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Involvement of the cannabimimetic compound, *N*-palmitoyl-ethanolamine, in inflammatory and neuropathic conditions: Review of the available pre-clinical data, and first human studies

Nissar A. Darmani[a,1], Angelo A. Izzo[b,1], Brian Degenhardt[a], Marta Valenti[c], Giuseppe Scaglione[d], Raffaele Capasso[b], Italo Sorrentini[d], Vincenzo Di Marzo[c,*]

[a]*Department of Pharmacology and the A. T. Still Research Institute, Kirksville College of Osteopathic Medicine, A. T. Still University of Health Sciences, Kirksville, MO, USA*
[b]*Department of Experimental Pharmacology, University of Naples Federico II, via D. Montesano 49, 80131 Naples, Italy*
[c]*Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli (NA), Italy*
[d]*Gastroenterologia, A.O. "Rummo", Benevento, Italy*

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Abstract

The endogenous cannabimimetic compound, and anandamide analogue, *N*-palmitoyl-ethanolamine (PEA), was shown to exert potent anti-inflammatory and analgesic effects in experimental models of visceral, neuropathic and inflammatory pain by acting via several possible mechanisms. However, only scant data have been reported on the regulation of PEA levels during pathological conditions in animals or, particularly, humans. We review the current literature on PEA and report the results of three separate studies indicating that its concentrations are significantly increased during three different inflammatory and neuropathic conditions, two of which have been assessed in humans, and one in a mouse model. In patients affected with chronic low back pain, blood PEA levels were not significantly different from those of healthy volunteers, but were significantly and differentially increased (1.6-fold, $P<0.01$, $N=10$ per group) 30 min following an osteopathic manipulative treatment. In the second study, the paw skin levels of PEA in mice with streptozotocin-induced diabetic neuropathic pain were found to be significantly higher (1.5-fold, $P<0.005$, $N=5$) than those of control mice. In the third study, colonic PEA levels in biopsies from patients with ulcerative colitis were found to be 1.8-fold higher ($P<0.05$, $N=8-10$) than those in healthy subjects. These heterogeneous data, together with previous findings reviewed here, substantiate the hypothesis that PEA is an endogenous mediator whose levels are increased following neuroinflammatory or neuropathic conditions in both animals and humans, possibly to exert a local anti-inflammatory and analgesic action.

Keywords: Cannabinoid; Vanilloid; Receptor; FAAH; Entourage; Pain; Inflammation

Abbreviations: NAE, *N*-acyl-ethanolamine; PEA, *N*-palmitoyl-ethanolamine; OEA, *N*-oleoyl-ethanolamine; SEA, *N*-stearoyl-ethanolamine; AEA, *N*-arachidonoyl-ethanolamine; FAAH, fatty acid amide hydrolase; TRPV1, transient receptor potential vanilloid type 1 channel; CLBP, chronic low back pain; OMT, osteopathic manipulative treatment; IBD, inflammatory bowel disorder; STZ, streptozotocin.

* Corresponding author. Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Comprensorio Olivetti, Palazzina 70, 80078 Pozzuoli (NA), Italy. Tel.: +39 081 8675093; fax: +39 081 8041770.
E-mail address: vdimarzo@icmib.na.cnr.it (V. Di Marzo).
[1] These two authors are listed in alphabetical order as they contributed equally to this work.

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1. Introduction

The *N*-acyl-ethanolamines (NAEs) represent a class of endogenous bioactive amides sharing common biosynthetic and metabolic pathways. One of the members of this family, the anti-inflammatory compound, *N*-palmitoyl-ethanolamine (PEA), has been known since the late 1950s (see Schmid et al., 1990 for review). However, it was not until the discovery of the endocannabinoid *N*-arachidonoyl-ethanolamine (AEA, anandamide) (Devane et al., 1992) that interest in these fatty acid amides was revived. Since it was soon clear that only long chain polyunsaturated NAEs are capable of binding to cannabinoid receptors (Di Marzo, 1998), different molecular targets were looked for: (1) *N*-stearoyl-ethanolamine (SEA), to explain its pro-apoptotic effects in vitro and central cannabimimetic actions in vivo (Maccarrone et al., 2002a); (2) *N*-oleoyl-ethanolamine (OEA), to provide a mechanism for its anorexic actions (Rodriguez de Fonseca et al., 2001); and (3) PEA, to explain its anti-inflammatory, neuroprotective and analgesic properties (see Lambert et al., 2002 for review). It has been recently suggested that while SEA has its own binding sites in the brain (Maccarrone et al., 2002a), OEA binds with high affinity to the peroxisome proliferator receptor-α (PPAR-α) (Fu et al., 2003). Regarding PEA, at least part of its neuroprotective or anti-inflammatory effects that are insensitive to cannabinoid receptor antagonists (Lambert et al., 2002) might be due to the interaction with a possibly new G-protein coupled receptor in microglial cells (Franklin et al., 2003) or to PPAR-α (Lo Verme et al., 2005), respectively. However, it had also been proposed that PEA could act as an agonist for the cannabinoid receptor type 2 (Facci et al., 1995), since some of its analgesic effects are antagonized by SR144528, a selective CB_2 receptor blocker (Jaggar et al., 1998a; Calignano et al., 1998). Yet, PEA, like OEA and SEA, exhibits very little, if any, affinity for the cloned CB_1 and CB_2 cannabinoid receptors from the rat, mouse or man (Sheskin et al., 1997; Lambert et al., 1999). Several possibilities were proposed to explain the SR144528-sensitive analgesic effects of PEA, including the activation of a "CBn" receptor, very similar to the CB_2 receptor, and an action as "entourage" compound, i.e. by enhancing the activity and/or by inhibiting the degradation of endogenous agonists of CB_2 receptors (Mechoulam et al., 1998). Indeed, apart from being synthesized from different precursors through the action of the same enzyme, the recently cloned *N*-arachidonoyl-phosphatidyl-ethanolamine-selective phospholipase D (Okamoto et al., 2004), PEA and AEA are hydrolyzed by the same amidase enzymes. There are two examples of such enzymes identified so far: (i) the "fatty acid amide hydrolase" (FAAH) (see Fowler et al., 2001 for review), and (ii) a lysosomal hydrolase with tissue distribution different from that of FAAH (Ueda et al., 2001). In the latter case, the catalytic efficacy of the enzyme is higher with PEA than with AEA, and, in principle, it allows PEA to efficiently inhibit AEA degradation by substrate competition. In the case of FAAH, instead, chronic PEA was shown to down-regulate its expression, thereby enhancing some pharmacological actions of AEA (Di Marzo et al., 2001a).

By acting independently of FAAH, PEA also enhances those effects of AEA that are mediated by the vanilloid TRPV1 receptor (De Petrocellis et al., 2001). This is a non-selective cation channel expressed in C-fibers and acting as a molecular transducer of nociceptive stimuli, gated by protons, heat and plant toxins such as capsaicin and resiniferatoxin (Caterina et al., 1997). AEA also can gate TRPV1, particularly when certain regulatory events occur (see Di Marzo et al., 2002 for review). It was shown recently that PEA can enhance the TRPV1-mediated actions on intracellular Ca^{2+} of AEA, in part by increasing its affinity for the channel in specific binding assays (De Petrocellis et al., 2001). Furthermore, PEA was found to enhance the anti-proliferative effects on cancer cells of vanilloid compounds in vitro (De Petrocellis et al., 2002).

Although the metabolic pathways of PEA have been extensively investigated, very few studies have examined the regulation of PEA levels under physiological or pathological conditions (Lambert et al., 2002, for a review). Furthermore, no such study has ever been carried out in humans during conditions of inflammation and pain. Here, we report the results of three studies, two of which have been carried out in humans, and one in an animal model, showing that PEA levels may increase during inflammatory or neuropathic pain.

2. Materials and methods

2.1. Patients for the back pain determinations

Twenty Caucasian participants (mean age = 38 ± 9 years; range 24–53 years) from a mid-western rural community in the USA were enrolled in this study: 10 with chronic low back pain (CLBP) and 10 control subjects with no low back pain (7 males and 3 females for each group). Low back pain was defined as pain, muscle tension, or stiffness localized below the posterior costal margin and above the inferior gluteal folds. Those in the CLBP group had pain in the small of the back for a minimum of 5 days a week for at least 3 months. Subjects were excluded if they had manual treatment of the spine within the 8 weeks preceding entry into the study, were taking steroids, opioids, muscle relaxants, or anticonvulsants, were experiencing current acute back pain, diagnosed with an autoimmune disease, or had infections or inflammatory processes at the initiation of the study.

Within a 5 week, seasonally stable time period, the entire protocol was completed. Each subject was scheduled to have venous blood collections at the same time of day between 9 and 11:00 a.m. for four consecutive days. On days 1, 2 and 3, the participants rested at least 5–10 min prior to having their blood drawn. Blood samples (5 ml) were collected by vein suction in k_2EDTA (5 mM final concentration) and processed within 1 h after blood was withdrawn. On the fourth day of the protocol, participants presented to the clinic 1 h before the scheduled blood draw. For 5–10 min, the participant's musculoskeletal system was examined using routine osteopathic palpatory tests. Areas of somatic dysfunction (sites of muscle hypertonicity, tenderness and joint restriction) were identified. All subjects, regardless of group placement, had a unique collection of somatic dysfunctions at various locations within their bodies. Those with CLBP had a higher frequency and severity of somatic dysfunction in the lumbar, sacral and pelvic regions compared to the control group. For the next 20–25 min, all participants received an osteopathic manipulative treatment (OMT) directed to specific sites of dysfunction for each patient using commonly used, non-thrust techniques (Table 1, Ward, 2003). Based on the physical findings, the CLBP group received more intensive treatment of their lumbar, sacral and pelvic regions compared to the control group. After the treatment, the subjects rested approximately 30 min before a blood sample was taken. The baseline blood PEA levels on days 1, 2 and 3 were not significantly different from each other. For comparative purposes, only day 1 baseline PEA values were used. To compare day 1 baseline PEA values with 30-min post-osteopathic manipulative treatment (Post-OMT) values obtained on day 4 within the same subjects, both paired *t*-tests and Wilcoxon Signed Rank tests were used. To compare the CLBP and no low back pain control groups on the change in PEA levels, a two-sample *t*-test and a Mann–Whitney *U*-test were used.

2.2. Induction and assessment of diabetic neuropathy in mice

These experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC). Insulin-dependent diabetes mellitus was induced in male ICR mice (22–26 g, Harlan Italy, Corezzana, MI, Italy) by using streptozotocin (STZ). Mice received a single high i.p. dose of 200 mg/kg STZ (prepared freshly by dissolving it in saline adjusted to pH 4.5 in 0.1 N citrate buffer) (Gabra and Sirois, 2002). The induction of diabetes was confirmed by measuring the blood glucose level 96 h after STZ administration (Katovich et al., 1995). Glucose levels were determined using the glucose test kit with an automatic analyzer (Accu-Chek® Active, Roche) in blood samples obtained from tail vein. Only mice with a plasma glucose concentration greater than 300 mg/dl (16.7 mM) were considered as diabetic (Rashid et al., 2003). Hyperalgesia in diabetic mice was confirmed using the hot plate test after 7 days from the injection of STZ (Gabra and Sirois, 2002). Briefly, nociception was measured in both control and diabetic mice using the hot plate test (Eddy and Leimbach, 1953). The plate was adjusted to a temperature of 55 ± 0.5 °C. When the pain threshold is reached, the animal starts to react by licking its hind paw or to jump, and the reaction time is recorded with a timer, with a maximum cutoff time of 30 s to avoid tissue damage. Pretreatment latencies were determined three times with an interval of 24 h starting 3 days before the injection of STZ or vehicle, and the mean was calculated in order to obtain a stable pre-drug response (Gabra and Sirois, 2002). For PEA and AEA determinations, diabetic mice were killed with CO_2 7 days after STZ and the skin paw from either control or diabetic mice were removed and tissue specimens were weighed immediately, immersed into liquid nitrogen, and stored at −70 °C until assay. PEA and AEA contents, expressed as nmol/g wet tissue weight, were compared by ANOVA followed by the Bonferroni's test (as per Statmost®).

2.3. Patients for the ulcerative colitis determinations

Biopsies were obtained in agreement with current Italian healthcare rules after previous informed consent from the subjects, during colonoscopy and by means of biopsy forceps on the colonic mucosa from 8 control patients (5 males and 3 females, average age 41.5 ± 17.5),

Table 1
Definitions of osteopathic techniques applied

Technique	Definition
Articulatory	A joint is carried through its full motion with the therapeutic goal of increased freedom and range of movement using a gentle springing or thrust
Muscle energy	The patient is placed in a specific position by the physician and instructed to voluntarily move the body against a defined resistance by the physician
Soft tissue	Involves lateral stretching, linear stretching, deep pressure, traction, or compression while monitoring tissue response and motion changes by palpation
Strain-counterstrain	The physician specifically places the patient in a position that relieves point tenderness and maintains the position until the tenderness remains eliminated after the patient is returned to a neutral position

submitted to colonoscopy for either hematochezia ($n=3$), colon carcinoma screening ($n=3$, all negative), or abdominal pain ($n=2$); and on the inflamed mucosa of 8 untreated patients with mild to moderate ulcerative colitis at the first diagnostic work-up (6 males and 2 females, average age 49.8±17.1 years, means±SD). Small aliquots (10–15 mg wet weight) of each biopsy sample were kept at −80 °C until processing, while the rest of the sample was subjected to histological analysis. Since tissue aliquots were too small to be weighed without being defrosted, PEA and AEA contents were expressed as pmol/mg lipid extracts, and were compared by ANOVA followed by the Bonferroni's test.

2.4. PEA and AEA extraction and quantification

Tissues were Dounce-homogenized with chloroform/methanol/Tris–HCl 50 mM, pH 7.4 (1/1/1 by vol.) containing 50 pmol of d_8-AEA and 50 pmol of d_4-PEA as internal standards. Lipid-containing organic phase was dried down, weighed and pre-purified by open-bed chromatography on silica gel, and analyzed by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (LC-APCI-MS) using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2010) quadrupole MS via a Shimadzu APCI interface. MS analyses were carried out in the selected ion monitoring (SIM) mode as described previously (Di Marzo et al., 2001b). Temperature of the APCI source was 400 °C; HPLC column was a Phenomenex (5 μm, 150×4.5 mm) reverse phase column, eluted as described (Di Marzo et al., 2001b). Anandamide (retention time: 14.5 min) and PEA (retention time: 19.0 min) quasi-molecular ions ($m/z=348$ and 300, respectively) were quantified by isotope dilution with the above mentioned deuterated standards ($m/z=356$ and 304, respectively).

Peripheral blood samples were collected by vein suction in K_2EDTA (5 mM final concentration). For the LC-MS determinations, EDTA and phenyl-methyl-sulfonyl-fluoride (PMSF) (100 μM final concentration) were added to blood samples. PMSF is an inhibitor of fatty acid amide hydrolase (FAAH), and was added in order to prevent AEA and PEA degradation. Five milliliters of whole blood for each sample were carefully layered over 3 ml of HISTOPAQUE® 1077 solution (Sigma) in ACCUSPIN® tubes (Sigma) and centrifuged at 400×g for 30 min at room temperature. After centrifugation, erythrocytes and granulocytes sedimented at the bottom of the tube. After removal of the clear plasma layer from the top of the tube, the opaque layer containing the mononuclear cells was collected, resuspended by gentle aspiration in 10 ml of phosphate buffered saline (PBS) and centrifuged at 250×g for 10 min at room temperature. For quantitative determinations, the plasma and the mononucleate cell layers were collected together, the proteins precipitated by adding 3 vols. of acetone, the supernatants were collected and subjected to lipid extraction with methanol/chloroform. Enough of each solvent was added to reach a final ratio buffer/methanol/chloroform of 1:1:2 (v/v/v). Methanol containing 100 pmol of d_8-AEA and d_4-PEA was added as internal standard. The organic phase was then dried under nitrogen and purified by means of open bed chromatography on silica gel and analyzed by LC-MS as described above.

3. Results

3.1. PEA levels are enhanced in the blood of back pain patients after therapeutic manipulation

Baseline PEA blood levels in individuals with no back pain, monitored over a period of 3 days are shown in Fig. 1, and are very similar to those previously reported in humans (Maccarrone et al., 2002b). No significant difference was observed in either mean (±SEM) or median (q_1–q_3) baseline blood PEA levels on day 1, day 2 or day 3 between the no low back pain control group and chronic low back pain (CLBP) subjects. Although the mean blood PEA levels increased by 2.75±1.3 pmol/ml following OMT in the "no low back pain" control group, the increase in mean post-OMT PEA blood level (16.4±1.32 pmol/ml) just failed to show a significant change ($P=0.057$) from baseline mean PEA blood levels obtained on day 1 (13.65±1.02 pmol/ml). However, the

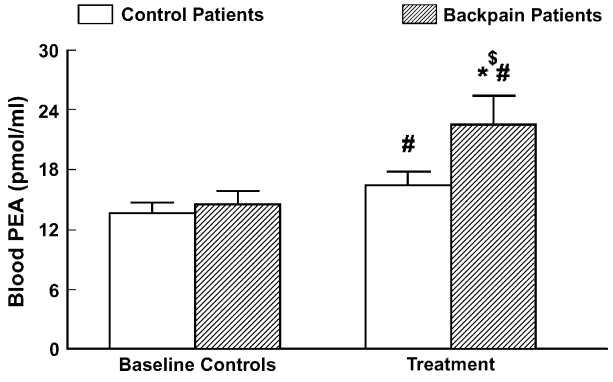

Fig. 1. The effect of a 30-min prior application of articulatory, muscle energy, soft tissue and strain-counterstrain osteopathic manipulative treatments on *N*-palmitoyl-ethanolamine (PEA) levels (±SEM, $N=10$) in circulating mononuclear cells plus serum of no back pain control subjects relative to patients suffering from chronic low back pain. *Significantly different from corresponding mean baseline blood concentration; #significantly different from corresponding median baseline blood concentration; §significantly larger increase in both mean and median blood PEA levels in chronic low back pain patients relative to no low back pain control subjects. See text for details on the statistical method used.

corresponding median increase in post-OMT PEA blood levels was significant versus the control median (i.e. 16.6 (12.7–20.1) versus 12.75 (11.6–15.7) pmol/ml, $P=0.03$) (Fig. 1). In the CLBP group, osteopathic manipulation significantly increased both the mean (22.48 ± 2.87 versus 14.46 ± 1.53, $P=0.003$) and the median (22.5 (18–28.3) versus 14.17 (11.9–15.9), $P=0.006$) blood PEA levels relative to its corresponding baseline pre-OMT control levels (Fig. 1). Furthermore, there was also a significantly larger increase in both mean and median blood PEA levels for the low back pain subjects relative to no low back pain control group following OMT ($\Delta=8.03\pm2$ versus 2.74 ± 1.26, $P=0.05$ and 8.36 (3.9–12.1) versus 1.42 (0.07–5.7) pmol/ml, respectively). No increase in AEA was observed in either control or back pain patients (1.7 ± 0.1 versus 1.7 ± 0.1 and 1.8 ± 0.1 versus 1.5 ± 0.1, respectively). The selectivity of the effect suggests that changes in PEA levels following OMT are unlikely to be mostly due to the slightly different treatments received by the two groups of patients.

3.2. PEA levels are enhanced in the skin of mice with streptozotocin-induced diabetic neuropathy

When the PEA content in the paw skin of mice treated with streptozotocin and exhibiting hyperalgesia on a hot plate was compared to that of the paw skin from untreated animals, a significant enhancement of PEA levels was observed in the former group of samples (from 0.53 ± 0.04 to 0.79 ± 0.03 nmol/g tissue weight, $P<0.05$, $N=5$) (Fig. 2). No statistically significant difference was observed for AEA levels, but only a trend to increase in diabetic mouse paws (from 0.070 ± 0.01 to 0.093 ± 0.003 nmol/g tissue weight, $P=0.08$, $N=4$, Fig. 2). The amounts of AEA and PEA found in control mouse paw skin are consistent with those previously reported for rat paw skin using a different quantification technique (Beaulieu et al., 2000).

3.3. PEA levels are enhanced in biopsies from patients with ulcerative colitis

Histological evaluation of the eight patients with ulcerative colitis showed mild to moderate activity. Average PEA levels in biopsies from the eight control patients were 2.6 ± 0.3 pmol/mg lipid extract. Significantly elevated levels of PEA were found in biopsies from the eight patients with ulcerative colitis (4.6 ± 0.7 pmol/mg lipid extract, $P<0.05$) (Fig. 3), where also the levels of AEA were significantly increased (from 0.5 ± 0.05 to 1.2 ± 0.2 pmol/mg lipid extract, $P<0.0005$, Fig. 3).

4. Discussion

The anti-inflammatory and analgesic properties of PEA have been thoroughly investigated in the 1950s and 1960s (Schmid et al., 1990 for review) as well as more recently (Lambert et al., 2002 for review). In particular, PEA was found to inhibit: (1) the bi-phasic response of formalin-induced nociception in rodents (Jaggar et al., 1998a; Calignano et al., 1998); (2) several typical inflammatory and nocifensive responses in rodents (Mazzari et al., 1996; Calignano et al., 2001; Costa et al., 2002; Conti et al., 2002); (3) visceral pain in two different experimental models (Jaggar et al., 1998a; Farquhar-Smith and Rice, 2001; Farquhar-Smith et al., 2002); and (4) resiniferatoxin-induced sensory neuropeptide release in vivo and neuropathic hyperalgesia in

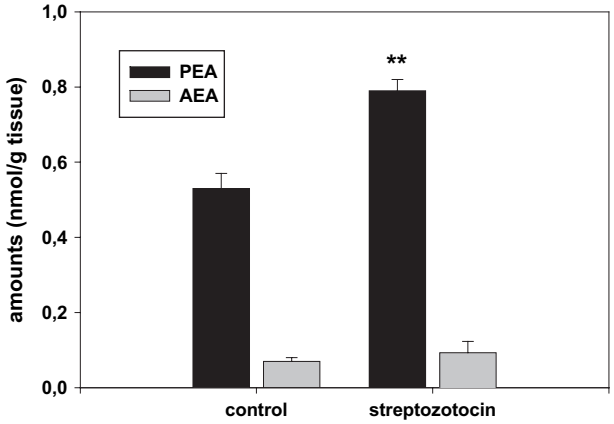

Fig. 2. *N*-Palmitoyl-ethanolamine (PEA) and anandamide (AEA) levels ($\pm$SEM, $N=4,5$) in the paw skin of mice treated with either vehicle or streptozotocin, after development of thermal hyperalgesia. $^{**}P<0.005$ as calculated by ANOVA followed by the Bonferroni's test.

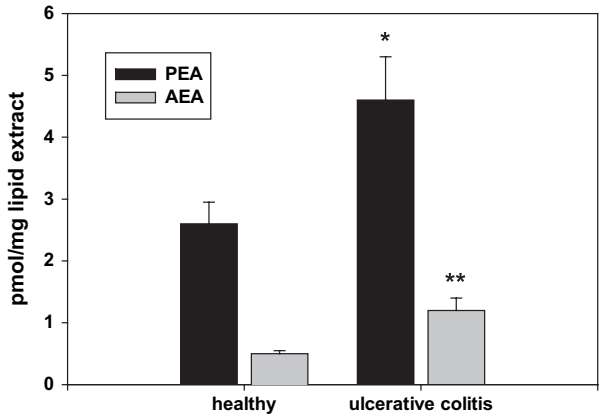

Fig. 3. *N*-Palmitoyl-ethanolamine (PEA) and anandamide (AEA) levels ($\pm$SEM, $N=8-10$) in bioptic colonic samples from control subjects and patients with ulcerative colitis. $^{*}P<0.05$; $^{**}P<0.0005$, as calculated by ANOVA followed by the Bonferroni's test. See text for details on the patients.

the rat (Helyes et al., 2003). Thus, an analgesic and anti-inflammatory action under conditions of inflammatory, visceral and neuropathic pain has been demonstrated for this compound. PEA, together with the endocannabinoid AEA, with which it shares many of the pharmacological actions mentioned above, was found to be produced from both neuronal and immune cells (Di Marzo et al., 1994; Bisogno et al., 1997), and therefore it represents an ideal mediator for neuro-immune interactions during neurogenic pain. Nevertheless, only scant studies have appeared in the literature describing changes of PEA levels during pathological conditions in either experimental models or in the clinic. Thus, it has been shown that the levels of PEA are enhanced in one clinical case of stroke (Schabitz et al., 2002) and following ischemia and stroke in animals (Moesgaard et al., 2000; Berger et al., 2004), in agreement with its putative neuroprotective action (Skaper et al., 1996; Lambert et al., 2001; Hansen et al., 2002 for a review), and during cadmium-induced rat testis inflammation (Kondo et al., 1998). By converse, to the best of our knowledge, no study has been carried out to investigate changes of PEA levels under conditions of inflammatory, visceral and neuropathic pain, except for one study showing no change in PEA (and AEA) levels during the formalin-induced nocifensive response in the rat (Beaulieu et al., 2000). For this reason, we have carried out the present clinical and experimental studies aiming at assessing whether tissue or blood levels of PEA correlate with diverse peripheral neuropathic and visceral pain conditions.

Chronic low back pain (CLBP) is defined as pain, muscle tension, or stiffness localized below the posterior costal margin and above the inferior gluteal folds. CLBP is very prevalent and has numerous causes. Its clinical features are best explained by both mechanical and biochemical (e.g. inflammatory mediator-mediated) abnormalities (Borenstein, 2001; Saal, 1995). There are numerous treatment approaches addressing CLBP, all generally having less than ideal outcomes. Considering the large region involved in CLBP and its diverse origins, effective manual treatment for this condition cannot be rigidly standardized but instead treatment principles can be consistently applied based on the specific collection of findings present for each participant. Such an approach, as described in previously published studies (Mills et al., 2003; Andersson et al., 1999), was utilized in this study. The treatments given were consistent in that all subjects in both groups received articulatory, muscle energy, indirect and soft tissue techniques to dysfunctions found in the cervical spine, rib cage, and low back within a uniform length of time. The CLBP subjects received a greater percentage of treatment to the lumbosacral region since there was more dysfunction present in that area. Assessing OMT has been hampered by a lack of objective outcomes. With age and gender matched controls, the primary differences between the groups were the presence or absence of CLBP and the severity of dysfunction particularly in the lumbar and sacral regions. The present study indicates that daily baseline PEA blood levels are stable and do not differentiate between control and CLBP groups. Although the small and unavoidable minor differences in the treatments received by the two groups may have biased in part some of the results observed, it appears that OMT differentially increased blood PEA concentrations in control and CLBP groups. While the OMT-induced 20% increase in the mean blood PEA levels over the corresponding baseline value in no low back pain subjects failed to achieve significance, the observed increase (30%) in the level of its median was significant. Furthermore, relatively significantly larger increases (56 and 59%, respectively) in both mean and median PEA, but not AEA, levels were induced by OMT in the blood of patients suffering from low back pain. These findings seem to suggest that osteopathic manipulation causes significantly greater increases (193%) in PEA levels in persistent pathological inflammatory conditions such as chronic low back pain relative to the normal physiological state. Accordingly, it has been suggested that PEA becomes an effective analgesic only when an inflammatory state is established (Jaggar et al., 1998a, 1998b).

A second type of neuroinflammatory pain that may involve PEA is diabetic neuropathy. This is a common complication of diabetes mellitus affecting to an equal extent both type 1 and type 2 patients, and is associated with considerable morbidity (painful polyneuropathy, neuropathic ulceration) and even mortality (autonomic neuropathy) (Vinik et al., 1992). The epidemiology and natural history of diabetic neuropathy is still largely uncertain, mostly due to confusion in the definition and measurement of this disorder. Various mechanisms, including metabolic and vascular abnormalities, have been proposed to explain the progressive pathological changes that occur in peripheral nerve and eventually result in the clinical manifestations of diabetic neuropathy. A typical experimental model of diabetic neuropathy is represented by the streptozotocin-induced autoimmune diabetes in rodents, which is followed by hyperalgesia and allodynia. This may be partly due to sensitization/up-regulation of TRPV1 receptors in sensory afferents (Kamei et al., 2001; Rashid et al., 2003; Hong and Wiley, 2004), and can be counteracted by stimulation of cannabinoid CB_1 receptors (Ellington et al., 2002). Given the capability of PEA to influence both TRPV1 and cannabinoid receptor-mediated signalling, we reasoned that it would be of interest to assess whether PEA levels in the paw skin of streptozotocin-treated, hyperalgesic mice were altered in comparison with those of untreated mice. We found a significant elevation of PEA, but not AEA, levels during this

pathological condition. Unlike AEA (Ellington et al., 2002), which was inactive, the pharmacological effects of PEA in this model have not been investigated, although previous experiments carried out in other models of neuropathic pain (Mazzari et al., 1995; Helyes et al., 2003) suggest that PEA might play an analgesic action also in this case. This might be effected either via yet-to-be characterized CB_2-like receptors on sensory efferents and mast cells, to inhibit the production of inflammatory and nociceptive mediators, or through enhanced activation of AEA actions on TRPV1, with subsequent desensitization of these receptors (De Petrocellis et al., 2001). Furthermore, since a PPAR-α/γ agonist was shown to be beneficial against the pathogenesis and complications of type 2 diabetes (Shibata et al., 2000), it is possible that PEA is produced also to counteract this disorder through its direct action at PPAR-α (Lo Verme et al., 2005).

Finally, given the possible role of PEA in visceral pain and inflammation, we have examined its levels in patients with ulcerative colitis, an inflammatory disorder of the gut whose exact pathogenesis has not yet been conclusively established. Alterations of the immune system, possibly induced by gut infections, psychological causes, and participation of the central nervous system have been proposed (for examples see Geissler et al., 1995; Caprilli et al., 2000), together with the involvement of autonomic and sensory neurons in both the symptoms (Straub et al., 2002; Arnold et al., 2003; Abo and Kawamura, 2002) and etiology (Peck and Wood, 2000; Kemler et al., 1999) of this and other inflammatory bowel disorders (IBDs). Mast cells, which are deeply involved in ulcerative colitis (Winterkamp et al., 2002; Kim et al., 2003), produce high amounts of PEA (Bisogno et al., 1997), which potently inhibits mouse small intestine motility (Capasso et al., 2001) and visceral pain, thus suggesting for this mediator a protective action during IBDs. Blockade of the vanilloid TRPV1 receptor in enteric sensory afferents (Ward et al., 2003; Coutts, 2004), one of the putative molecular targets of PEA, was proposed as a possible therapeutic strategy to treat IBDs (Kihara et al., 2003; Geppetti and Trevisani, 2004). Also cannabinoid CB_1 receptors, whose activity can be indirectly modulated by PEA (see Section 1), have been implicated in gut inflammation, and FAAH-deficient mice, which contain higher tissue levels of FAAH NAE substrates such as AEA and PEA, were found to be less sensitive to developing intestinal inflammation (Izzo et al., 2001; Massa et al., 2004). CB_1 receptors are present in both autonomic and sensory cholinergic neurons, with extensive co-localization with TRPV1 receptors (Kulkarni-Narla and Brown, 2001; Coutts, 2004), as well as in epithelial cells of the mucosa (Izzo et al., 2003; Ligresti et al., 2003). Our finding of significantly elevated levels of PEA in colonic biopsies of patients with ulcerative colitis strengthens the hypothesis that this compound participates in the control of visceral pain and intestine motility during neuroinflammatory conditions. Since we have observed that the levels also of AEA were significantly elevated in this case, it can be hypothesized that the molecular mode of action of PEA in this pathological state is that of a "reinforcer" of AEA actions at CB_1 and TRPV1 receptors, as previously suggested for this compound in studies carried out in vitro (Di Marzo et al., 2001a; De Petrocellis et al., 2001).

In conclusion, we have shown here that PEA levels are enhanced in peripheral blood or tissues affected by neuropathic and inflammatory pain, both in animals and humans. Although, due to technical reasons, we could not investigate whether increased PEA levels were due to increased biosynthesis or decreased degradation, our new data support the hypothesis that PEA is an endogenous mediator potentially affording protection against inflammatory and neuropathic pain, and should foster further studies on its potential use as a therapeutic drug.

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