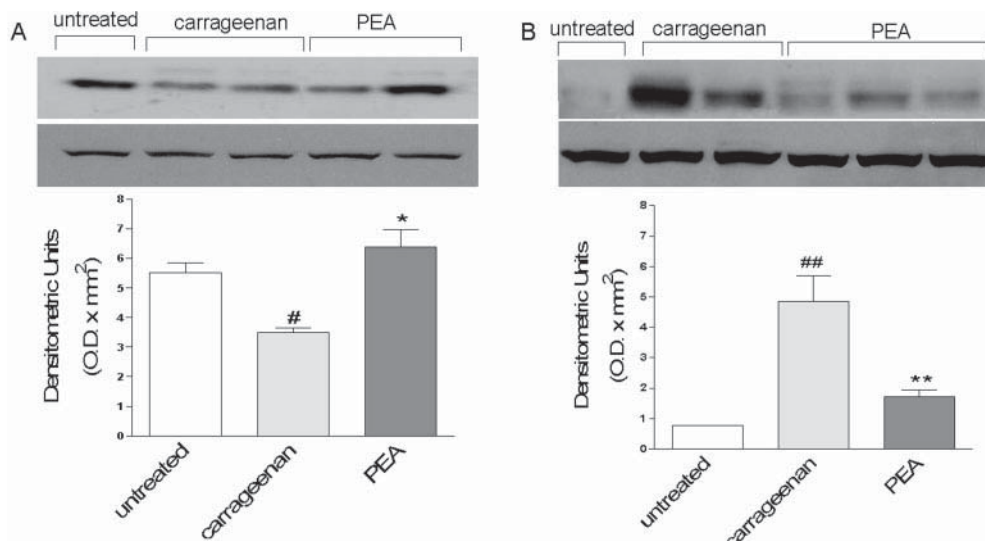


Fig. 8. IκB-α and NF-κB p65 expression on the spinal cord. A, Western blot analysis of IκB-α levels in the cytosolic fractions of spinal cord tissue lysates from mice treated with 1 μg of PEA (PEA) or vehicle i.c.v. (carrageenan) 30 min before carrageenan treatment. Spinal cord tissue lysates from mice treated with subplantar saline and vehicle i.c.v. injections are also reported (untreated). The animals were sacrificed 5 h after paw edema induction. A representative blot of lysates obtained is shown. Equal loading was confirmed by α-tubulin staining. #, $p < 0.05$ versus untreated group; *, $p < 0.05$ versus carrageenan group. B, Western blot analysis of NF-κB p65 levels in the nuclear fractions of spinal cord tissue lysates from mice treated with 1 μg of PEA (PEA) or vehicle i.c.v. (carrageenan) 30 min before carrageenan treatment. Spinal cord tissue lysates from mice treated with subplantar saline injections and vehicle i.c.v. are also reported (untreated). The animals were sacrificed 5 h after paw edema induction. A representative blot of lysates obtained is shown. Equal loading was confirmed by α-tubulin staining. ##, $p < 0.01$ versus untreated group; **, $p < 0.01$ versus carrageenan group.

2000; Samad et al., 2001). Moreover, it has been reported that in mice peripheral inflammation results in a dramatic increase of COX-2 expression in neurons of laminae I–VI and X in the dorsal horn (Maihöfner et al., 2000). Here, we reported that i.c.v. injection of PEA reduced COX-2 and iNOS levels in the spinal cord, resulting in a reduction of paw edema. The involvement of spinal PPAR-α in modulating peripheral inflammation is strongly indicated by our data demonstrating a down-regulation of the receptor after subplantar carrageenan administration. Intracerebroventricular administration of PEA restored PPAR-α receptor levels in the spinal cord. Thus, the increased PPAR-α levels and the reduced expression of COX-2 and iNOS are the major effects observed after i.c.v. injection of PEA, and they support the anti-edemagenic effects observed in the carrageenan-induced paw edema. The possibility that i.c.v. PEA, administered at the low doses used in the present study, could reach the spinal cord is unlikely. PEA inactivation primarily consists of its intracellular hydrolysis by lipid hydrolases (Schmid et al., 1985). Indeed, PEA undergoes active and rapid metabolism (Cravatt et al., 1996; Ueda et al., 2001; Bracey et al., 2002). Thus, a possible interpretation of our results is that central PPAR-α stimulation results in a PPAR-α-mediated effect at the spinal level, which is able to control carrageenan-induced COX-2 and iNOS synthesis. Glucocorticoids are potent hormones in regulating COX-2 and iNOS expression during inflammatory process. Therefore, we explored the possibility that adrenal-derived glucocorticoids could be involved in the anti-inflammatory effects of PEA. However, our results demonstrate that in adrenalectomized mice, PEA was still active, excluding the possible involvement of glucocorticoid release in controlling COX-2 and iNOS expression.

Many different stimuli, such as proinflammatory cytokines, including interleukin-1β, can activate NF-κB, causing this nuclear factor to regulate the expression of proinflam-

matory cytokines and factors such as iNOS and COX-2 (Auphan et al., 1995; George et al., 2000). To further characterize the anti-inflammatory activity of PEA in the spinal cord, we asked whether the activation of PPAR-α by PEA could be related to the inhibition of NF-κB activity. We focused our attention on IκB-α phosphorylation and degradation (Grilli et al., 1996; DiDonato et al., 1997). The appearance of IκB-α and NF-κB p65 in cytosolic or nuclear fractions of spinal cord was investigated by Western blot assay. We found that the anti-inflammatory effects of PEA occurred, at least in part, via prevention of carrageenan-mediated IκB-α degradation 5 h after paw inflammation induction. In accordance with this result, the appearance of NF-κB p65 in the nuclear fractions was also significantly reduced by PEA, and it supports our finding of the roles of COX-2 and iNOS expression in the spinal cord 6 h after carrageenan injection. Accordingly, it has been reported that activated NF-κB in the spinal cord affects pain behavior and the expressions of COX-2 mRNA following peripheral inflammation (Lee et al., 2004).

However, the role played by spinal iNOS during peripheral inflammation is controversial (Dolan et al., 2000; Tao et al., 2003; Sekiguchi et al., 2004). Our result clearly indicates that iNOS increases about 3-fold versus control 6 h following carrageenan administration. It is likely that spinal iNOS could be a preferential isoform in spinal signaling of peripheral damage. Nevertheless, the question requires further study.

Our results indicate that central PPAR-α is involved in the control of proinflammatory enzymes at the spinal level, which is the first neural structure in which primary afferent stimuli are processed before reaching the CNS. Our data add further support to the broad spectrum of anti-inflammatory effects induced by PPAR-α agonists, by demonstrating a centrally mediated component for these drugs.

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