

REVIEW

The expanding field of cannabimimetic and related lipid mediators

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The discovery of the endogenous cannabimimetic lipid mediators, anandamide and 2-arachidonoyl glycerol, opened the door to the discovery of other endogenous lipid mediators similar in structure and function. The majority of these compounds do not bind appreciably to known cannabinoid receptors; yet some of them produce cannabimimetic effects while others exert actions through novel mechanisms that remain to be elucidated. This review explores the growing diversity of recently discovered putative lipid mediators and their relationship to the endogenous cannabinoid system. The possibility that there remain many unidentified signalling lipids coupled with the evidence that many of these yield bioactive metabolites due to actions of known enzymes (e.g. cyclooxygenases, lipoxygenases, cytochrome P450s) suggests the existence of a large and complex family of lipid mediators about which only little is known at this time. The elucidation of the biochemistry and pharmacology of these compounds may provide therapeutic targets for a variety of conditions including sleep dysfunction, eating disorders, cardiovascular disease, as well as inflammation and pain.

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Abbreviations: 2-AG, 2-arachidonoyl glycerol; anandamide, *N*-arachidonoyl ethanolamide; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; FAAH, fatty acid amide hydrolase; GABA, arachidonoyl- γ -aminobutyric acid; NADA, *N*-arachidonoyl dopamine; NAGly, *N*-arachidonoyl glycine; noladin ether, 2-arachidonoyl glycerol ether; OEA, oleoyl ethanolamide; OLDA, oleoyl dopamine; PALDA, palmitoyldopamine; PEA, palmitoyl ethanolamide; SEA, stearoyl ethanolamide; STEARDA, stearoyldopamine; TRPV1, transient receptor potential type vanilloid 1 receptor

Introduction

Our understanding of a physiological role of cannabinoids in memory, cardiovascular function, cognition, pain, reproduction, motor control, and immune function has grown rapidly in the last decade. These have been the subject of recent reviews in this journal (Ross, 2003; Randall *et al.*, 2004; Walter & Stella, 2004). The landmark works that identified the endogenous compounds, arachidonoyl ethanolamide (anandamide, Devane *et al.*, 1992) and 2-arachidonoyl glycerol (2-AG; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995), paved the way for the search for other endogenous lipids that are associated with cannabinoid system physiology. Historically, the complexities and limitations of lipid chemistry hampered the identification and characterization of endocannabinoids and related lipid mediators. Hence, the increased availability of sensitive techniques for the study of lipids is a primary factor in the recent advances in this field. Additional endogenous lipids discovered that show affinity for cannabinoid receptors include dihomogamma-linolenoyl ethanolamide (Hanus *et al.*, 1993), docosatetraenoyl ethanolamide (Hanus *et al.*, 1993), 2-arachidonoyl glycerol ether (noladin ether; Hanus *et al.*, 2001), and *N*-arachidonoyl dopamine (NADA) (Huang *et al.*, 2002). Many other endogenous lipids similar to endocannabinoids in structure and metabolism have been identified. These com-

pounds do not bind appreciably to known cannabinoid receptors, and yet some of them demonstrate cannabimimetic effects. These novel lipid mediators will likely prove to be important to the function and regulation of cannabinoid neurophysiology or operate in parallel *via* overlapping signaling pathways. The following minireview provides a brief overview of these compounds and their relationship to the endogenous cannabinoid system.

Acyl glycerols

2-linoleoyl glycerol and 2-palmitoyl glycerol share structural homology with the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG) in that the only difference is the fatty acid length and saturation (Figure 1). Noladin ether likewise consists of arachidonic acid and glycerol with the exception that the linkage to the glycerol moiety is an ether *versus* an ester as is the case for the other compounds in this class (Figure 1). Noladin ether was identified by Hanus *et al.* (2001) and confirmed by Fezza *et al.* (2002) in which it was demonstrated to occur in relatively high amounts in dissected thalamus. Oka *et al.* (2003), however, failed to measure noladin ether in nervous tissue. If noladin ether is in fact an endogenous compound, its location in the thalamus suggests a role in sensory processing, but its localization to sensory areas of the thalamus has not been established. Additionally, noladin ether occurs in very low levels in the spinal cord,

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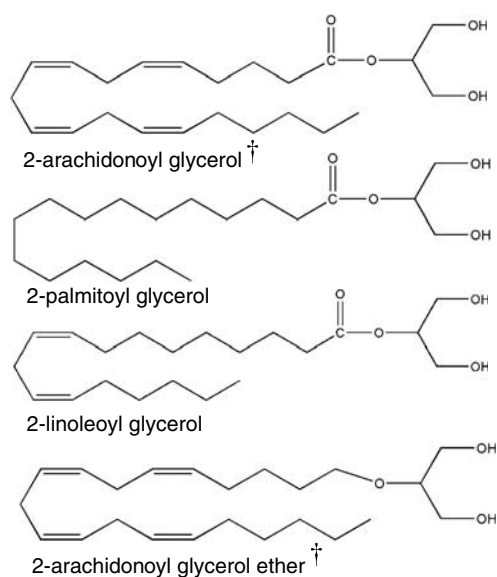


Figure 1 Chemical structures of bioactive acyl glycerols. †Indicates compounds with activity at either CB1 or CB2 receptors.

raising a question as to any important role in spinal neurotransmission. Noladin ether possesses biological activity. Hanus *et al.* (2001) showed that the compound produces analgesic effects in the hot plate test following systemic administration in mice (20 mg kg⁻¹, i.p.), binds to CB1 but not CB2 receptors, produces hypothermia, catalepsy, and decreases in locomotor activity. Additionally, noladin ether was more effective and demonstrated a more persistent response in decreasing intraocular pressure than either anandamide or 2-AG (Laine *et al.*, 2002).

2-Linoleoyl glycerol and 2-palmitoyl glycerol were isolated in mouse gut (Mechoulam *et al.*, 1995), brain (Sugiura *et al.*, 1995), and spleen (Ben-Shabat *et al.*, 1998). Whereas neither compound binds appreciably to CB2 receptors, when combined with 2-AG in the same percentages measured in tissue, these compounds markedly potentiated the binding of 2-AG to CB2 receptors causing a decrease in the K_i for 2-AG from 1640 ± 260 to 273 ± 22 nM (Ben-Shabat *et al.*, 1998). The same synergistic effects were demonstrated in the aforementioned behavioral tests (Ben-Shabat *et al.*, 1998).

These enhancements of cannabinoid activity by congeners that lack binding affinity have been termed entourage effects (Mechoulam *et al.*, 1998).

Acyl ethanolamides

The first identified endogenous cannabinoid, arachidonoyl ethanolamide (Devane *et al.*, 1992), was given the name, anandamide, which is derived from the Indian Sanskrit term *ananda* for bliss. Seven additional bioactive *N*-acyl ethanolamides were subsequently identified (Figure 2). Although dihomono- γ -linolenylethanolamide, and docosatetraenylethanolamide bind to CB1 receptors (Hanus *et al.*, 1993), none of the others in this group show appreciable binding. Each of these compounds has been identified in various mammalian and invertebrate tissues (Di Marzo *et al.*, 1996; Maccarrone *et al.*, 2001; Schuel *et al.*, 2002; Salzet & Stefano, 2002) and are

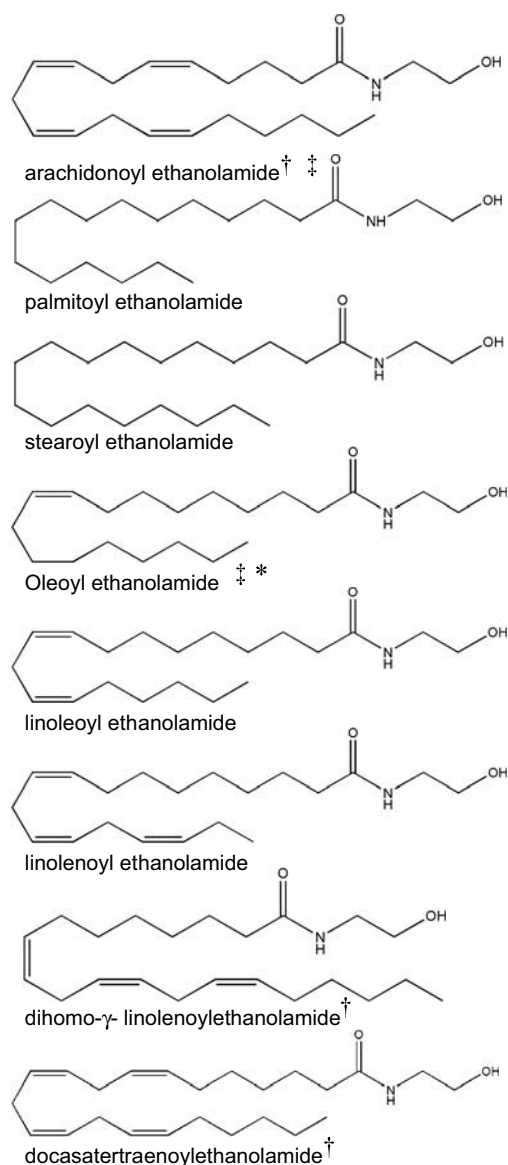


Figure 2 Chemical structures of bioactive acyl ethanolamides. †Indicates compounds with activity at either CB1 or CB2 receptors. ‡Indicates compounds with activity at the TRPV1 receptor. *Indicates compounds with activity at the PPAR α receptor.

hypothesized to possess cannabinimetic activity possibly due to entourage effects (Mechoulam *et al.*, 1998).

The oldest of these compounds, palmitoyl ethanolamide (PEA), was identified nearly 5 decades ago as the principle anti-inflammatory agent in lipid extracts of various natural products (Kuehl *et al.*, 1957). Extensive reviews of PEA have recently been published (Lambert *et al.*, 2002; Schmid & Berdyshev, 2002). PEA produces anti-inflammatory (Facci *et al.*, 1995; Mazzari *et al.*, 1996) and antinociceptive effects (Calignano *et al.*, 1998; Jaggar *et al.*, 1998) when administered exogenously. Synergistic effects in antinociception were observed with coadministration of anandamide and PEA, and abolished by either CB1 or CB2 antagonists, respectively (Calignano *et al.*, 1998; 2001). PEA produced a two-fold decrease in the K_i value for anandamide binding at the transient receptor potential type vanilloid 1 receptor (TRPV1),

an effect that was not due to inhibition of anandamide hydrolysis (De Petrocellis *et al.*, 2001). Nor does it appear that this effect was caused by blocking the putative anandamide transporter (Rakhshan *et al.*, 2000). Although PEA exhibits poor affinity for CB1 and CB2 receptors (Sheskin *et al.*, 1997; Lambert *et al.*, 1999), the antinociceptive effects of PEA were blocked by the CB2 antagonist SR144528, suggesting possible activation of a non-CB2 receptor of which the molecular nature, location, and signal transduction mechanisms are unknown (Calignano *et al.*, 1998; 2001).

In contrast to PEA, oleoyl ethanolamide (OEA) inhibited anandamide uptake (Rakhshan *et al.*, 2000) and degradation (Karava *et al.*, 2001). Like anandamide, OEA has been implicated in the neural regulation of feeding behaviors (Rodriguez de Fonseca *et al.*, 2001) by acting on peripheral sensory fibers. Furthermore, OEA levels were significantly decreased during starvation (Rodriguez de Fonseca *et al.*, 2001). By contrast, anandamide levels increased during starvation (Gomez *et al.*, 2002), suggesting a reciprocal effect of the two compounds within this system. OEA has negligible affinity for both CB1 and CB2 receptors. OEA activates the nuclear receptor, peroxisome proliferator-activated receptor α (PPAR- α ; Fu *et al.*, 2003; Guzman *et al.*, 2004), which may explain its effects on feeding (Fu *et al.*, 2003). OEA also activates the TRPV1 receptor in a PKC-dependent manner (Ahern, 2003).

Even though OEA does not bind to CB1 receptors, measurements of the endogenous levels of OEA revealed significant increases in cortical levels in CB1 knockout mice relative to wild-type mice at 2 months of age. At 6 months of age, there were no differences between the wild type and the knockouts (Maccarrone *et al.*, 2001). Conversely, the levels of OEA in the hippocampus of CB1 knockout mice were significantly lower than the wild type at 2 months with a further reduction at 6 months (Maccarrone *et al.*, 2001). PEA and stearoyl ethanolamide (SEA) showed similar changes in levels in this CB1 knockout model. These data add to the evidence that OEA, PEA, and SEA may function in concert with endocannabinoids to regulate feeding.

Linoleoyl ethanolamide, PEA, SEA, and OEA were isolated from mouse J774 macrophages and N18 neuroblastoma cells (Di Marzo *et al.*, 1996) as well as RBL-2H3 leukocytes (Bisogno *et al.*, 1997). The levels of these compounds were significantly increased by addition of ionomycin in each system (Di Marzo *et al.*, 1996; Bisogno *et al.*, 1997). Linoleoyl ethanolamide inhibits fatty acid amide hydrolase (FAAH; Maurelli *et al.*, 1995; Maccarrone *et al.*, 1998), and was shown to inhibit sea urchin fertilization (Berdyshev, 1999).

Acyl dopamines

Another class of acyl amides is the acyl dopamines. These compounds (Figure 3) were recently identified in bovine and rat brain (Huang *et al.*, 2002; Chu *et al.*, 2003). Like the compounds discussed above, they comprised a fatty acid chain with a moiety attached at the carboxyl end *via* an amide linkage. The moiety in this group of compounds is dopamine, a naturally occurring aromatic amine. These compounds share a structural similarity with the potent TRPV1 agonist, capsaicin (Figure 3).

NADA activates CB1 receptors (K_i $0.5 \pm 0.2 \mu\text{M}$) and induces analgesia following systemic administration (but not

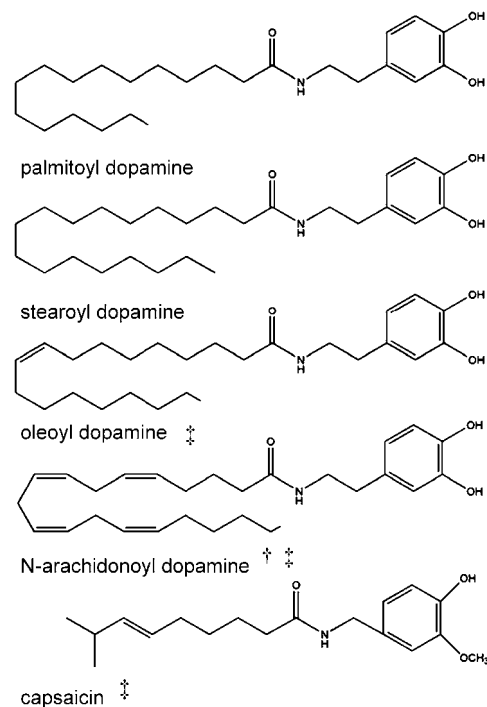


Figure 3 Chemical structures of bioactive acyl dopamines. The TRPV1 agonist, capsaicin, is shown at the bottom to demonstrate the structural similarity to these compounds. †Indicates compounds with activity at either CB1 or CB2 receptors. ‡Indicates compounds with activity at the TRPV1 receptor.

tested with a cannabinoid antagonist) (Bisogno *et al.*, 1997; Huang *et al.*, 2002). Like anandamide, NADA mobilizes intracellular calcium *via* activation of TRPV1 receptors (Huang *et al.*, 2002; Toth *et al.*, 2003; Gavva *et al.*, 2004). Premkumar *et al.* (2004) hypothesize that it is acting on TRPV1 in a PKC-dependent manner by demonstrating that NADA-induced currents could be blocked by the PKC inhibitor, bisindolylmaleimide. They also demonstrated that NADA-induced changes in current were increased ~ 30 -fold by applying NADA intracellularly, suggesting that the increased access to the TRPV1 receptor facilitated this change (Premkumar *et al.*, 2004). The distribution of endogenous NADA in various brain areas differs from that of anandamide with the highest levels found in the striatum and hippocampus (Huang *et al.*, 2002). It also occurs in the dorsal root ganglion, suggesting that it may serve a role in pain and sensory modulation. Patch-clamp studies of cultured DRG neurons showed that NADA elicited immediate and reversible responses, which were blocked by both the CB1 antagonist, SR141617A, and the TRPV1 antagonist, capsazepine (Sagar *et al.*, 2004).

Electrophysiological recordings from the dorsal horn in anesthetized rats showed that neuronal responses to mechanical stimulation were inhibited by $5 \mu\text{g}$ of NADA. When low levels of mechanical pressure were applied, the effect was blocked by SR141716A. Conversely, the TRPV1 antagonist iodo-resiniferatoxin (IRTX) blocked the effects of NADA when higher levels of mechanical pressure were tested (Sagar *et al.*, 2004). In behavioral experiments using non-anesthetized animals, a $5 \mu\text{g}$ dose of NADA caused thermal hyperalgesia when administered peripherally in rats (Huang *et al.*, 2002)

and primates (Butelman *et al.*, 2003). Harrison *et al.* (2003) showed that it initiates contractions in both pig bronchi and urinary bladder in a manner similar to that of anandamide and capsaicin. Additionally, NADA was shown to inhibit T-cell activation, IL-2 and TNF- α gene activation, as well as inhibit NF- κ B-dependent transcriptional activity (Sancho *et al.*, 2004). Given that NADA is capable of eliciting analgesia upon systemic administration, hyperalgesia upon intradermal injection, inhibition of neuronal responses to mechanical stimulation, inhibition of immune responses, and initiating smooth muscle contraction, it is possible that endogenous NADA may activate TRPV1, CB1, or an additional as yet unknown receptor depending on location and circumstance.

Oleoyle dopamine (OLDA) shows only a modest affinity for the CB1 receptor (K_i $1.6 \pm 0.4 \mu\text{M}$) and it possesses the highest potency of any putative endovanilloid identified to date in the mobilization of intracellular calcium in TRPV1-transfected HEK cells (Chu *et al.*, 2003; Gavva *et al.*, 2004). Additionally, OLDA appeared to be recognized by the putative anandamide transporter (Chu *et al.*, 2003), whereas, it did not inhibit anandamide degradation through FAAH. Like NADA, OLDA was shown to inhibit early and late events in T-cell activation; however, its effects on IL-2, TNF- α , and NF- κ B were not examined (Sancho *et al.*, 2004). Behaviorally, it induces thermal hyperalgesia that is reversed by IRTX (Chu *et al.*, 2003). In other behavior studies, the μ -opioid receptor agonists, loperamide and fentanyl, both prevented OLDA-induced allodynia in the primate (Butelman *et al.*, 2004).

Palmitoyldopamine (PALDA) and stearoyldopamine (STEARDA) possess very low TRPV1 activity, and failed to inhibit anandamide degradation and transport (Chu *et al.*, 2003). However, when preincubated for 5 min with TRPV1-transfected HEK-293 cells, both compounds dose-dependently enhanced NADA's TRPV1-mediated mobilization of intracellular calcium (De Petrocellis *et al.*, 2004). This same effect on intracellular calcium was observed for anandamide after preincubation with PALDA and STEARDA (De Petrocellis *et al.*, 2004). Behaviorally, only STEARDA potentiated the induction of thermal hyperalgesia by NADA (De Petrocellis *et al.*, 2004).

N-acyl amides

Oleamide, linoleamide, and arachidonamide are the simplest forms of fatty-acid amides, as the amide linkage is to an ammonium ion (Figure 4). These compounds produce a variety of physiological effects and they share with anandamide the same degradatory enzyme, FAAH (Maurelli *et al.*, 1995; Cravatt *et al.*, 1996). Oleamide and linoleamide were isolated in brain CSF, and appear to be involved in sleep regulation (Cravatt *et al.*, 1995; Boger *et al.*, 1998a; Huang & Jan, 2001). Using multiple reaction monitoring on a triple quadrupole mass spectrometer, we found evidence for endogenous arachidonamide in the brain (data not shown). Oleamide and arachidonamide have been reported to affect gap junctions (Boger *et al.*, 1998b; 1999), and oleamide has been shown to affect GABAergic, dopaminergic, and serotonergic neurotransmission (Huidobro-Toro & Harris, 1996; Guan *et al.*, 1997; Thomas *et al.*, 1997; Yost *et al.*, 1998; Fedorova *et al.*, 2001). Additionally, i.p. injections of oleamide were shown to increase food intake for up to 3 h (Martinez-Gonzalez *et al.*, 2004). It remains to be determined if

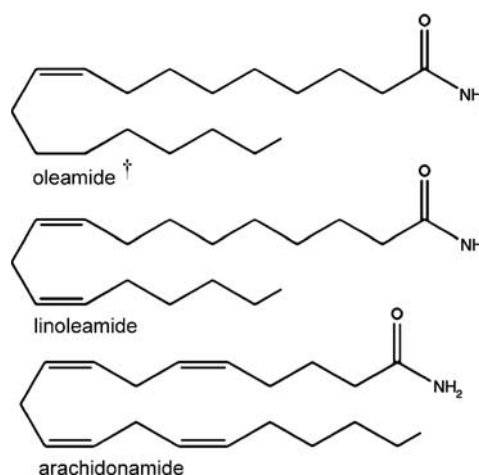


Figure 4 Chemical structures of bioactive acyl amides. †Indicates compounds with possible activity at the CB1 receptor. None have shown any activity at CB2 or TRPV1 receptors.

linoleamide and archidonamide show similar behavioral effects.

Two reports indicated that oleamide, linoleamide, and arachidonamide do not interact appreciably with cannabinoid receptors (Pinto *et al.*, 1994; Sheskin *et al.*, 1997). However, a recent report by Leggett *et al.* (2004) provided evidence that oleamide inhibits agonist and antagonist ligand binding to CB1 but not CB2 receptors, and increases GTP γ S via CB1 activation. The concentrations needed for these effects (~ 3 – $100 \mu\text{M}$) may not be in the physiological range (Fowler, 2004). Nevertheless, cannabinimetic effects such as antinociception and hypothermia were observed after systemic administration of oleamide, and the antinociceptive effects were blocked by the CB1 antagonist SR141716A (Fedorova *et al.*, 2001).

Acyl amino acids

The final class of acyl amides discussed here comprises the acyl amino acids. *N*-arachidonoyl glycine (NAGly; Figure 5), which differs from anandamide by a single oxygen moiety, was first synthesized by Sheskin *et al.* (1997) as part of a structure-activity study of anandamide-like compounds. Burstein (1999) suggested that this compound may be produced *in vivo* by oxidative metabolism of anandamide. We noted that the compound could be formed by conjugation of two naturally occurring molecules, arachidonic acid and glycine. This led us to search for it in the brain (Huang *et al.*, 2001). NAGly was identified in bovine brain extracts and found to occur in rat spinal cord, intestine, testes, skin, blood, kidneys, heart (Huang *et al.*, 2001), and more recently the reproductive tract (Bradshaw *et al.*, 2003). This compound produces antinociceptive effects in rats, reduces edema produced by local injections of arachidonic acid, and inhibits NF- κ B, a transcription factor that regulates chemokine production (Burstein *et al.*, 2002). The effects of NAGly may be due, in part, to its ability to increase the levels of anandamide through inhibition of FAAH. However, a recent study by Cascio *et al.* (2004) showed that the inhibition of FAAH by NAGly is species-dependent and varies among cell and tissue types. The effects of NAGly may result, in part, from metabolites produced by cyclooxygenase-2 (COX-2). Prusakiewicz *et al.*

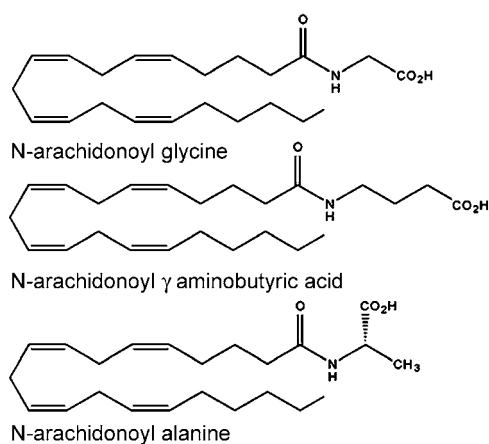


Figure 5 Chemical structure of bioactive acyl amino acids. None have shown any activity at CB1, CB2, or TRPV1 receptors.

(2002) showed that like the effects on anandamide and 2-AG (Yu *et al.*, 1997; Kozak *et al.*, 2002), COX-2 metabolizes NAGly to PGH₂ glycine and hydroxyeicosatetraenoic glycine, suggesting the existence of a new class of eicosanoids derived from NAGly.

In addition to NAGly, Huang *et al.* (2001) identified two additional arachidonoyl amino acids: arachidonoyl alanine and arachidonoyl- γ -aminobutyric acid (GABA), the latter of which produced antinociceptive effects. Recently, Mechoulam's laboratory identified *N*-arachidonoyl serine in a mammalian brain extract and showed that it is a potent activator of the abnormal cannabidiol receptor (Jarai *et al.*, 1999; Milman *et al.*, 2004). Further experiments in our laboratory using precursor ion scans and multiple reaction monitoring on a triple quadrupole mass spectrometer provided evidence for the existence of other arachidonoyl amino acids including the conjugates of arachidonic acid with valine, cysteine, and glutamine, but more work is needed to prove their existence. Similar experiments have further suggested the existence of

glycine and GABA conjugates of palmitic, oleic, and stearic acid, but again, additional work is needed to verify the existence of these compounds.

Summary

In all, 20 putative lipid mediators were reviewed and their involvement in pain, immune function, reproduction, and appetite were discussed here. The majority of these compounds do not bind appreciably to CB1 or CB2 receptors but many exhibit cannabimimetic effects. These can be attributed, in part, to interference with inactivation of endocannabinoids thus enhancing their actions at cannabinoid receptors (MacCarrone *et al.*, 1998; Di Marzo *et al.*, 2001; Jonsson *et al.*, 2001; Karava *et al.*, 2001), and there remains the possibility that some of these compounds act downstream potentiating cannabinoid receptor signaling. Recent reports indicate that some of these compounds act on known signaling molecules such as PPAR α , TRPV1 and probably others that remain to be identified. The diversity of the lipids identified to date, which seem to occur in a combinatorial fashion (any of many different acyl groups coupled to any of many different small polar molecules), indicates the high likelihood that there are numerous unidentified endogenous signaling lipids. This coupled with the evidence that many such compounds yield bioactive metabolites provides a hypothetical framework for a large and complex system of neural and immune signaling systems that have yet to be discovered. One may reasonably hope that as techniques for the study of lipid mediators become more sensitive, this somewhat indistinct landscape of lipid neurotransmitters and neuromodulators may resolve into a well-defined terrain.

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References

- AHERN, G.P. (2003). Activation of TRPV1 by the satiety factor oleylethanolamide. *J. Biol. Chem.*, **278**, 30429–30434.
- BEN-SHABAT, S., FRIDE, 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. *Eur. J. Pharmacol.*, **353**, 23–31.
- BERDYSHEV, E.V. (1999). Inhibition of sea urchin fertilization by fatty acid ethanolamides and cannabinoids. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.*, **122**, 327–330.
- BISOGNO, T., VENTRIGLIA, M., MILONE, A., MOSCA, M., CIMINO, G. & DI MARZO, V. (1997). Occurrence and metabolism of anandamide and related acyl-ethanolamides in ovaries of the sea urchin *Paracentrotus lividus*. *Biochim. Biophys. Acta*, **1345**, 338–348.
- BOGER, D.L., HENRIKSEN, S.J. & CRAVATT, B.F. (1998a). Oleamide: an endogenous sleep-inducing lipid and prototypical member of a new class of biological signaling molecules. *Curr. Pharm. Des.*, **4**, 303–314.
- BOGER, D.L., PATTERSON, J.E., GUAN, X., CRAVATT, B.F., LERNER, R.A. & GILULA, N.B. (1998b). Chemical requirements for inhibition of gap junction communication by the biologically active lipid oleamide. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 4810–4815.
- BOGER, D.L., SATO, H., LERNER, A.E., GUAN, X. & GILULA, N.B. (1999). Arachidonic acid amide inhibitors of gap junction cell–cell communication. *Bioorg. Med. Chem. Lett.*, **9**, 1151–1154.
- BRADSHAW, H.B., KREY, J.K. & WALKER, J.M. (2003). Levels of endocannabinoids and related lipid mediators in the female reproductive tract change as a function of estrous cycle. *Soc. Neuro.*, Abstracts Online Prog. Number 464.3.
- BURSTEIN, S.H. (1999). The cannabinoid acids: nonpsychoactive derivatives with therapeutic potential. *Pharmacol. Ther.*, **82**, 87–96.
- BURSTEIN, S.H., HUANG, S.M., PETROS, T.J., ROSSETTI, R.G., WALKER, J.M. & ZURIER, R.B. (2002). Regulation of anandamide tissue levels by *N*-arachidonoylglycine. *Biochem. Pharmacol.*, **64**, 1147–1150.
- BUTELMAN, E.R., BALL, J.W., HARRIS, T.J. & KREEK, M.J. (2003). Topical capsaicin-induced allodynia in unanesthetized primates: pharmacological modulation. *J. Pharmacol. Exp. Ther.*, **311**, 155–163.
- BUTELMAN, E.R., HARRIS, T.J. & KREEK, M.J. (2004). Antiallodynic effects of loperamide and fentanyl against topical capsaicin-induced allodynia in unanesthetized primates. *J. Pharmacol. Exp. Ther.*, **306**, 1106–1114.
- CALIGNANO, A., LA RANA, G., GIUFFRIDA, A. & PIOMELLI, D. (1998). Control of pain initiation by endogenous cannabinoids. *Nature*, **394**, 277–281.

- CALIGNANO, A., LA RANA, G. & PIOMELLI, D. (2001). Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *Eur. J. Pharmacol.*, **419**, 191–198.
- CASCIO, M.G., MINASSI, A., LIGRESTI, A., APPENDINO, G., BURSTEIN, S. & DI MARZO, V. (2004). A structure-activity relationship study on *N*-arachidonoyl-amino acids as possible endogenous inhibitors of fatty acid amide hydrolase. *Biochem. Biophys. Res. Comm.*, **314**, 192–196.
- CHU, C.J., HUANG, S.M., DE PETROCELLIS, L., BISOGNO, T., EWING, S.A., MILLER, J.D., ZIPKIN, R.E., DADDARIO, N., APPENDINO, G., DIMARZO, V. & WALKER, J.M. (2003). *N*-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. *J. Biol. Chem.*, **278**, 13633–13639.
- 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**, 83–87.
- CRAVATT, B.F., PROSPERO-GARCIA, O., SIUZDAK, G., GILULA, N.B., HENRIKSEN, S.J., BOGER, D.L. & LERNER, R.A. (1995). Chemical characterization of a family of brain lipids that induce sleep. *Science*, **268**, 1506–1509.
- DE PETROCELLIS, L., DAVIS, J.B. & DI MARZO, V. (2001). Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. *FEBS Lett.*, **506**, 253–256.
- DE PETROCELLIS, L., CHU, C.J., MORIELLO, A.S., KELLNER, J.C., WALKER, J.M. & DI MARZO, V. (2004). Actions of two naturally occurring saturated *N*-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. *Br. J. Pharmacol.*, **143**, 251–256.
- 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**, 1946–1949.
- DI MARZO, V., DE PETROCELLIS, L., SEPE, N. & BUONO, A. (1996). Biosynthesis of anandamide and related acylethanolamides in mouse J774 macrophages and N18 neuroblastoma cells. *Biochem. J.*, **316** (Part 3), 977–984.
- 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. *Biochem. J.*, **358**, 249–255.
- 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. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 3376–3380.
- FEDOROVA, I., HASHIMOTO, A., FECIK, R.A., HEDRICK, M.P., HANUS, L.O., BOGER, D.L., RICE, K.C. & BASILE, A.S. (2001). Behavioral evidence for the interaction of oleamide with multiple neurotransmitter systems. *J. Pharmacol. Exp. Ther.*, **299**, 332–342.
- FEZZA, F., BISOGNO, T., MINASSI, A., APPENDINO, G., MECHOULAM, R. & DI MARZO, V. (2002). Noladin ether, a putative novel endocannabinoid: inactivation mechanisms and a sensitive method for its quantification in rat tissues. *FEBS Lett.*, **513**, 294–298.
- FOWLER, C.J. (2004). Oleamide: a member of the endocannabinoids family? *Br. J. Pharmacol.*, **141**, 195–196.
- FU, J., GAETANI, S., OVEISI, F., LO VERME, J., SERRANO, A., DE FONSECA, F.R., 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- α . *Nature*, **425**, 90–93.
- GAVVA, N.R., KLIONSKY, L., QU, Y., SHI, L., TAMIR, R., EDENSON, S., ZHANG, T.J., VISWANDADHAN, V.N., TOTH, A., PEARCE, L.V., VANDERAH, T.W., PORRECA, F., BLUMBERG, M., LILE, J., SUN, Y., WILD, K., LOUIS, J.-C. & TREANOR, J.S. (2004). Molecular determinants of vanilloid sensitivity in TRPV1. *J. Biol. Chem.*, **279**, 20283–20295.
- GOMEZ, R., NAVARRO, M., FERRER, B., TRIGO, J.M., BILBAO, A., DEL ARCO, I., CIPPITELLI, A., NAVA, F., PIOMELLI, D., RODRIGUEZ, D.E. & FONSECA, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J. Neurosci.*, **22**, 9612–9617.
- GUAN, X., CRAVATT, B.F., EHRLING, G.R., HALL, J.E., BOGER, D.L., LERNER, R.A. & GILULA, N.B. (1997). The sleep-inducing lipid oleamide deconvolutes gap junction communication and calcium wave transmission in glial cells. *J. Cell. Biol.*, **139**, 1785–1792.
- GUZMAN, M., LO VERME, J., FU, J., OVEISI, F., BLAZQUEZ, C. & PIOMELLI, D. (2004). Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor α (PPAR- α). *J. Biol. Chem.*, **279**, 27849–27854.
- HANUS, L., ABU-LAFI, S., FRIDE, E., BREUER, A., VOGEL, Z., SHALEV, D.E., KUSTANOVICH, I. & MECHOULAM, R. (2001). 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 3662–3665.
- HANUS, L., GOPHER, A., ALMOG, S. & MECHOULAM, R. (1993). Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J. Med. Chem.*, **36**, 3032–3034.
- HARRISON, S., DE PETROCELLIS, L., TREVISANI, M., BENVENUTI, F., BIFULCO, M., GEPPETTI, P. & DIMARZO, V. (2003). Capsaicin-like effects of *N*-arachidonoyl-dopamine in the isolated guinea pig bronchi and urinary bladder. *Eur. J. Pharmacol.*, **475**, 107–114.
- HUANG, J.K. & JAN, C.R. (2001). Linoleamide, a brain lipid that induces sleep, increases cytosolic Ca²⁺ levels in MDCK renal tubular cells. *Life Sci.*, **68**, 997–1004.
- HUANG, S.M., BISOGNO, T., PETROS, T.J., CHANG, S.Y., ZAVITSANOS, P.A., ZIPKIN, R.E., SIVAKUMAR, R., COOP, A., MAEDA, D.Y., DE PETROCELLIS, L., BURSTEIN, S., DI MARZO, V. & WALKER, J.M. (2001). Identification of a new class of molecules, the arachidonoyl amino acids, and characterization of one member that inhibits pain. *J. Biol. Chem.*, **276**, 42639–42644.
- HUANG, S.M., BISOGNO, T., TREVISANI, M., AL-HAYANI, A., DE PETROCELLIS, L., FEZZA, F., TOGNETTO, M., PETROS, T.J., KREY, J.F., CHU, C.J., MILLER, J.D., DAVIES, S.N., GEPPETTI, P., WALKER, J.M. & DI MARZO, V. (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 8400–8405.
- HUIDOBRO-TORO, J.P. & HARRIS, R.A. (1996). Brain lipids that induce sleep are novel modulators of 5-hydroxytryptamine receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 8078–8082.
- JAGGAR, 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**, 189–199.
- JARAI, Z., WAGNER, J.A., VARGA, K., LAKE, K.D., COMPTON, D.R., MARTIN, B.R., ZIMMER, A.M., BONNER, T.I., BUCKLEY, N.E., MEZEY, E., RAZDAN, R.K., ZIMMER, A. & KUNOS, G. (1999). Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 14136–14141.
- JONSSON, K.O., VANDEVOORDE, S., LAMBERT, D.M., TIGER, G. & FOWLER, C.J. (2001). Effects of homologues and analogues of palmitoylethanolamide upon the inactivation of the endocannabinoid anandamide. *Br. J. Pharmacol.*, **133**, 1263–1275.
- KARAVA, V., FASIA, L. & SIAFAKA-KAPADAI, A. (2001). Anandamide amidohydrolase activity, released in the medium by *Tetrahymena pyriformis*. Identification and partial characterization. *FEBS Lett.*, **508**, 327–331.
- KOZAK, K.R., CREW, B.C., MORROW, J.D., WANG, L.H., MA, Y.H., WEINANDER, R., JAKOBSSON, P.J. & MARNETT, L.J. (2002). Metabolism of the endocannabinoids, 2-arachidonoylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J. Biol. Chem.*, **277**, 44877–44885.
- 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. *J. Am. Chem. Soc.*, **79**, 5577–5578.
- LAINE, K., JARVINEN, K., MECHOULAM, R., BREUER, A. & JARVINEN, T. (2002). Comparison of the enzymatic stability and intraocular pressure effects of 2-arachidonoylglycerol and noladin ether, a novel putative endocannabinoid. *Invest. Ophthalmol. Vis. Sci.*, **43**, 3216–3222.

- LAMBERT, D.M., DIPAOLO, F.G., SONVEAUX, P., KANYONYO, M., GOVAERTS, S.J., HERMANS, E., BUEB, J., DELZENNE, N.M. & TSCHIRHART, E.J. (1999). Analogues and homologues of *N*-palmitoylethanolamide, a putative endogenous CB(2) cannabinoid, as potential ligands for the cannabinoid receptors. *Biochim. Biophys. Acta*, **1440**, 266–274.
- LAMBERT, D.M., VANDEVOORDE, S., JONSSON, K.O. & FOWLER, C.J. (2002). The palmitoylethanolamide family: a new class of anti-inflammatory agents? *Curr. Med. Chem.*, **9**, 663–674.
- LEGGETT, J.D., ASPLEY, S., BECKETT, S.R., D'ANTONA, A.M. & KENDALL, D.A. (2004). Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *Br. J. Pharmacol.*, **141**, 253–262.
- MACCARRONE, M., ATTINA, M., BARI, M., CARTONI, A., LEDENT, C. & FINAZZI-AGRO, A. (2001). Anandamide degradation and *N*-acylethanolamines level in wild-type and CB1 cannabinoid receptor knockout mice of different ages. *J. Neurochem.*, **78**, 339–348.
- MACCARRONE, M., VANDER STELT, M., ROSSI, A., VELDINK, G.A., Vliegenthart, J.F. & AGRO, A.F. (1998). Anandamide hydrolysis by human cells in culture and brain. *J. Biol. Chem.*, **273**, 32332–32339.
- MARTINEZ-GONZALEZ, D., BONILLA-JAIME, H., MORALES-OTAL, A., HENRIKSON, S.J., VELAZQUEZ-MOCTEZUMA, J. & PROSPERO-GARCIA, O. (2004). Oleamide and anandamide effects on food intake and sexual behavior of rats. *Neuro. Lett.*, **364**, 1–6.
- MAURELLI, S., BISOGNO, T., DE PETROCELLIS, L., DILUCCIA, A., MARINO, G. & DI MARZO, V. (1995). Two novel classes of neuroactive fatty acid amides are substrates for mouse neuroblastoma 'anandamide amidohydrolase'. *FEBS Lett.*, **377**, 82–86.
- 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. *Eur. J. Pharmacol.*, **300**, 227–236.
- MECHOULAM, R., BEN-SHABAT, S., HANUS, L., LIGUMSKY, M., KAMINSKI, N.E., SCHATZ, A.R., GOPHER, A., ALMOG, S., MARTIN, B.R., COMPTON, D.R., PERTWBEE, R.G., GRIFFIN, G., BAYEWITCH, M., BARG, J. & VOGEL, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **50**, 83–90.
- MECHOULAM, R., FRIDE, E. & DIMARZO, V. (1998). Endocannabinoids. *Eur. J. Pharmacol.*, **359**, 1–18.
- MILMAN, G., MAOR, Y., HOROWITZ, M., GALLILY, R., HANUS, L. & MECHOULAM, R. (2004). Arachidonoyl-serine, an endocannabinoids-like bioactive constituent of rat brain. ICRS Abstracts Online 133.
- OKA, S., TSUCHIE, A., TOKUMURA, A., MURAMATSU, M., SUHARA, Y., TAKAYAMA, H., WAKU, K. & SUGIURA, T. (2003). Ether-linked analogue of 2-arachidonoylglycerol (noladin ether) was not detected in the brains of various mammalian species. *J. Neurochem.*, **85**, 1374–1381.
- PINTO, J.C., POTIE, F., RICE, K.C., BORING, D., JOHNSON, M.R., EVANS, D.M., WILKEN, G.H., CANTRELL, C.H. & HOWLETT, A.C. (1994). Cannabinoid receptor binding and agonist activity of amides and esters of arachidonic acid. *Mol. Pharmacol.*, **46**, 516–522.
- PREMKUMAR, L.S., QI, Z.-H., VAN BUREN, J. & RAISINGHANI, M. (2004). Enhancement of potency and efficacy of NAD A by PKC-mediated phosphorylation of vanilloid receptor. *J. Neurophys.*, **91**, 1442–1449.
- PRUSAKIEWICZ, J.J., KINGSLEY, P.J., KOZAK, K.R. & MARNETT, L.J. (2002). Selective oxygenation of *N*-arachidonoylglycine by cyclooxygenase-2. *Biochem. Biophys. Res. Commun.*, **296**, 612–617.
- RAKSHAN, F., DAY, T.A., BLAKELY, R.D. & BARKER, E.L. (2000). Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. *J. Pharmacol. Exp. Ther.*, **292**, 960–967.
- RANDALL, M.D., KENDALL, D.A. & O'SULLIVAN, S. (2004). The complexities of the cardiovascular actions of cannabinoids. *Br. J. Pharmacol.*, **142**, 20–26.
- RODRIGUEZ DE FONSECA, F., NAVARRO, M., GOMEZ, R., ESCURDO, L., NAVA, F., FU, J., MURILLO-RODRIGUEZ, E., GIUFFRIDA, A., LOVERME, J., GAETANI, S., KATHURIA, S., GALL, C. & PIOMELLI, D. (2001). An anorexic lipid mediator regulated by feeding. *Nature*, **414**, 209–212.
- ROSS, R.A. (2003). Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.*, **140**, 790–801.
- SAGAR, D.R., SMITH, P.A., MILLNS, P.J., SMART, D., KENDALL, D.A. & CHAPMAN, V. (2004). TRPV1 and CB1 receptor-mediated effects of the endovanilloid/endocannabinoids *N*-arachidonoyl-dopamine on primary afferent fibre and spinal cord neuronal responses in the rat. *Eur. J. Neurol.*, **20**, 175–184.
- SALZET, M. & STEFANO, G.B. (2002). The endocannabinoid system in invertebrates. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 353–361.
- SANCHO, R., MACHO, A., DE LA VEGA, L., CALZADO, M.A., FIEBICH, B.L., APPENDINO, G. & MUNOZ, E. (2004). Immunosuppressive activity of endovanilloids *N*-arachidonoyl-dopamine inhibits activation of the NF- κ B, NFAT, and activator protein 1 signaling pathways. *J. Immunol.*, **172**, 2341–2351.
- SCHMID, H.H. & BERDYSHEV, E.V. (2002). Cannabinoid receptor-inactive *N*-acylethanolamines and other fatty acid amides: metabolism and function. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 363–376.
- SCHUEL, H., BURKMAN, L.J., LIPPES, J., CRICKARD, K., FORESTER, E., PIOMELLI, D. & GIUFFRIDA, A. (2002). *N*-acylethanolamines in human reproductive fluids. *Chem. Commun.*, **215**, 211–227.
- SHEKIN, T., HANUS, L., SLAGER, J., VOGEL, Z. & MECHOULAM, R. (1997). Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. *J. Med. Chem.*, **40**, 659–667.
- SUGIURA, T., KONDO, S., SUKAGAWA, A., NAKANE, S., SHINODA, A., ITOH, K., YAMASHITA, A. & WAKU, K. (1995). 2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.*, **215**, 89–97.
- THOMAS, E.A., CRAVATT, B.F., DANIELSON, P.E., GILULA, N.B. & SUTCLIFFE, J.G. (1997). Fatty acid amide hydrolase, the degradative enzyme for anandamide and oleamide, has selective distribution in neurons within the rat central nervous system. *J. Neurosci. Res.*, **50**, 1047–1052.
- TOTH, A., KEDEI, N., WANG, Y. & BLUMBERG, P.M. (2003). Arachidonoyl dopamine as a ligand for the vanilloid receptor VR1 of the rat. *Life Sci.*, **73**, 487–498.
- WALTER, L. & STELLA, N. (2004). Cannabinoids and neuroinflammation. *Br. J. Pharmacol.*, **141**, 775–785.
- YOST, C.S., HAMPSON, A.J., LEONOUKAKIS, D., KOBLIN, D.D., BORNHEIM, L.M. & GRAY, A.T. (1998). Oleamide potentiates benzodiazepine-sensitive gamma-aminobutyric acid receptor activity but does not alter minimum alveolar anesthetic concentration. *Anesth. Analg.*, **86**, 1294–1300.
- YU, M., IVES, D. & RAMESHA, C.S. (1997). Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J. Biol. Chem.*, **272**, 21181–21186.

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