



## The search for the palmitoylethanolamide receptor

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### Abstract

Palmitoylethanolamide (PEA), the naturally occurring amide of ethanolamine and palmitic acid, is an endogenous lipid that modulates pain and inflammation. Although the anti-inflammatory effects of PEA were first characterized nearly 50 years ago, the identity of the receptor mediating these actions has long remained elusive. We recently identified the ligand-activated transcription factor, peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ), as the receptor mediating the anti-inflammatory actions of this lipid amide. Here we outline the history of PEA, starting with its initial discovery in the 1950s, and discuss the pharmacological properties of this compound, particularly in regards to its ability to activate PPAR- $\alpha$ .

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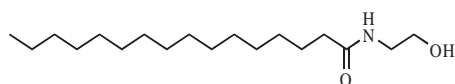
**Keywords:** Palmitoylethanolamide; Peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ); Lipid; Inflammation; Pain; Epilepsy

### Discovery of PEA

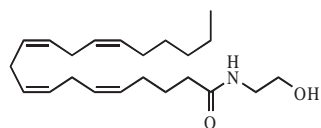
The discovery of naturally occurring fatty acid ethanolamides (FAEs) (Fig. 1) stems from an interesting clinical finding in the early 1940s, when investigators noted that supplementing the diets of

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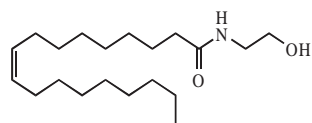
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Palmitoylethanolamide (PEA)



Arachidonylethanolamide (AEA)



Oleoylethanolamide (OEA)

Fig. 1. Chemical structures of various fatty acid ethanolamides, palmitoylethanolamide, oleoylethanolamide, and arachidonylethanolamide (anandamide).

underprivileged children with dried chicken egg yolk prevented recurrences of rheumatic fever, despite continued streptococcal infections (Coburn and Moore, 1943). In a continuation of this research, it was demonstrated that lipid fractions purified from egg yolk (Coburn et al., 1954; Long and Martin, 1956), as well as peanut oil and soybean lecithin (Long and Miles, 1950), exerted anti-allergic effects in the guinea pig (see Fig. 2 for a timeline of events). Soon thereafter, PEA (*N*-(2-hydroxyethyl)hexadecanamide, *N*-palmitoylethanolamine, LG 2110/1) was isolated as the agent responsible for these anti-inflammatory properties (Kuehl et al., 1957). This work ultimately led to its identification in mammalian tissues in 1964 (Bachur et al., 1965).

### PEA in the clinic

The years following the discovery of PEA's anti-inflammatory effects (Benvenuti et al., 1968; Perlik et al., 1971) witnessed a series of interesting clinical studies on this compound, which was tested under the trade name of Impulsin by the Czech pharmaceutical company, SPOFA. It is not clear what prompted these clinical trials; nonetheless, PEA was found to reduce the severity and duration of symptoms caused by the influenza virus in school children and soldiers (Kahlich et al., 1979; Masek et al., 1974). The apparent success of these trials led to the clinical use of PEA in the former Czechoslovakia for acute respiratory diseases during the late 1970s. After several years on the market, PEA was withdrawn from clinical use for unknown reasons, which do not appear to be related to toxicity. PEA is currently used to restore skin reactivity in animals in a veterinary composition, marketed under the trade name Redonyl® by the Italian company, Innovet. The termination of PEA in the clinic and the failure to identify its molecular target caused a period of research stasis that lasted more than 20 years. It was the discovery that another fatty acid amide, anandamide (arachidonoy-

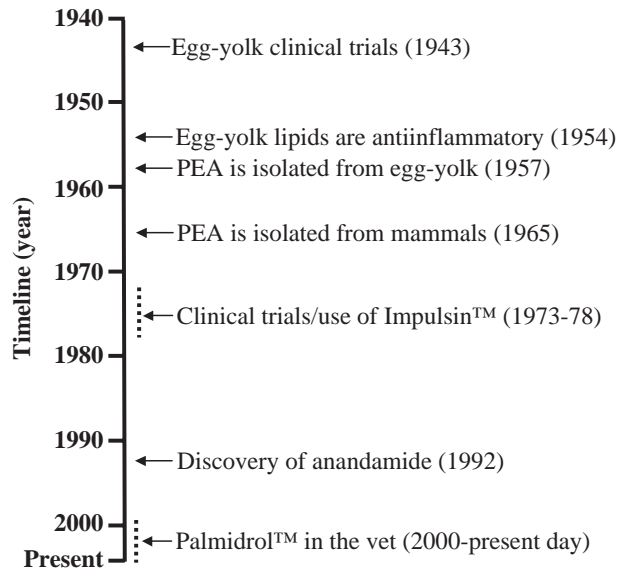


Fig. 2. PEA: a historical perspective. The discovery of PEA dates back to 1957, when it was found to be the active anti-inflammatory constituent of egg yolk. PEA was identified in mammalian tissues in 1965 and was used in the clinic during the 1970s for the treatment of influenza. PEA is currently used as a veterinary anti-inflammatory medication as part of a nutritional composition sold under the trade name of Redonyl®.

lethanolamide), is an endogenous agonist for cannabinoid receptors (Devane et al., 1992) that caused a renewal of interest in PEA.

## Cannabinoid receptors

Cannabinoid receptors, the molecular targets of the psychoactive component in marijuana, delta-9-tetrahydrocannabinol (THC), belong to the G-protein-coupled receptor super family (Matsuda et al., 1990; Munro et al., 1993). Activation of either CB<sub>1</sub> receptors (which are primarily localized to the brain but are also present in the periphery) or CB<sub>2</sub> receptors (which are predominantly found in cells of the immune system) results in G<sub>i</sub>-mediated reduction of adenylyl cyclase activity and subsequent inhibition of cAMP-mediated responses. In the brain, CB<sub>1</sub> receptors are mostly found on presynaptic endings of GABAergic and glutamatergic neurons (for a comprehensive review, see Piomelli, 2003). Behavioral effects produced by activation of central CB<sub>1</sub> receptors include reduced locomotion and body temperature, catalepsy, alterations in memory, sedation, and euphoria—effects that are also commonly observed with recreational marijuana use (Adams and Martin, 1996). In the periphery, activation of CB<sub>1</sub> receptors on nerve endings produces analgesia (Calignano et al., 1998; Jaggar et al., 1998), reduces cough (Calignano et al., 2000), and inhibits intestinal transit (Capasso et al., 2001). The functions served by CB<sub>2</sub> receptors are less clear, but recent evidence suggests that they may modulate immune cell function (Klein et al., 2001) to produce peripheral analgesia and anti-inflammation (Ibrahim et al., 2003; Malan et al., 2003; Quartilho et al., 2003)—although the mechanism by which these phenomena occur remains enigmatic.

## Pharmacological properties of PEA

Since the discovery of anandamide, the properties of PEA have been explored with growing interest (Fig. 3). In addition to its known anti-inflammatory activity, PEA also produces analgesia (Calignano et al., 1998, 2001; Jaggar et al., 1998), anti-epilepsy, and neuroprotection (Franklin et al., 2003; Lambert et al., 2001; Sheerin et al., 2004; Skaper et al., 1996). PEA also inhibits food intake (Rodríguez de Fonseca et al., 2001), reduces gastrointestinal motility (Capasso et al., 2001) and cancer cell proliferation (De Petrocellis et al., 2002; Di Marzo et al., 2001), and finally protects the vascular endothelium in the ischemic heart (Bouchard et al., 2003).

Following the early experiments in the 1950s, a series of more recent studies has shown that PEA inhibits mast cell degranulation (Aloe et al., 1993; Mazzari et al., 1996) and pulmonary inflammation in mice (Berdyshev et al., 1998). These effects have been tentatively linked to reduced nitric oxide production by macrophages (Ross et al., 2000) and/or neutrophil influx (Farquhar-Smith and Rice, 2003). For example, in one study, animals treated with PEA for 3 days (10 mg kg<sup>-1</sup>, orally, once daily) following a carrageenan-induced inflammatory insult displayed significantly lower levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) (Costa et al., 2002)—a genomic action reminiscent of steroidal anti-inflammatory drugs. Other studies have demonstrated that PEA reduces inflammation in a matter of hours (Conti et al., 2002; LoVerme et al., 2005), implying that a non-genomic mechanism of action may also exist.

Broad-spectrum analgesia by PEA has been documented in a variety of pain models. PEA reduces pain behaviors elicited by formalin (Calignano et al., 1998; Jaggar et al., 1998), magnesium sulfate (Calignano et al., 2001), carrageenan (Conti et al., 2002; Mazzari et al., 1996), nerve growth factor (Farquhar-Smith and Rice, 2003), and turpentine (Farquhar-Smith and Rice, 2001; Jaggar et al., 1998). Moreover, PEA was found to inhibit hyperalgesia after sciatic nerve ligation, a model of neuropathic pain (Helyes et al., 2003). Because PEA-induced analgesia is rapid and precedes the compound's anti-inflammatory actions, it has been suggested that PEA may function as an endogenous regulator of nociception (Calignano et al., 1998).

Less understood are the functions of PEA in the central nervous system (CNS), where PEA is present at high levels (Cadas et al., 1997). Increasing evidence points to an antiepileptic and neuroprotective action of PEA (Lambert et al., 2001; Sheerin et al., 2004). For example, in one study, intraperitoneal administration of the compound was shown to inhibit electroshock-induced and chemically induced seizures with a half-maximally effective dose (ED<sub>50</sub>) of 9 mg kg<sup>-1</sup>, i.p. (Lambert et al., 2001). Other neuroprotective actions have also been reported; for example, in a separate study, PEA dose-dependently protected cultured mouse cerebellar granule cells from glutamate toxicity (Skaper et al., 1996). In yet

<u>Anti-Inflammation</u>	<u>Analgesia</u>	<u>Neuroprotection</u>	<u>Anti-viral</u>
↓ Mast cell activation	↓ Acute pain	↓ Convulsions	↓ Incidences of acute respiratory diseases
↓ iNOS expression	↓ Inflammatory pain	↓ Excitotoxicity	
↓ COX-2 expression	↓ Neuropathic pain		
↓ Neutrophil influx	▪ SR144528 inhibitable		

Fig. 3. Pharmacological consequences of PEA administration. PEA produces a broad range of actions including anti-inflammation, analgesia, neuroprotection, and anticonvulsant activity. It may also exert prophylactic actions towards respiratory tract infections.

another study, PEA reduced histamine-induced cell death in hippocampal cultures (Skaper et al., 1996). Finally, PEA was more recently shown to enhance microglial cell motility (Franklin et al., 2003).

### PEA biosynthesis and inactivation

Unlike classical neurotransmitters and hormones which are stored in and released from intracellular secretory vesicles, the production of FAEs occurs through on-demand synthesis within the lipid bilayer (Cadas et al., 1996; Schmid et al., 1990). In mammalian tissues, two concerted and independent biochemical reactions are responsible (Fig. 4). The first is the transfer of a fatty acid from membrane-bound phospholipids to phosphatidylethanolamine (PE), catalyzed by a calcium ion and cyclic-AMP regulated *N*-acyltransferase (NAT), to form the FAE precursor *N*-acyl phosphatidylethanolamine (NAPE). Different FAE precursors are generated according to which fatty acid is initially transferred to PE (i.e., the initial transfer of palmitic acid will yield a PEA precursor, while that of arachidonic acid will yield an anandamide precursor). The second step in FAE synthesis is the cleavage of membrane-bound NAPE to release free PEA, which is mediated by a NAPE-specific phospholipase D (PLD). This lipid hydrolase shares little sequence homology to other members of the PLD family and recognizes multiple NAPE species, producing PEA along with other FAEs (Okamoto et al., 2004).

Alternatively, a separate mechanism of synthesis has been proposed involving a similar two-step reaction: (1) the hydrolysis of NAPE to *N*-palmitoyl-lysoPE (lyso-NAPE) by soluble phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) and (2) the subsequent cleavage of lyso-NAPE by a lysophospholipase D (lyso-PLD) (Natarajan et al., 1984). The activities of these two enzymes are highest in the stomach, brain, and testis (Sun et al., 2004). The relative contribution of each of these synthetic pathways is unknown at present.

PEA inactivation primarily consists of its intracellular hydrolysis by lipid hydrolases (Fig. 4) (Schmid et al., 1985). One of these enzymes, called fatty acid amide hydrolase (FAAH), has been molecularly cloned (Cravatt et al., 1996) and extensively characterized (Bracey et al., 2002), and selective inhibitors that block its activity *in vivo* have been developed (Kathuria et al., 2003). A second enzyme, referred to as PEA-preferring acid amidase (PAA), has also been identified (Ueda et al., 2001).

FAAH, a membrane-bound intracellular serine hydrolase, for which PEA is an excellent substrate (Désarnaud et al., 1995; Hillard et al., 1995; Ueda et al., 1995), is present in all mammalian tissues, but is particularly abundant in brain and liver (Cravatt and Lichtman, 2002). In fact, mice lacking the *faah* gene have dramatically reduced PEA hydrolysis and increased PEA levels in brain and liver tissues (Cravatt et al., 2004; Lichtman et al., 2002; Patel et al., 2004). In contrast to FAAH, PAA activity is most abundant in the rodent intestine, spleen, and lung (Ueda et al., 2001). PAA recognizes all FAEs, suggesting that it may play a broad role in the deactivation of these compounds by intact cells; however, in the presence of detergent, this activity displays a marked preference for PEA as a substrate (Ueda et al., 2001).

### Physiological regulation of PEA levels

The physiological stimuli that regulate PEA levels in mammalian tissues are largely unknown; however, multiple studies indicate that this lipid accumulates during cellular stress, particularly following tissue injury. For example, PEA and other FAEs increase postmortem in the pig and mouse brain (Patel et

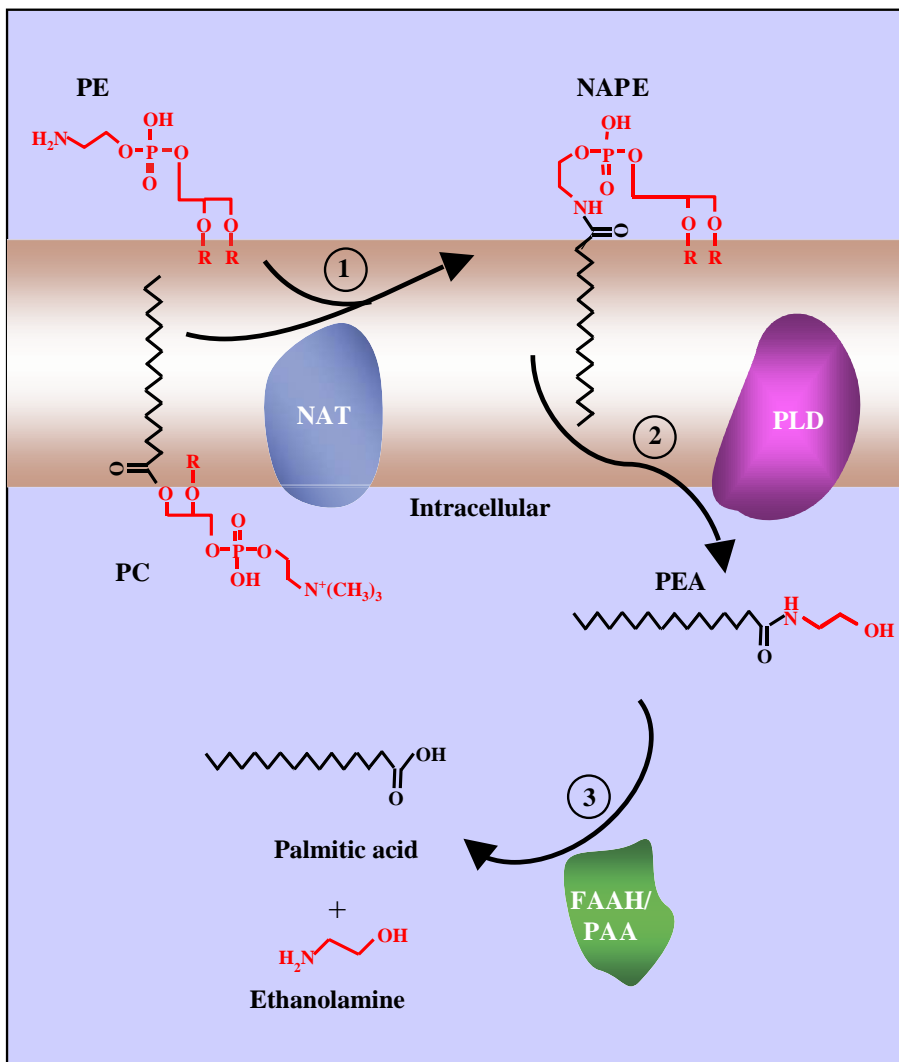


Fig. 4. PEA biosynthesis and inactivation. The biosynthesis of PEA occurs in a two steps. (1) The calcium- and cAMP-dependent transfer of palmitic acid from phosphatidylcholine (PC) to phosphatidylethanolamine (PE) to form *N*-acylphosphatidylethanolamine (NAPE). (2) The cleavage of NAPE to release PEA, mediated by a NAPE-specific phospholipase D (PLD). (3) PEA is hydrolyzed by fatty acid amide amidohydrolase (FAAH) or PEA-preferring acid amidase (PAA) to form palmitic acid and ethanolamine.

al., 2004; Schmid et al., 1995). In rat testis, PEA levels were elevated nearly 40-fold following  $CdCl_2$ -induced tissue degeneration (Kondo et al., 1998). Similar elevations of PEA have been observed in the ischemic heart (Epps et al., 1979) and brain (Franklin et al., 2003; Schabitz et al., 2002). Isolated cells, including brain neurons, macrophages (J774), basophils (RBL-2H3), and neuroblastoma ( $N_{18}TG_2$ ) cells, have also been reported to generate PEA in response to ionomycin or digestion with exogenous PLD (Bisogno et al., 1997; Cadas et al., 1997; Di Marzo et al., 1994). PEA levels also increase in response to ultraviolet-B irradiation in mouse epidermal (JB6  $P^+$ ) cells (Berdyshev et al., 2000).

As discussed, most studies have focused on PEA production *in vitro*, or when performed *in vivo*, aggressive inflammatory agents were used, which in some cases induce extensive tissue degeneration (Kondo et al., 1998). In contrast, the regulation of PEA during inflammatory states is less clear. Capasso et al. (2001) demonstrated that during croton oil-induced intestine inflammation, levels of PEA significantly decreased in the mouse small intestine. While this manuscript was under review, Darmani et al. (2005) reported contrasting results demonstrating that PEA levels increased during neuroinflammatory conditions in both humans and rats. We found that phorbol esters/protein kinase C activators that exert profound proinflammatory effects decrease PEA levels in mouse abdominal skin. Using an established protocol (LoVerme et al., 2005; Sheu et al., 2002), we applied the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (0.06% wt vol<sup>-1</sup>, 50  $\mu$ l) in acetone to the abdomens of male CD1 mice and quantified PEA levels by high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS) in inflamed skin 4 and 18 h following TPA administration. PEA levels in vehicle (acetone)-treated skin were comparable to those measured in untreated skin, whereas PEA levels in TPA-treated animals were twofold lower (Fig. 5). These results suggest that the reduction of PEA levels may contribute to the normal inflammatory response, albeit in an unknown capacity.

## The PEA receptor

Despite its potential clinical significance, the cellular receptor responsible for the actions of PEA has long remained unidentified, and a great deal of controversy has surrounded its identity. The structural and functional similarities between anandamide and PEA first suggested that these two lipid mediators might share the same receptor. In support of this idea, PEA was initially reported to displace the binding of the high-affinity cannabinoid agonist [<sup>3</sup>H]WIN55,212-2 with a half-maximal inhibitory concentration (IC<sub>50</sub>) of 1.0 nM from RBL-2H3 cell membranes, which are known to express CB<sub>2</sub> mRNA (Facci et al., 1995). However, these results have not been subsequently replicated (Jacobsson and Fowler, 2001; Lambert et al., 2002; Lambert and Di Marzo, 1999; Showalter et al., 1996; Sugiura et al., 2000) and it is

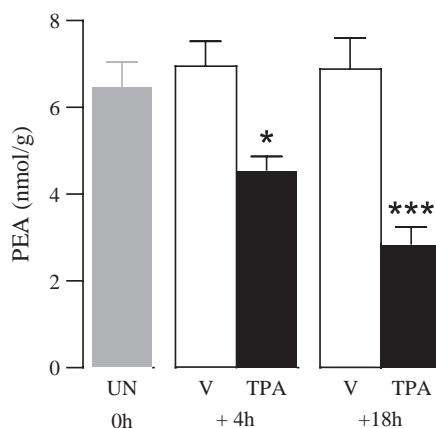


Fig. 5. Effects of inflammation on endogenous PEA levels. PEA levels measured in untreated (UN), vehicle (acetone)-treated (V), or TPA (12-*O*-tetradecanoylphorbol-13-acetate, 0.06% wt vol<sup>-1</sup>, 50  $\mu$ l)-treated abdominal skin of male CD1 mice (25 g), 0, 4, or 18 h following administration. \* $p < 0.05$ , \*\*\* $p < 0.001$  vs. V ( $n = 10$ – $11$ ); ANOVA followed by a Bonferroni post test.



now generally accepted that PEA does not bind to CB<sub>2</sub> receptors. Adding to the PEA mystery, the CB<sub>2</sub> antagonist/inverse agonist SR144528 inhibits many, but not all, of the pharmacological actions of PEA (Calignano et al., 1998, 2001; Conti et al., 2002; Jaggar et al., 1998). In particular, the analgesic actions of PEA are blocked by SR144528 (Calignano et al., 1998; Farquhar-Smith and Rice, 2001, 2003; Helyes et al., 2003), whereas its anti-peristaltic effects are not (Capasso et al., 2001). Further confounding the issue, it appears that the anti-inflammatory actions of PEA are only sensitive to SR144528 in acute models of inflammation (Conti et al., 2002; Costa et al., 2002; LoVerme et al., 2005).

In light of these apparent discrepancies, several possible scenarios have been proposed to describe the mechanism of action of PEA (Fig. 6). The first possibility is that PEA binds to an unidentified receptor for which SR144528 is a functional antagonist (i.e., a compound that either directly interacts with the receptor, or indirectly blocks a downstream effector of this receptor (CB<sub>2</sub> receptors)). A second possibility is that PEA acts through a so-called “entourage effect” (Ben-Shabat et al., 1998; Lambert and Di Marzo, 1999). According to this theory, PEA may increase anandamide levels in tissues by competing with this compound for FAAH-mediated hydrolysis, resulting in a potentiation of anandamide’s actions. However, “entourage effects” have only been observed *in vitro*. In contrast, administration of PEA *in vivo* has the opposite result of decreasing endogenous anandamide levels (LoVerme et al., 2005). Finally, Di Marzo et al. (2001) suggested that in human breast cancer cells, PEA may work by inhibiting FAAH expression, which should lead to an increase in anandamide levels. This hypothesis does not account, however, for the very rapid actions of PEA, which occur within minutes of its administration.

### PPAR- $\alpha$ is the anti-inflammatory target of PEA

Prior work in our laboratory has shown that OEA, a lipid amide structurally related to PEA (Fig. 1), regulates feeding behavior and lipid metabolism in rodents by activating the nuclear receptor peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ) (Fu et al., 2003; Gaetani et al., 2003; Guzmán et al., 2004;

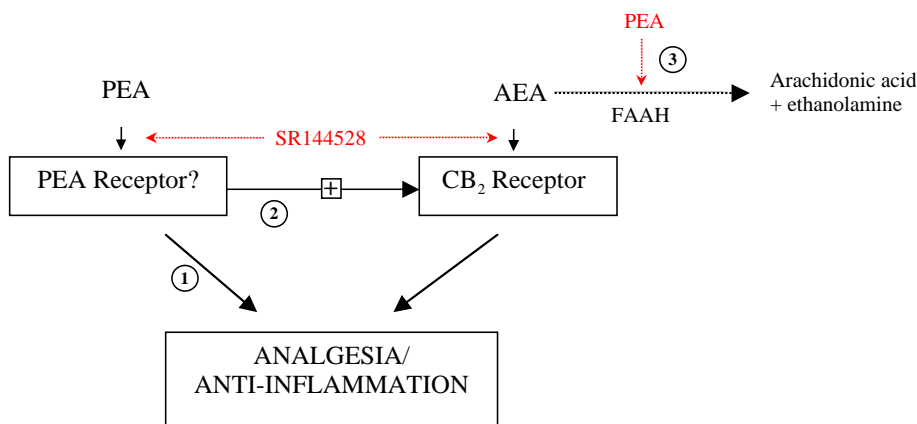


Fig. 6. Proposed mechanisms of action for PEA. PEA may exert its effects through an unknown receptor, which may either be sensitive to SR144528 (1) or engage CB<sub>2</sub> receptors as downstream effectors (2). (3) PEA may act as an “entourage compound,” effectively inhibiting FAAH activity, and thus increasing local anandamide levels (see text for further details).



Rodríguez de Fonseca et al., 2001). A growing body of evidence has implicated PPAR- $\alpha$  in the control of inflammatory responses and these receptors are expressed in various cells of the immune system (for review, see Daynes and Jones, 2002). Mice lacking the gene encoding for PPAR- $\alpha$  display prolonged inflammatory responses (Devchand et al., 1996) and synthetic agonists of this receptor cause profound anti-inflammatory effects (Chinetti et al., 2000; LoVerme et al., 2005; Sheu et al., 2002). These effects are accompanied by reduced expression of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and various inflammatory cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), and tumor necrosis factor alpha (TNF- $\alpha$ )—effects reminiscent of those executed by PEA (Costa et al., 2002). In addition, PPAR- $\alpha$  activators have been shown to inhibit monocyte differentiation and neutrophil function, including endothelial extravasation. Although the precise mechanism of PPAR- $\alpha$  anti-inflammation is unclear, the receptor has been linked to a non-genomic inhibition of the pro-inflammatory signaling pathways mediated by nuclear factor  $\kappa$ B (NF- $\kappa$ B) and activated protein-1 (AP-1). Thus, multiple lines of evidence suggest that PPAR- $\alpha$  receptors and their ligands are important modulators of the inflammatory process.

Recent evidence has shown that, like its structural analogue OEA, PEA directly activates PPAR- $\alpha$  with a half-maximal effective concentration (EC<sub>50</sub>) of approximately 3  $\mu$ M (LoVerme et al., 2005)—a potency comparable to that of the synthetic PPAR- $\alpha$  agonist Wy-14643, which produces robust anti-inflammatory actions (Sheu et al., 2002). However, unlike its structural analogue OEA, which activates both PPAR- $\alpha$  and PPAR- $\beta/\delta$  (Fu et al., 2003), PEA does not engage either PPAR- $\beta/\delta$  or PPAR- $\gamma$  (LoVerme et al., 2005). Moreover, when administered in a topical formulation to inflamed mouse skin, PEA inhibits inflammation and induces the expression of PPAR- $\alpha$  mRNA—an effect characteristic of synthetic high-affinity PPAR- $\alpha$  ligands (Fu et al., 2003; LoVerme et al., 2005).

Most importantly, PEA does not elicit anti-inflammatory effects in mutant PPAR- $\alpha$ -null mice (PPAR- $\alpha$ <sup>-/-</sup> mice). Indeed, when assessed in either the carrageen hindpaw or phorbol ester ear pinna tests, PEA reduced inflammation in wild-type, but not in PPAR- $\alpha$ <sup>-/-</sup> mice (LoVerme et al., 2005). Collectively, these findings indicate that PEA produces its anti-inflammatory actions by acting as a PPAR- $\alpha$  agonist (Fig. 7).

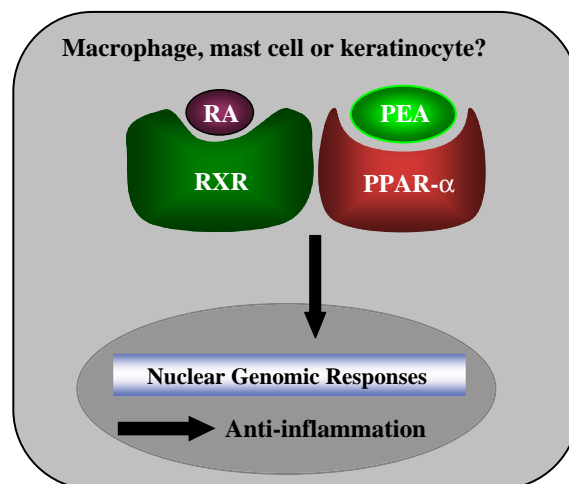


Fig. 7. PEA activates PPAR- $\alpha$  to produce its anti-inflammatory effects. According to this hypothetical model, ligation of PPAR- $\alpha$  by PEA induces a heterodimerization event with ligated retinoic acid receptors (RXR) forming the activated receptor complex, which translocates to the nucleus to bind a PPAR- $\alpha$  response element (PPRE) and reduce inflammation.

## Concluding remarks

The discovery that PPAR- $\alpha$  mediates the anti-inflammatory effects of PEA (LoVerme et al., 2005) raises several important questions. Is PPAR- $\alpha$  responsible for all other effects of PEA, including analgesia and neuroprotection? And if so, what is the relationship between PPAR- $\alpha$  and CB<sub>2</sub> receptors? How does endogenous PEA interact with PPAR- $\alpha$  to regulate physiological inflammatory processes? Our data suggest that part of this response may involve a reduction in PEA levels in inflamed tissues, which might in turn serve to remove an endogenous anti-inflammatory tone. Alternatively, it has been reported that PEA levels may increase during inflammation to produce local anti-inflammatory and analgesic actions (Darmani et al., 2005). These possibilities remain, however, to be explored. A final question is whether non-cannabinoid FAEs, such as stearoylethanolamide (Maccarrone et al., 2002; Terrazzino et al., 2004), also activate PPAR- $\alpha$  or related transcription factors. As these questions are progressively answered, we may not only gain insights on the PEA signaling system, but also identify new potential targets for analgesic and anti-inflammatory medicines.

## References

- Adams, I.B., Martin, B.R., 1996. Cannabis: pharmacology and toxicology in animals and humans. *Addiction* 91 (11), 1585–1614.
- Aloe, L., Leon, A., Levi-Montalcini, R., 1993. A proposed autacoid mechanism controlling mastocyte behaviour. *Agents and Actions* 39, C145–C147 (Spec No.).
- Bachur, N.R., Masek, K., Melmon, K.L., Udenfriend, S., 1965. Fatty acid amides of ethanolamine in mammalian tissues. *Journal of Biological Chemistry* 240 (3), 1019–1024.
- Ben-Shabat, S., Frideri, E., Sheskin, T., Tamiri, T., Rhee, M.H., Vogel, Z., Bisogno, T., De Petrocellis, L., Di Marzo, V., Mechoulam, R., 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *European Journal of Pharmacology* 353 (1), 23–31.
- Benvenuti, F., Lattanzi, F., De Gori, A., Tarli, P., 1968. Activity of some derivatives of palmitoylethanolamide on carragenin-induced edema in the rat paw. *Bollettino della Società Italiana di Biologia Sperimentale* 44 (9), 809–813.
- Berdyshev, E., Boichot, E., Corbel, M., Germain, N., Lagente, V., 1998. Effects of cannabinoid receptor ligands on LPS-induced pulmonary inflammation in mice. *Life Sciences* 63 (8), L125–L129.
- Berdyshev, E.V., Schmid, P.C., Dong, Z., Schmid, H.H., 2000. Stress-induced generation of *N*-acylethanolamines in mouse epidermal JB6 P<sup>+</sup> cells. *Biochemical Journal* 346 (Part 2), 369–374.
- Bisogno, T., Maurelli, S., Melck, D., De Petrocellis, L., Di Marzo, V., 1997. Biosynthesis, uptake, and degradation of anandamide and palmitoylethanolamide in leukocytes. *Journal of Biological Chemistry* 272 (6), 3315–3323.
- Bouchard, J.F., Lepicier, P., Lamontagne, D., 2003. Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sciences* 72 (16), 1859–1870.
- Bracey, M.H., Hanson, M.A., Masuda, K.R., Stevens, R.C., Cravatt, B.F., 2002. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* 298 (5599), 1793–1796.
- Cadas, H., Gaillet, S., Beltramo, M., Venance, L., Piomelli, D., 1996. Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *Journal of Neuroscience* 16 (12), 3934–3942.
- Cadas, H., di Tomaso, E., Piomelli, D., 1997. Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. *Journal of Neuroscience* 17 (4), 1226–1242.
- Calignano, A., La Rana, G., Giuffrida, A., Piomelli, D., 1998. Control of pain initiation by endogenous cannabinoids. *Nature* 394 (6690), 277–281.
- Calignano, A., Kátóna, I., Désarnaud, F., Giuffrida, A., La Rana, G., Mackie, K., Freund, T.F., Piomelli, D., 2000. Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* 408 (6808), 96–101.
- Calignano, A., La Rana, G., Piomelli, D., 2001. Antinociceptive activity of the endogenous fatty acid amide, palmitoylethanolamide. *European Journal of Pharmacology* 419 (2–3), 191–198.

- Capasso, R., Izzo, A.A., Fezza, F., Pinto, A., Capasso, F., Mascolo, N., Di Marzo, V., 2001. Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. *British Journal of Pharmacology* 134 (5), 945–950.
- Chinetti, G., Fruchart, J.C., Staels, B., 2000. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflammation Research* 49 (10), 497–505.
- Coburn, A., Moore, L., 1943. *American Journal of Diseases of Children* 65 (744).
- Coburn, O.H., Graham, C.E., Haninger, J., 1954. The effect of egg yolk in diets on anaphylactic arthritis (passive Arthus phenomenon) in the guinea pig. *Journal of Experimental Medicine* 100 (5), 425–435. (November 1).
- Conti, S., Costa, B., Colleoni, M., Parolaro, D., Giagnoni, G., 2002. Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. *British Journal of Pharmacology* 135 (1), 181–187.
- Costa, B., Conti, S., Giagnoni, G., Colleoni, M., 2002. Therapeutic effect of the endogenous fatty acid amide, palmitoylethanolamide, in rat acute inflammation: inhibition of nitric oxide and cyclo-oxygenase systems. *British Journal of Pharmacology* 137 (4), 413–420.
- Cravatt, B.F., Lichtman, A.H., 2002. The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chemistry and Physics of Lipids* 121 (1–2), 135–148.
- Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B., 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384 (6604), 83–87.
- Cravatt, B.F., Saghatelian, A., Hawkins, E.G., Clement, A.B., Bracey, M.H., Lichtman, A.H., 2004. Functional disassociation of the central and peripheral fatty acid amide signaling systems. *Proceedings of the National Academy of Sciences of the United States of America* 101 (29), 10821–10826.
- Darmani, N.A., Izzo, A.A., Degenhardt, B., Valenti, M., Scaglione, G., Capasso, R., Sorrentini, I., Di Marzo, V., 2005. Involvement of the cannabimimetic compound, *N*-palmitoylethanolamine, in inflammatory and neuropathic conditions: review of the available pre-clinical data, and first human studies. *Neuropharmacology* 48 (8), 1154–1163.
- Daynes, R.A., Jones, D.C., 2002. Emerging roles of PPARs in inflammation and immunity. *Nature Reviews. Immunology* 2 (10), 748–759.
- De Petrocellis, L., Bisogno, T., Ligresti, A., Bifulco, M., Melck, D., Di Marzo, V., 2002. Effect on cancer cell proliferation of palmitoylethanolamide, a fatty acid amide interacting with both the cannabinoid and vanilloid signalling systems. *Fundamental & Clinical Pharmacology* 16 (4), 297–302.
- Désarnaud, F., Cadas, H., Piomelli, D., 1995. Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *Journal of Biological Chemistry* 270 (11), 6030–6035.
- Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258 (5090), 1946–1949.
- Devchand, P.R., Keller, H., Peters, J.M., Vazquez, M., Gonzalez, F.J., Wahli, W., 1996. The PPAR alpha-leukotriene B4 pathway to inflammation control. *Nature* 384 (6604), 39–43.
- Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., Piomelli, D., 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372 (6507), 686–691.
- Di Marzo, V., Melck, D., Orlando, P., Bisogno, T., Zagoory, O., Bifulco, M., Vogel, Z., De Petrocellis, L., 2001. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. *Biochemical Journal* 358 (Pt. 1), 249–255.
- Epps, D.E., Schmid, P.C., Natarajan, V., Schmid, H.H., 1979. *N*-acylethanolamine accumulation in infarcted myocardium. *Biochemical and Biophysical Research Communications* 90 (2), 628–633.
- Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S.D., Leon, A., 1995. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proceedings of the National Academy of Sciences of the United States of America* 92 (8), 3376–3380.
- Farquhar-Smith, W.P., Rice, A.S., 2001. Administration of endocannabinoids prevents a referred hyperalgesia associated with inflammation of the urinary bladder. *Anesthesiology* 94 (3), 507–513.
- Farquhar-Smith, W.P., Rice, A.S., 2003. A novel neuroimmune mechanism in cannabinoid-mediated attenuation of nerve growth factor-induced hyperalgesia. *Anesthesiology* 99 (6), 1391–1401.
- Franklin, A., Parmentier-Batteur, S., Walter, L., Greenberg, D.A., Stella, N., 2003. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *Journal of Neuroscience* 23 (21), 7767–7775.

- Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez de Fonseca, F., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., Piomelli, D., 2003. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR- $\alpha$ . *Nature* 425 (6953), 90–93.
- Gaetani, S., Oveisi, F., Piomelli, D., 2003. Modulation of meal pattern in the rat by the anorexic lipid mediator oleylethanolamide. *Neuropsychopharmacology* 28 (7), 1311–1316.
- Guzmán, M., Lo Verme, J., Fu, J., Oveisi, F., Blazquez, C., Piomelli, D., 2004. Oleylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor  $\alpha$  (PPAR- $\alpha$ ). *Journal of Biological Chemistry* 279 (27), 27849–27854.
- Helyes, Z., Nemeth, J., Than, M., Bolcskei, K., Pinter, E., Szolcsanyi, J., 2003. Inhibitory effect of anandamide on resiniferatoxin-induced sensory neuropeptide release in vivo and neuropathic hyperalgesia in the rat. *Life Sciences* 73 (18), 2345–2353.
- Hillard, C.J., Wilkison, D.M., Edgmond, W.S., Campbell, W.B., 1995. Characterization of the kinetics and distribution of *N*-arachidonylethanolamine (anandamide) hydrolysis by rat brain. *Biochimica et Biophysica Acta* 1257 (3), 249–256.
- Ibrahim, M.M., Deng, H., Zvonok, A., Cockayne, D.A., Kwan, J., Mata, H.P., Vanderah, T.W., Lai, J., Porreca, F., Makriyannis, A., Malan Jr., T.P., 2003. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proceedings of the National Academy of Sciences of the United States of America* 100 (18), 10529–10533.
- Jacobsson, S.O., Fowler, C.J., 2001. Characterization of palmitoylethanolamide transport in mouse Neuro-2a neuroblastoma and rat RBL-2H3 basophilic leukaemia cells: comparison with anandamide. *British Journal of Pharmacology* 132 (8), 1743–1754.
- Jaggat, S.I., Hasnie, F.S., Sellaturay, S., Rice, A.S., 1998. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* 76 (1–2), 189–199.
- Kahlich, R., Klima, J., Cihla, F., Frankova, V., Masek, K., Rosicky, M., Matousek, F., Bruthans, J., 1979. Studies on prophylactic efficacy of *N*-2-hydroxyethyl palmitamide (Impulsin) in acute respiratory infections. Serologically controlled field trials. *Journal of Hygiene, Epidemiology, Microbiology and Immunology* 23 (1), 11–24.
- Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. *Nature Medicine* 9 (1), 76–81.
- Klein, T.W., Newton, C.A., Friedman, H., 2001. Cannabinoids and the immune system. *Pain Research & Management* 6 (2), 95–101.
- Kondo, S., Sugiura, T., Kodaka, T., Waku, K., Tokumura, A., 1998. Accumulation of various *N*-acylethanolamines including *N*-arachidonylethanolamine (anandamide) in cadmium chloride-administered rat testis. *Archives of Biochemistry and Biophysics* 354 (2), 303–310.
- Kuehl, F.A., Jacob, T.A., Ganley, O.H., Ormond, R.E., Meisinger, M.A.P., 1957. The identification of *N*-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. *Journal of the American Chemical Society* 79, 5577–5578.
- Lambert, D.M., Di Marzo, V., 1999. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? *Current Medicinal Chemistry* 6 (8), 757–773.
- Lambert, D.M., Vandevorde, S., Diependaele, G., Govaerts, S.J., Robert, A.R., 2001. Anticonvulsant activity of *N*-palmitoylethanolamide, a putative endocannabinoid, in mice. *Epilepsia* 42 (3), 321–327.
- Lambert, D., Vandevorde, S., Jonsson, K., Fowler, J., 2002. The palmitoylethanolamide family: a new class of anti-inflammatory agents? *Current Medicinal Chemistry* 9 (6), 663–674.
- Lichtman, A.H., Hawkins, E.G., Griffin, G., Cravatt, B.F., 2002. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. *Journal of Pharmacology and Experimental Therapeutics* 302 (1), 73–79.
- Long, D., Martin, A., 1956. Factors in arachis oil depressing sensitivity to tuberculin in BCG infected guinea pigs. *Lancet* 270 (6921), 464–466.
- Long, D., Miles, A., 1950. *Lancet* 492.
- LoVerme, J.L., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A., Piomelli, D., 2005. The nuclear receptor peroxisome proliferator-activated receptor- $\alpha$  mediates the anti-inflammatory actions of palmitoylethanolamide. *Molecular Pharmacology* 67 (1), 15–19.

- Maccarrone, M., Cartoni, A., Parolaro, D., Margonelli, A., Massi, P., Bari, M., Battista, N., Finazzi-Agro, A., 2002. Cannabimimetic activity, binding, and degradation of stearoylethanolamide within the mouse central nervous system. *Molecular and Cellular Neurosciences* 21 (1), 126–140.
- Malan Jr., T.P., Ibrahim, M.M., Lai, J., Vanderah, T.W., Makriyannis, A., Porreca, F., 2003. CB2 cannabinoid receptor agonists: pain relief without psychoactive effects? *Current Opinion in Pharmacology* 3 (1), 62–67.
- Masek, K., Perlik, F., Klima, J., Kahlich, R., 1974. Prophylactic efficacy of *N*-2-hydroxyethyl palmitamide (Impulsin) in acute respiratory tract infections. *European Journal of Clinical Pharmacology* 7 (6), 415–419.
- Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346 (6284), 561–564.
- Mazzari, S., Canella, R., Petrelli, L., Marcolongo, G., Leon, A., 1996. *N*-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *European Journal of Pharmacology* 300 (3), 227–236.
- Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365 (6441), 61–65.
- Natarajan, V., Schmid, P.C., Reddy, P.V., Schmid, H.H., 1984. Catabolism of *N*-acylethanolamine phospholipids by dog brain preparations. *Journal of Neurochemistry* 42 (6), 1613–1619.
- Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., Ueda, N., 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. *Journal of Biological Chemistry* 279 (7), 5298–5305.
- Patel, S., Carrier, E.J., Ho, W.S., Rademacher, D.J., Cunningham, S., Reddy, D.S., Falck, J.R., Cravatt, B.F., Hillard, C.J., 2004. The post-mortal accumulation of brain *N*-arachidonylethanolamine (anandamide) is dependent upon fatty acid amide hydrolase activity. *Journal of Lipid Research* 1 December, Electronic publication.
- Perlik, F., Raskova, H., Elis, J., 1971. Anti-inflammatory properties of *N*-(2-hydroxyethyl) palmitamide. *Acta Physiologica Academiae Scientiarum Hungaricae* 39 (4), 395–400.
- Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. *Nature Reviews. Neuroscience* 4 (11), 873–884.
- Quartilho, A., Mata, H.P., Ibrahim, M.M., Vanderah, T.W., Porreca, F., Makriyannis, A., Malan Jr., T.P., 2003. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* 99 (4), 955–960.
- Rodríguez de Fonseca, F., Navarro, M., Gómez, R., Escuredo, L., Nava, F., Fu, J., Murillo-Rodríguez, E., Giuffrida, A., LoVerme, J., Gaetani, S., Kathuria, S., Gall, C., Piomelli, D., 2001. An anorexic lipid mediator regulated by feeding. *Nature* 414 (6860), 209–212.
- Ross, R.A., Brockie, H.C., Pertwee, R.G., 2000. Inhibition of nitric oxide production in RAW264.7 macrophages by cannabinoids and palmitoylethanolamide. *European Journal of Pharmacology* 401 (2), 121–130.
- Schabitz, W.R., Giuffrida, A., Berger, C., Aschoff, A., Schwaninger, M., Schwab, S., Piomelli, D., 2002. Release of fatty acid amides in a patient with hemispheric stroke: a microdialysis study. *Stroke* 33 (8), 2112–2114.
- Schmid, P.C., Zuzarte-Augustin, M.L., Schmid, H.H., 1985. Properties of rat liver *N*-acylethanolamine amidohydrolase. *Journal of Biological Chemistry* 260 (26), 14145–14149.
- Schmid, H.H., Schmid, P.C., Natarajan, V., 1990. *N*-acylated glycerophospholipids and their derivatives. *Progress in Lipid Research* 29 (1), 1–43.
- Schmid, P.C., Krebsbach, R.J., Perry, S.R., Dettmer, T.M., Maasson, J.L., Schmid, H.H., 1995. Occurrence and postmortem generation of anandamide and other long-chain *N*-acylethanolamines in mammalian brain. *FEBS Letters* 375 (1–2), 117–120.
- Sheerin, A.H., Zhang, X., Saucier, D.M., Corcoran, M.E., 2004. Selective antiepileptic effects of *N*-palmitoylethanolamide, a putative endocannabinoid. *Epilepsia* 45 (10), 1184–1188.
- Sheu, M., Fowler, A.J., Kao, J., Schmuth, M., Schoonjans, K., Auwerx, J., Fluhr, J., Man, M., Elias, P., Feingold, K., 2002. Topical peroxisome proliferator activated receptor- $\alpha$  activators reduce inflammation in irritant and allergic contact dermatitis models. *Journal of Investigative Dermatology* 118 (1), 94–101.
- Showalter, V.M., Compton, D.R., Martin, B.R., Abood, M.E., 1996. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *Journal of Pharmacology and Experimental Therapeutics* 278 (3), 989–999.
- Skaper, S.D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L., Leon, A., 1996. The aliamide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proceedings of the National Academy of Sciences of the United States of America* 93 (9), 3984–3989.

- Sugiura, T., Kondo, S., Kishimoto, S., Miyashita, T., Nakane, S., Kodaka, T., Suhara, Y., Takayama, H., Waku, K., 2000. Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *Journal of Biological Chemistry* 275 (1), 605–612.
- Sun, Y.X., Tsuboi, K., Okamoto, Y., Tonai, T., Murakami, M., Kudo, I., Ueda, N., 2004. Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochemical Journal* 380 (Part 3), 749–756.
- Terrazzino, S., Berto, F., Dalle Carbonare, M., Fabris, M., Guiotto, A., Bernardini, D., Leon, A., 2004. Stearoylethanolamide exerts anorexic effects in mice via down-regulation of liver stearyl-coenzyme A desaturase-1 mRNA expression. *FASEB Journal* 18 (13), 1580–1582.
- Ueda, N., Kurahashi, Y., Yamamoto, S., Tokunaga, T., 1995. Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *Journal of Biological Chemistry* 270 (40), 23823–23827.
- Ueda, N., Yamanaka, K., Yamamoto, S., 2001. Purification and characterization of an acid amidase selective for *N*-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *Journal of Biological Chemistry* 276 (38), 35552–35557.