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Fatty acid amide signaling molecules

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ABSTRACT

Key studies leading to the discovery and definition of the role of endogenous fatty acid amide signaling molecules are summarized.

Work conducted over the last twenty years has provided compelling evidence that the fatty acid amides serve as a new and additional class of endogenous signaling molecules. Herein, we review these studies and key elements of the work resulting in their discovery, biosynthesis, degradation, and fundamental endogenous role. These may be grouped largely into two classes, the fatty acid ethanolamides, of which anandamide is the prototypical member, and the fatty acid primary amides, of which oleamide is the most explored member. Because of their overlapping endogenous and signaling roles, a brief introduction to key structurally related fatty acid esters and ethers is also provided.

Fatty acid ethanolamides. Although isolated, identified and physiologically characterized as early as the mid 1900's,[1] the notion that the fatty acid ethanolamides serve as key fundamental signaling molecules gained substance with the discovery that anandamide represents an endogenous ligand for the newly identified cannabinoid receptors.

Anandamide. *Isolation/identification*. Shortly following the identification and characterization of the cannabinoid receptors in 1988 (CB1) and 1993 (CB2),[2] Mechoulam and co-workers isolated, identified, and characterized anandamide as an endogenous agonist of the receptors in 1992 (Fig. 1).[3] Although received with some skepticism at the time given the simplicity of the structure and the lack of precedent for this class of signaling molecules, anandamide, also known as *N*-arachidonoyl ethanolamide (AEA), is now widely accepted as an endogenous cannabinoid neurotransmitter. Its name is derived from the Indian Sanskrit word, *ananda*, which means 'bringer of eternal bliss and tranquillity'. Anandamide is part of a large family of signaling lipids, the *N*-acylethanolamines (NAEs). It was the first endogenous ligand identified in a screen for ligands for the cannabinoid receptor shortly after their identification[2] and was isolated from porcine brain extracts.[3] This endocannabinoid inhibited the specific binding of a radiolabeled cannabinoid probe [^{3}H]HU-243 to synaptosomal membranes in a manner typical of competitive ligands and produced a concentration-dependent inhibition of the electrically evoked twitch response of the mouse vas deferens, a characteristic effect of psychotropic cannabinoids.

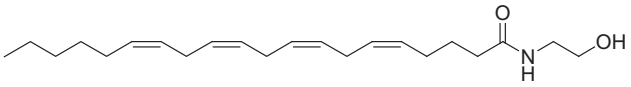

Figure 1. Structure of anandamide.

Biosynthesis/metabolism. Despite over 20 years of study, the biosynthesis of anandamide and other NAEs is not yet fully characterized. It is generally accepted that *N*-acyl phosphatidylethanolamines (NAPEs) are the precursors for NAEs, but the precise enzymatic steps leading to release of NAEs from NAPEs are unclear. Several postulated routes for their synthesis are reported and discussed in the literature.[4] The original model for the biosynthesis of NAEs follows the sequential action of (1) a calcium-dependent transacylase (CDTA) that transfers the *sn*-1 acyl chain of phospholipids onto the primary amine of phosphatidylethanolamine (PE) to generate *N*-acyl phosphatidylethanolamines (NAPEs), and (2) a D-type phospholipase that hydrolyzes NAPEs to produce NAEs (Scheme 1).[5] Initial studies indicated that this two-step pathway might also contribute to the biosynthesis of anandamide. Anandamide, along with its NAE congeners and their respective NAPE precursors, is produced by neurons in a calcium-dependent manner,[6] and a brain CDTA activity is capable of producing the anandamide precursor *N*-arachidonoyl PE in vitro.[7] Subsequent characterization of an NAPE-selective phospholipase D (NAPE-PLD) revealed that this enzyme can convert *N*-arachidonoyl PE to anandamide in vitro.[8] It was assumed

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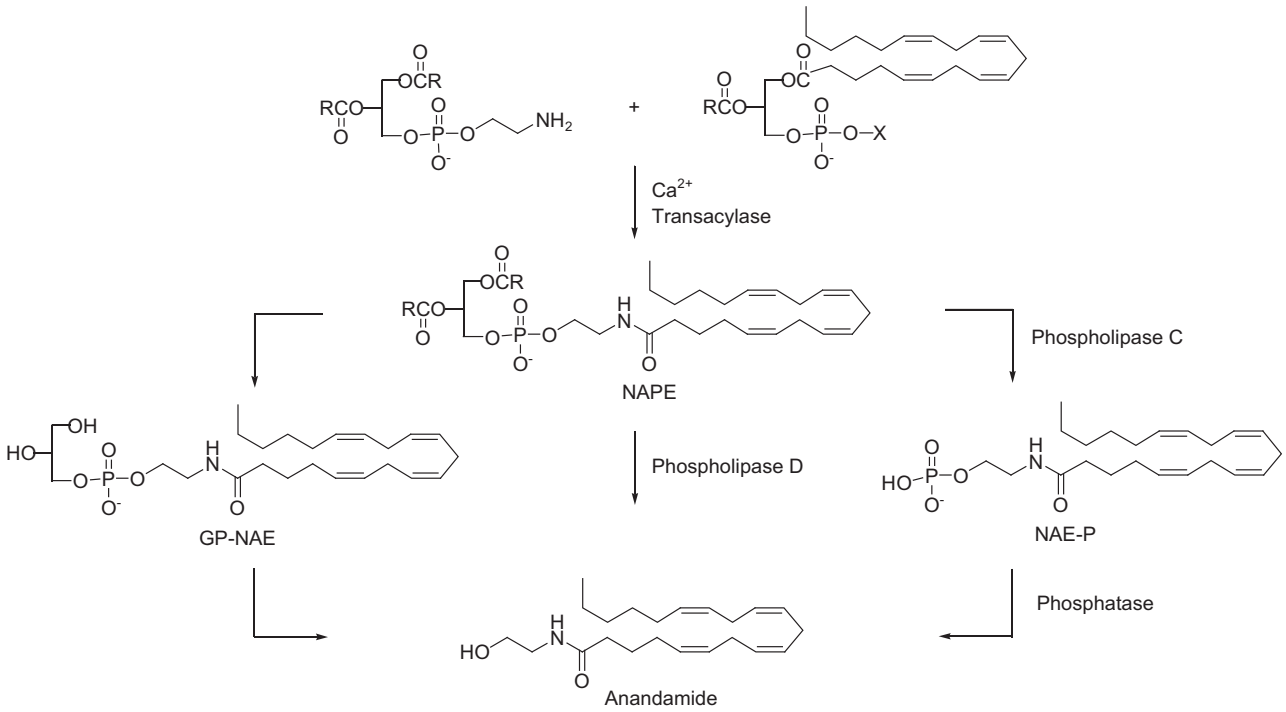

Scheme 1.

that most, if not all NAEs, were biosynthesized in a common enzymatic pathway. However, investigations into the regulated production of NAEs suggested the existence of other biosynthetic pathways for all members of this lipid class. The generation and characterization of mice lacking the *NAPE-PLD* gene provided convincing evidence to support the existence of multiple biosynthetic pathways for NAEs in the nervous system.[9] For the NAPE-PLD-independent pathways, two intermediates have been reported to date. The first is a glycerophospho-NAE intermediate, where the *sn*-1 or *sn*-2 *O*-acyl chains of NAPEs or both are first hydrolyzed to generate lyso-NAPEs and glycerophospho(GP)-NAEs, respectively,[10] followed by cleavage of the phosphodiester bond of these intermediates to generate NAEs.[11] The second involves the phospholipase C-dependent conversion of NAPEs to phospho-NAEs, followed by phosphatase-mediated hydrolysis of these intermediates to generate NAEs.[12] Anandamide is not stored in cells but is formed when needed, then released from neurons on depolarization and rapidly inactivated. Its principle, if not exclusive, mechanism of inactivation at its sites of action is enzymatic hydrolysis by the membrane-bound serine hydrolase fatty acid amide hydrolase (FAAH).[13,14]

The metabolism of anandamide by human liver and kidney microsomes and the formation of epoxyeicosatrienoic ethanolamides and hydroxyeicosatetraenoic acid ethanolamides have also been studied and reported.[15]

Function. Anandamide binds to the central CB1 and peripheral CB2 cannabinoid receptors through which it is thought to exhibit its analgesic and anti-inflammatory effects.[16] It has been reported to bind with higher affinity to CB1 (K_i = 89 nM)[17] than to CB2 (K_i = 371 nM)[18] in CHO cells in a radioligand binding assay using [^{3}H]CP55940 and was also measured in other cell lines. Anandamide has been shown to behave as an agonist with greater efficacy at CB1 than CB2.[19] Conflicting reports of its potency have been reported. Since anandamide is such an effective substrate for FAAH,[13a] such measurements in cell-based assays need to be carried out in the presence of a potent and selective FAAH inhibitor[20] to insure accurate concentrations are maintained in the binding or functional assays. It also has effects, particularly in the vasculature, that cannot be explained by actions at either the CB1 or CB2 receptors. These effects may be mediated by novel G protein-coupled receptors, but genome searching has not yet revealed a strong candidate. Several approaches have suggested that an orphan receptor, GPR55, is a target for anandamide, but the pharmacology of this receptor is such that it cannot yet be categorically classified as a cannabinoid receptor.[21]

Anandamide is also reported to be an endogenous ligand for the vanilloid receptor (TRPV1) that is involved in the transduction of acute and inflammatory pain signals, activating the receptor in a PKC-dependent (protein kinase C-dependent) manner, leading first to the perception of pain and then desensitization providing what may be a second site of action for its analgesic effects.[22]

Anandamide modulates distinct and diverse physiological processes, including nociception,[23] anxiety,[24] inflammation,[25] appetite,[26] learning and memory.[27] A study also reported that anandamide inhibits breast cancer cell proliferation.[28]

Palmitoyl ethanolamide. *Isolation/identification*. Palmitoyl ethanolamide, or *N*-(2-hydroxyethyl)hexadecanamide (PEA), was first identified more than 50 years ago (Fig. 2). It was isolated from egg yolk, hexane-extracted peanut meal, and soybean lecithin.[29] This endogenous compound is present in the rat brain, liver and skeletal muscle.[1]

Biosynthesis/metabolism. PEA and anandamide are synthesized from different precursors through the action of the same enzyme, the *N*-arachidonoyl-phosphatidyl-ethanolamine-selective phospholipase D[30] and are hydrolyzed by the same amidase enzymes.

In addition to FAAH,[13] an additional enzyme has been purified and characterized that exhibits a higher catalytic efficiency for palmitoyl ethanolamide than with anandamide.[31] This enzyme is a

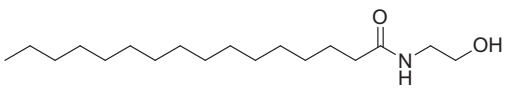

Figure 2. Structure of palmitoyl ethanolamide.

lysosomal hydrolase with optimal activity at acidic pH and a different tissue distribution than FAAH. Esters, retroesters and retroamides of 16:0 palmitic acid were reported as selective inhibitors of the palmitoyl ethanolamide amidase.[32]

Function. Palmitoyl ethanolamide was shown to reduce allergic reactions and inflammation[33,34] in animals along with influenza symptoms in humans.[35] It was found to inhibit peripheral inflammation[36,37] and mast-cell degranulation,[38] as well as to exert neuroprotective[39] and anti-nociceptive effects in rats and mice.[40,23a] These actions are accompanied by changes in nitric oxide production,[41] neurotrophil influx,[42] and expression of pro-inflammatory proteins such as inducible nitric-oxide synthase and cyclooxygenase-2.[43] The nuclear receptor peroxisome proliferator-activated receptor-α (PPAR-α) was indentified as the molecular target responsible for the anti-inflammatory properties of PEA.[44] It was also reported that PEA has an anti-inflammatory effect on human adipocytes and could be a potentially interesting candidate molecule in the prevention of obesity-associated insulin resistance.[45] Its anticonvulsant activity in mice has been reported, however, its precise mechanism of action remains to be elucidated.[39]

Analogues of palmitoyl ethanolamide, varying the fatty acid chain length from caproyl to stearoyl and the nature of the amide substituent, were evaluated for affinity to cannabinoid receptors and, like PEA itself, were reported to be inactive.[46]

An enhancement of the hypotensive effects of intrathecally (i.t.) injected endocannabinoids in the spinal cord by palmitoyl ethanolamide has been reported. The facilitative action of palmitoyl ethanolamide affects the vanilloid TRPV1 as well as the cannabinoid CB1 receptor-mediated effects of endocannabinoids on blood pressure control.[47,48]

Levels of palmitoyl ethanolamide along with other endogenous neuroprotective substrates were measured in different brain areas of R2/6 mice, a transgenic model of Huntington's disease, versus wild-type animals.[49] These studies suggest that drugs inhibiting endocannabinoid degradation might be useful to treat this disease.

Oleoyl ethanolamide. *Isolation/biosynthesis*. Oleoyl ethanolamide (OEA) is a natural analog of the endogenous cannabinoid anandamide (Fig. 3). It is produced, like anandamide, in cells in a stimulus-dependent manner and is rapidly eliminated by enzymatic hydrolysis,[13] suggesting a function in cellular signaling.[6] OEA, along with AEA and PEA, has been shown to be present in human seminal plasma, mid-cycle oviductal fluid, follicular fluid, amniotic fluid, milk, and fluids from malignant ovarian cysts.[50]

Function. Oleoyl ethanolamide mainly modulates feeding and energy homeostasis and is thought to act by binding to peroxisome proliferator-activated receptor-α (PPAR-α).[26,51] It is reported to not bind to or activate cannabinoid receptors. OEA was found to excite sensory neurons and produce visceral increased sensitivity to pain via activation of the TRPV1 receptor.[52–54] However, a recent study described this agent as an anti-nociceptive substance in two models of visceral and inflammatory pain in both mouse and rat.[55]

It has also been reported that the appetite suppressant activity of OEA may be derived from its action as an efficacious agonist of GPR119,[56] a highly expressed receptor in pancreatic islets and in the colon[57] that has a distant similarity to biogenic amine and cannabinoid receptors (~40% identity in the transmembrane regions). However, weight loss mediated by OEA is not seen in mice lacking PPAR-α, but remains fully intact in mice lacking GPR119.[58]

Long chain saturated and unsaturated alkyl sulfonamide and propyl sulfonamide derivatives, analogs of oleoyl ethanolamide, have been evaluated in vivo and in vitro as PPAR-α activators. Additionally, the anorexic effects of the compounds have been studied in vivo in food-deprived rats. Among the active compounds, *N*-octadecyl-*N*′-propylsulfonamide has been identified as a potent hypolipidemic compound, a potent feeding suppressant, and a concentration dependent activator of PPAR-α.[59]

Steaoryl ethanolamide. *Identification*. Stearoyl ethanolamide (SEA) is a fully saturated C18 *N*-acyl ethanolamide (Fig. 4). It has been shown to accompany anandamide in many tissues including rat central neurons,[6] brain,[60] and testis,[61] mouse neuroblastoma,[62] murine basophiles and macrophages.[63] Palmitoyl and stearoyl ethanolamides have been found to be the two most abundant *N*-acyl ethanolamides in most tissues.

Function. The endogenous role of stearoyl ethanolamide has yet to be fully elucidated. It does not bind cannabinoid receptors, however it can affect cell signaling and elicit biological effects potentially through targets other than cannabinoid receptors. Although these pathways are not yet understood, stearoyl ethanolamide has been shown to enhance AP-1 transcriptional activity mediated by the extracellular-signed-regulated protein kinase (ERK) mitogen-activated protein kinase (MAP kinase) pathway. In 2001, steaoryl ethanolamide was shown to stimulate AP-1 activity in mouse epidermal JB6 P^+ cells through the ERK MAP kinase pathway.[64]

It is known that high levels of saturated versus unsaturated ethanolamides accumulate in injured tissue.[65] By employing a murine model of passive IgE-induced cutaneous anaphylaxis, stearoyl ethanolamide was shown to possess anti-inflammatory properties in vivo. The results demonstrated that an acute systemic administration of stearoyl ethanolamide markedly conteracts the edema in the pinna (ipsilateral ear) of adult mice caused by cutaneous anaphylaxis.

Linoleoyl ethanolamide. *Identification/biosynthesis*. As with other members of this class, linoleoyl ethanolamide (Fig. 5) was detected in porcine brain and murine peritoneal macrophages.[66] In addition, linoleoyl ethanolamide also has been isolated from mouse J774 macrophages and N18 neuroblastoma cells[62] as well as RBL-2H3 leukocytes.[67] The biosynthesis has not been studied in detail, but is presumed to be analogous to that of more frequently studied *N*-acyl ethanolamides, including anandamide.

Function. Linoleoyl ethanolamide is approximately 4-fold less potent than anandamide at causing catalepsy in mice and it does not prolong sleep time.[68] Hanus and co-workers reported that it binds to cannabinoid receptors and inhibits the electrically evoked twitch response of mouse isolated vas deferense similar to anandamide and other *N*-acyl ethanolamides.[69] However, linoleoyl ethanolamide has been found to only weakly bind CB1 and CB2 receptors, inhibiting the binding of [^{3}H]CP-55,940 with K_i values of 10 and 25 μM, respectively.[70]

In addition, linoleoyl ethanolamide may be involved in regulation of food intake by selective prolongation of feeding latency and post-meal interval. It appears to be formed locally in the intestine, where it activates PPAR-α.[71]

Fatty acid primary amides. Less appreciated, but equally important, the endogenous fatty acid primary amides[72] emerged as candidate signaling molecules with the discovery,[73] disclosure,[74] and surprisingly structural selectivity[75] that oleamide displays in exerting a fundamental role in regulating sleep. In this work and key to the field, its rapid enzymatic inactivation by hydrolysis led to the detection,[74] characterization,[13] and study of fatty acid amide hydrolase (FAAH), which regulates the activity

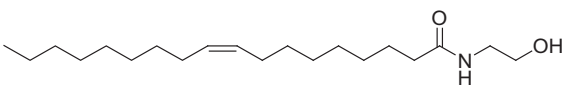

Figure 3. Structure of oleoyl ethanolamide.

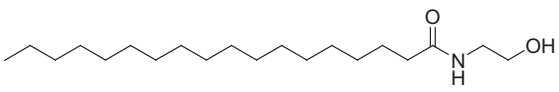

Figure 4. Structure of stearoyl ethanolamide.

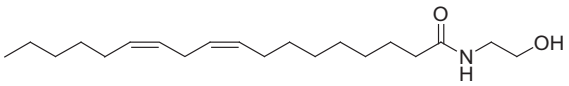

Figure 5. Structure of linoleoyl ethanolamide.

of fatty acid primary amides at their sites of action. An especially attractive feature of this class of fatty acid amide signaling molecules[76] is the fact that they are capped as a primary amide analogous to the widely recognized peptide signaling molecules suggesting conserved strategies for their biosynthesis, precursor storage, and release. Often overlooked in the screening for endogenous ligands and because of their rapid degradation (hydrolysis) by fatty acid amide hydrolase (FAAH),[13a] it is likely that the most important fundamental endogenous role of many members of this class remain to be defined.

Oleamide. *Identification*. Fatty acid primary amides form a group of endogenous lipid messengers of growing interest. In 1995, groups at Scripps isolated a novel lipid in the cerebrospinal fluid of sleep-deprived cats.[73] It was shortly thereafter identified as oleamide, the primary amide of oleic acid.[74,75] Oleamide or *cis*-9,10-octadecenamide has since attracted wide interest being the first fatty acid primary amide to be identified as a signaling molecule (Fig. 6). In addition to serving as a chemical messenger signaling sleep,[74,77] it exhibits cannabinoid-like activity,[78] and has been shown to have direct agonist action at CB1 cannabinoid receptors.[78,79] Oleamide has also been observed to interact directly with voltage-gated Na^+ channels and allosterically with $GABA_A$ and several 5-hydroxytryptamine (5-HT) receptor subtypes.

Biosynthesis. One of the key unanswered questions is how endogenous oleamide is produced. Currently there are several proposed pathways for its biosynthesis that have some experimental support. Glutamine can serve as an ammonia source for many amination reactions in vivo. A modest glutamine-dependent biosynthesis of oleamide from oleic acid was observed in rat brain microsomes[61] and similar observations in mouse neuroblastoma cells have been reported.[80] A second pathway has been suggested in which oleamide can be endogenously derived from its glycine adduct. This biosynthetic route entails the production of the amide of oleic acid with glycine or the *N*-terminal glycine of a peptide by an unidentified enzyme, followed by the oxidative cleavage of this acyl glycine by peptidylglycine α-amidating monooxygenase (PAM). PAM is a well-characterized enzyme involved in the production of C-terminally amidated neuropeptides.[81] Recent in vitro studies have demonstrated that PAM efficiently generates oleamide from its corresponding glycine adduct.[82,83] Merkler and co-workers have also shown that the $N_{18}TG_2$ cell line can synthesize oleamide from oleic acid,[84] thereby demonstrating that these cells contain the necessary catalytic activities for generating oleamide.

Function. Most prominent among its effects is the ability of oleamide to induce natural physiological sleep. Unlike typical sleep aids that act as CNS depressants, oleamide induces sleep indistinguishable from physiological sleep without the side effects of such sedatives or hypnotics. A key feature to emerge from these studies was the observation that removal of the cis double bond, its conversion to a trans double bond, or even its movement along the fatty acid chain by a single carbon reduced or abolished the sleep-inducing effects of the compound.[74]

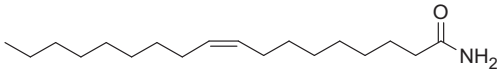

Figure 6. Structure of oleamide.

The characteristic tetrad of effects evoked by cannabinoid receptor agonists in vivo is hypothermia, hypoactivity, analgesia and catalepsy. Observations concerning the direct interaction of oleamide with cannabinoid receptors are conflicting. However, oleamide produces a dose-dependent hypothermia and a decrease in locomotor activity in both mice and rats.[77,85] It induces catalepsy in mice, but not in rats[86] and it produces antinoception in both species.[87] Studies using the selective CB1 antagonist SR141716A have also produced conflicting results. SR141716A was shown to reverse the effects of oleamide on sleep,[88] locomotor activity and antinoception, yet failed to reverse locomotion.[89] In both studies, SR141716A failed to reverse oleamide-induced hypothermia. An unusual, and likely irrelevant, 'entourage effect'[16d,90] of oleamide was proposed to account for its biological properties suggesting that they arise instead from the actions (concentration) of anandamide that are potentiated by competitive hydrolysis of oleamide by the enzyme fatty acid amide hydrolase (FAAH). In addition, Cheer[78] and more recently Leggett[79] demonstrated that oleamide does bind the CB1 receptor. Using radiolabeled ligand binding studies, it was shown that oleamide inhibits agonist [^{3}H]CP55940 binding to CB1 receptors. Oleamide also acts as an agonist at CB1 as shown by an increase in [^{35}S]GTPγS binding in rat brain slices in a pattern that mimicks that of the cannabinoid receptor agonist HU-210 and this receptor stimulation was blocked by the CB1 antagonist SR141716A. These studies indicate that oleamide is an endogenous cannabinoid receptor full agonist with selectivity for CB1 over CB2. Characteristic of the challenges in interpreting the results of such reports is the rapid hydrolysis and inactivation of oleamide by fatty acid amide hydrolase (FAAH).[13,91] Studies enlisting cell-based binding or functional assays should be carried out in the presence of a FAAH inhibitor in order to ensure maintenance of accurate concentrations of oleamide and such variations may account for the distinctions observed in many of the conflicting reports.

It is also likely that not all sites of action and perhaps not even the major site of action of oleamide have yet been identified. Consequently, oleamide's endogenous site of action remains unclear, but it has been shown to modulate both serotonergic and GABAergic receptor types in vitro, two neurotransmitter systems typically associated with the control of sleep-wake processes in vivo. Basile and co-workers quantified the changes in oleamide levels in the CSF of sleep-deprived rats, demonstrating a 3- to 4-fold increase in the compound's concentration upon sleep deprivation for 6 or more hours.[87] It has been shown that the $GABA_A$ receptor antagonist bicuculline reverses oleamide-induced hypothermia and analgesia and elimination of the β subunit of the $GABA_A$ receptor prevents oleamide-induced sleep.[92] Oleamide's endogenous and temporal associations are consistent with those required of cannabinoid, serotonergic, GABAnergic, or ion channel neurotransmission which may be involved in sleep induction.[93]

It has also been shown that inhibitory synaptic currents in rat $GABA_A$ receptors are sensitive to modulation by oleamide. Oleamide reversibly induces $GABA_A$ currents and depresses the frequency of spontaneous excitatory and inhibitory synaptic activity in cultured networks.[94] Synthetic depressant drugs are recognized as allosteric modulators of ion channel targets like the $GABA_A$ receptor and voltage-gated Na^+ channels. Oleamide has been found to be a nonselective modulator of inhibitory ionotropic receptors and has been shown to act indirectly at an allosteric site on the $GABA_A$ receptor in a fashion analogous to benzodiazepine binding.[95]

Studies have indicated that oleamide affects multiple neuropathway systems. Oleamide has been shown to modulate the signaling of several 5-hydroxytryptamine (5-HT) receptor subtypes, including $5\text{-}HT_{1A}$, $5\text{-}HT_{2A/C}$, and $5\text{-}HT_7$.[96] Previous studies by Huidroboro-Toro and Harris[97] indicate that oleamide potentiates $5\text{-}HT_{2A/C}$-mediated chlorine currents in frog oocyte systems. In this system, the chlorine

currents elicited by 5-$HT_{2A/C}$ result from a signaling cascade involving phosphoinositide hydrolysis and inositol trisphosphate stimulation. Thomas et al.[98] measured the effect of oleamide directly on phosphoinositide hydrolysis and demonstrated that oleamide substantially increases 5-HT-induced hydrolysis in P11 cells. Functional studies by Thomas et al.[99] and Hedlund[100] indicate that oleamide acts at an allosteric site on the 5-HT_7 receptor to influence G protein signaling regulating cyclic AMP formation. Oleamide has demonstrated a 50% increase in cyclic AMP production in HeLa cells expressing the 5-HT_7 receptor. In addition, oleamide induced a concentration dependent increase in cyclic AMP formation that could not be inhibited by clozapine suggesting that it acts at a site distinct from the primary 5-HT binding site. Oleamide has also been shown to activate 5-HT_7 neurons in mouse thalamus and hypothalamus.

Oleamide has also been reported to interact with gap junctions, and has been utilized as a tool to inhibit their function. It has been reported that oleamide blocks dye transfer between rat glial cells in culture[101] and blocks junctions formed by cells expressing Cx32 (β_1 connexin), but does not block Ca^{2+}-wave propagation between glial cells.[102,103] Other compounds traditionally used as inhititors of gap junction communication, like heptanol, block not only gap junction communication, but also intracellular Ca^{2+} signaling. Thus, oleamide might have selective effects on the permeability of gap junctions, an effect that can be exploited. In view of the importance of gap junctions in the cardiovascular system, the heart, endothelial cells, and vascular muscle, this aspect of its biology is of particular relevance.

Additional fatty acid primary amides. The primary amides of oleic (18:1^9), palmitic (16:0), palmitoleic (16:1^9), elaidic (18:1$^{9\text{-trans}}$), and linoleic (18:2^{9,12}) were identified in human plasma before physiological roles were established (Fig. 7).[104] Linoleamide was found to induce sleep and increase cytosolic Ca^{2+} levels in MDCK tubular cells.[105] Arachidonamide has been reported to affect gap junction communication.[101] Erucamide (22:1^{13}) has also been identified as the major angiogenic component in bovine mesenterial fluids stimulating new blood vessel formation and was reported to act as a modulator of water balance.[106] More recently, additional fatty acid amides including stearamide, palmitamide, and myristamide were isolated from human tear gland secretions.[107] However, the actions of these signaling molecules remain to be elucidated. The fact that arachidonamide, the primary amide of arachidonic acid, is the best substrate for FAAH being hydrolyzed and inactivated faster than oleamide (3-fold) or anandamide (2-fold),[13a] suggests that it represents a key signaling molecule in this class. Because its physiological role is yet to be defined, arachidonamide represents a prime candidate to examine in existing or new targets for the fatty acid amides and should be done so in the presence of FAAH inhibitors to block its rapid inactivation.

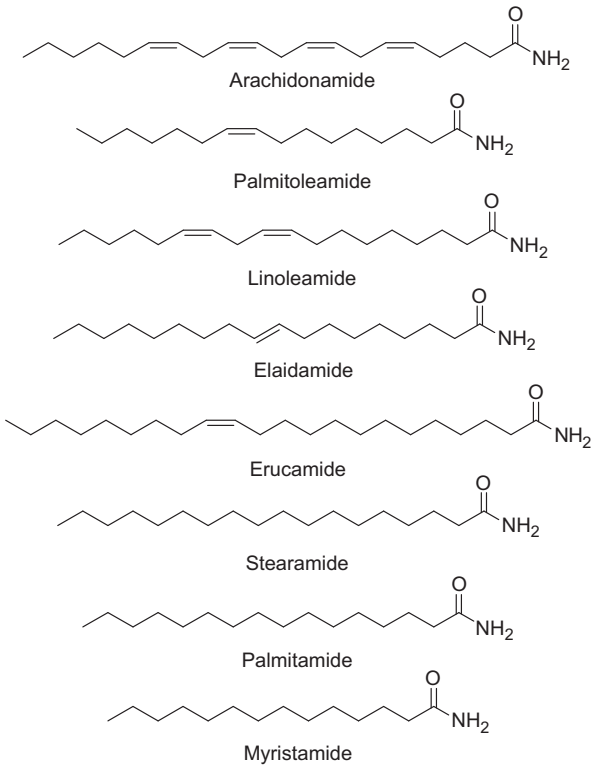

Figure 7. Additional endogenous fatty acid primary amides.

Glycine amides. An intriging series of *N*-acyl glycinamides bearing fatty acid acyl groups have been identified as endogenous fatty acid amides that are attracking increasing attention. At present, it is not yet clear whether they serve as chemical signaling molecules in their own right, or whether they are simply biosynthetic precursors to the active fatty acid primary amides.

***N*-Arachidonoylglycine**. *Isolation/identification*. *N*-Arachidonoylglycine (NAGly) has been isolated from cell cultures treated with anandamide (AEA),[108] from extracts of mammalian brain,[109] and has been synthesized as an analog of anandamide for structure–activity testing (Fig. 8).[110] Its biosynthesis is poorly understood to date, and two primary biosynthetic pathways have been proposed. One suggests that NAGly is formed by an enzymatically regulated conjugation of arachidonic acid and glycine. The other suggests that NAGly is an oxidative metabolite of AEA through the action of an alcohol dehydrogenase. In vivo and in vitro assays measuring metabolites with LC/MS/MS support the hypothesis that NAGly is a metabolite product of AEA by both oxidative metabolism of AEA and through the conjugation of glycine to arachidonic acid that is released during AEA hydrolysis by FAAH.[111] It is notable that endogenous levels of NAGly are greater than those of anandamide in the CNS.

Function. NAGly is a very poor ligand for the CB1 and CB2 receptors but has shown pain-relieving and anti-inflammatory effects in rodents.[112] Other signaling effects of NAGly have been indentified, including activation of the orphan G protein-coupled receptors GPR18[113] and GPR92.[114] An inhibitory interaction with the glycine transporter GLYT2a[115] and inhibition of AEA hydrolysis by FAAH have also been reported.[116] It is also a substrate of cyclooxygenase 2, producing an Gly amino acid conjugate of prostaglandins.[117] These observations have been interpreted to suggest that effects of NAGly may be derived from an increase in the concentration of AEA, or from modulating the ratio of prostaglandins from the pro-inflammatory PGE2 towards the inflammation-resolving J prostaglandins.[118]

***N*-Palmitoylglycine**. *Isolation/identification*. *N*-Palmitoylglycine (PalGly) is produced in high levels after cellular stimulation (KCl-induced depolarization of F-11 cells) in rat skin and spinal cord (Fig. 9). It is present in 100-fold greater amounts in skin and 3-fold greater amounts in brain compared to anandamide.[119]

Function. PalGly was up-regulated in FAAH knock-out mice suggesting a pathway for enzymatic regulation. It potently inhibits heat-evoked firing of nociceptive neurons in rat dorsal horn, and induced transient calcium influx in native adult dorsalroot ganglion (DRG) cells and a DRG-like cell line (F-11). It also contributed to the production of NO through calcium-sensitive nitric-oxide

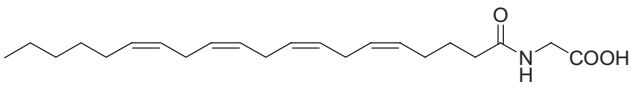

Figure 8. Structure of *N*-arachidonoylglycine.

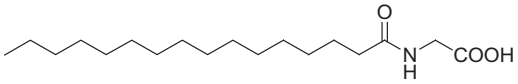

Figure 9. Structure of *N*-palmitoylglycine.

synthase enzymes present in F-11 cells and this activity was inhibited by the nitric-oxide synthase inhibitor 7-nitroindazole.[119]

***N*-Oleoylglycine**. *Identification/biosynthesis*. *N*-Oleoyglycine (OlGly) was first isolated from rat brain matrix and later detected in rat skin, lung, liver, kidney, heart, testes and spinal cord (Fig. 10).[120] The *N*-acyl glycines are produced in vivo from the fatty acyl-CoA thioesters and glycine by acyl-CoA:glycine *N*-acyltransferase (ACGNAT). Mueller and Driscoll demonstrated that cytochrome *c* catalyzes the formation of oleoylglycine from oleoyl-CoA, glycine and hydrogen peroxide.[121] Oleoylglycine has been proposed to be an important intermediate in the PAM-mediated biosynthesis of oleamide from oleic acid. In experiments with $N_{18}TG_2$ cells, Merkler detected oleoylglycine by mass spectroscopy as an intermediate in this biosynthetic pathway.[122]

Function. Chatuervedi suggested that oleoylglycine possesses biological activity that is independent of its conversion to oleamide. Oleoylglycine was found to be equipotent with oleamide in decreasing locomotion and body temperature.[123] However, the full extent of it's actions have yet to be elucidated.

Other *N*-acyl glycines. *Isolation*. Along with oleoylglycine, stearoyl (StrGly), linoleoyl (LinGly) and docosahexaenoyl glycine (DocGly) were also detected in rat brain, skin, liver, kidney, spinal cord, heart and testes (Fig. 11). Levels in the skin, lungs, and spinal cord were highest in stearoyl, oleoyl, and docosahexaenoyl glycine while levels of linoleoyl glycine in the spinal cord were lower than all the other *N*-acyl glycines measured.[120]

Function. Burstein demonstrated that docosahexaenoyl and linoleoyl glycine suppress proliferation of the murine macrophage cell line, RAW264.7, whereas oleoylglycine had no effect.[124] Many of these acyl glycines have yet to be carefully studied.

***N*-Acyl taurines**. Recent efforts using highly sensitive MS techniques and comparative global metabolomic profiling of FAAH knockout and wild type mice led to the identification of a new class of endogenous fatty acid amides in the CNS.[125]

Identification/biosynthesis. In 2004, Cravatt and co-workers discovered the presence and a 10-fold increase of long chain ($\geqslant$C20) saturated *N*-acyl taurines (NATs) in the central nervous system of FAAH knockout mice.[125,126] *N*-Acyl taurines isolated in the central nervous system were highly enriched in long chain saturated and monounsaturated *N*-acyl chains while those found in the kidney and liver were enriched in polyunsaturated *N*-acyl chains.[127]

The identity of the enzyme responsible for NAT biosynthesis remains to be elucidated. However, high levels of an activity capable of biosynthesizing NATs from fatty acyl CoA and taurine were detected in the liver and kidney.[128] The bile acid-CoA:amino acid *N*-acyltransferase (BAT) enzyme responsible for bile salt production is also enriched in the liver.[129] This enzyme could potentially catalyze the formation of NATs. Consistent with this premise, human BAT has been shown to form *N*-acyl glycines when incubated with fatty acyl CoA substrates in vitro.[130]

Function. *N*-Arachidonyl taurine (Fig. 12), in particular, was found to activate multiple members of the transient receptor potential (TRP) family of calcium channels, including TRPV1 and TRPV4,[131] both of which are expressed in the kidney. These channels have been proposed to play a role in the regulation of blood pressure and osmotic sensation. It has been noted that elevations in endogenous levels of NATs following acute or chronic inactivation of FAAH, suggests that NATs could form a major lipid signaling system, similar to *N*-acyl ethanolamides.[131]

Key structurally related fatty acid derived signaling molecules. 2-Arachidonylglycerol. *Isolation/identification*. 2-Arachidonylglycerol (2-AG) was isolated in 1995 from canine gut[132] and rat brain (Fig. 13).[133] It represents a second cannabinoid receptor ligand class and possesses an ester versus amide. It was the first putative endogenous cannabinoid receptor agonist isolated from peripheral tissue. Unlike anandamide, 2-AG is present at relatively high levels in the central nervous system (100-fold higher than anandamide) and it is the most abundant molecular species of monoacylglycerol found in mouse and rat brain. It has also been found in low amounts in the liver, spleen, lung and kidney.[134]

Function. The formation of 2-AG is calcium-dependent and is mediated by the activities of phospholipase C (PLC) and diacylglycerol lipase (DAGL).[133] The hydrolysis of 2-arachidonylglycerol to arachidonic acid and glycerol in the mouse brain, is mainly attributed to monoacylglycerol lipase, MAGL (~85%) with the remaining 15% mostly catalyzed by two uncharacterised enzymes alpha/beta-hydrolase domains 6 and 12 (ABHD6 and ABHD12).[135] FAAH was identified as the next largest contributor to 2-AG hydrolysis accounting for ~1% of total membrane activity.

2-Arachidonylglycerol binds both CB1 and CB2 and acts as a full agonist.[136] Despite substantial degradation, 2-AG has been shown to be a potent full efficacy agonist mediating CB1 receptor-dependent G-protein activation in rat cerebellar membranes. It has been reported that 2-AG was more potent than AEA in stimulating [^{35}S]GTPγS binding to rat cerebellar membranes.[137]

A series of conformationally constrained analogs at the glycerol moiety of 2-AG incorporating its key of pharmacophore features into a six-membered carbocyclic ring system were synthesized and were tested for their affinity for CB1 and CB2 receptors. All the compounds had affinity for the cannabinoid receptors comparable to 2-AG.[138]

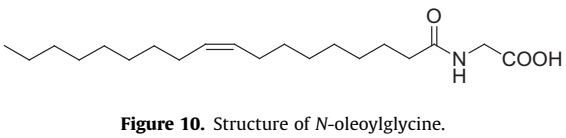

Figure 10. Structure of *N*-oleoylglycine.

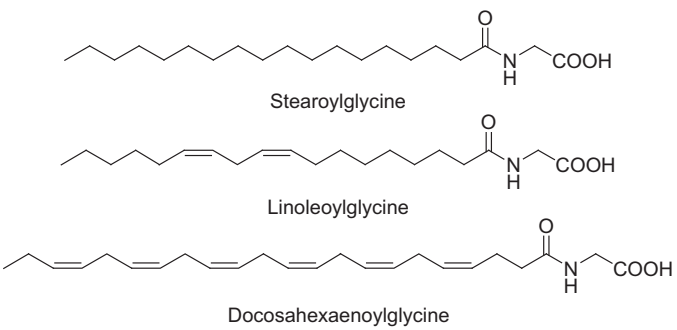

Figure 11. Additional *N*-acyl glycines.

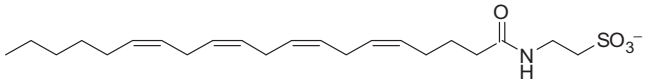

Figure 12. Structure of an *N*-acyl taurine.

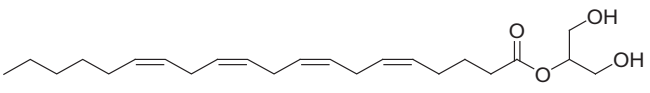

Figure 13. Structure of arachidonylglycerol.

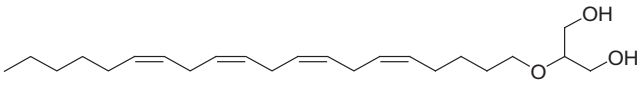

Figure 14. Structure of 2-arachidonoyl glyceryl ether.

2-Arachidonoyl glyceryl ether. *Isolation/identification*. Chemically, noladin ether (NE), or 2-arachidonoyl glyceryl ether (2-AGE), is the 2-glyceryl ether of arachidonyl alcohol and structurally resembles 2-AG (Fig. 14). It was initially extracted from porcine[139] and rat[140] brain in moderate concentrations. The presence of noladin in body tissue is disputed. Although a Japanese group could not detect it in the brains of mice, hamsters, guinea-pigs or pigs,[141] two other groups successfully detected it in animal tissues.[140,142]

Function. Noladin ether binds as a full agonist to CB1[139] and is a weaker binder to CB2.[143] It shows agonist behavior on both receptors and is a partial agonist for the TRPV1 receptor.[144] Upon binding CB2 receptors, it inhibits adenylate cyclase, stimulates ERK-MAPK, and regulates calcium transients.[143] It lowers intraocular pressure,[145] increases the uptake of GABA in the globus pallidus[145] and is neuroprotective by binding and activating PPAR-α.[146] In comparison to 2-AG, it is metabolically more stable resulting in a longer duration of action.[147]

The synthesis of dimethylheptyl (DMP) analogs, with a different tail length of 2-AG and 2-AGE have been reported and showed a distinct decrease of potency towards CB1 receptors.[148] Another series of mono- and diphosphate esters of NE have been reported and showed an enhancement in water-solubility compared to NE.[149] Two regioisomers and 13 analogs of noladin ether were synthesized and tested for their interaction with CB1 receptors in rat brain membrane. The results showed that a C-20 tetra-unsaturated moiety is necessary for high affinity.[150]

Virodhamine (*O*-arachidonoyl ethanolamine). *Identification*. Virodhamine was discovered in 2002 by Porter et al. during the development of a bioanalytical method to measure anandamide levels in tissue. A second peak with the same mass as anandamide but with a different retention time was detected and isolated and found to be arachidonic acid and ethanolamine joined by an ester linkage, in contrast to the amide linkage in anandamide (Fig. 15). Virodhamine has been isolated from rat brain and human hippocampus in levels similar to anandamide. In the peripheral tissues, levels of virodhamine isolated were 2- to 9-fold higher than anandamide.[151]

Biosynthesis. It is not yet defined how virodhamine is produced, stored, or degraded. Virodhamine could be generated from a fatty acid ethanolamine and arachadonic acid by a transphosphotidylation reaction catalyzed by phospholipase D. It is also possible that virodhamine could be produced from anandamide by an enzymatically catalyzed rearrangement of the ethanolamine portion of the molecule from an amide linkage to an ester.[152] Neither mechanism has been examined. It is also possible that it serves as an additional precursor of anandamide.

Virodhamine's ability to block anandamide transport, which is largely mediated by intracellular FAAH hydrolysis, suggests that it may be degraded in a manner similar to anandamide. Since FAAH has been shown to have both amidase and esterase activity, it could be responsible for virodhamine's hydrolysis in vivo.[153]

Function. Virodhamine produced dose-dependent hypothermia in mice. In a [^{35}S]GTPγS functional binding assay, EC_{50} values for virodhamine matched those reported for anandamide and WIN 55,212-2, both of which behave as agonists at CB1 and CB2 receptors, but it was found to be less efficacious than both anandamide and WIN 55,212-2. At CB2, virodhamine acted as a full agonist. However it acted as a partial agonist at CB1 with a maximal efficacy of 61% compared with anandamide.[62] Virodhamine has been shown to relax both human and rat mesenteric arteries through endothelial cannabinoid receptors.[154] Virodhamine has also been reported to have activity at the orphan receptor, GRP55.[155]

N-Acyl dopamine. *Identification/biosynthesis*. It was suggested that certain *N*-acyl dopamines may exist in mammalian tissues and serve as TRPV1 ligands.[156] *N*-Arachidonyl dopamine was first synthesized prior to its endogenous detection in 2002 in rat and bovine nervous tissues (Fig. 16).[157] More recently, several other *N*-acyl dopamines including, palmitoyl, stearoyl, and oleoyl dopamine have been detected.[158] These compounds share a structural similarity with the potent TRPV1 agonist, capsaicin.

A proposed biosynthesis of arachidonyl dopamine proceeds via condensation of arachidonic acid with tyrosine and the subsequent conversion of *N*-arachidonoyltyrosine to *N*-arachidonyl dopamine by tyrosine hydroxylase and L-aromatic amino-acid decarboxylase.[157]

Function. To date, only arachidonyl dopamine was found to have any significant biological activity. It enhances calcium influx in cultured dorsal root ganglion neurons and TRPV1-transfected human embryonic kidney (HEK) cells.[159] Patch-clamp studies of cultured dorsal root ganglion neurons showed that arachidonyl dopamine elicited reversible responses, which were blocked by both the CB1 antagonist SR141617 and the TRPV1 antagonist, capsazepine. In behavioral experiments using non-anesthesized rats, arachidonyl dopamine caused thermal hyperalgesia.[128] Whether arachidonyl dopamine or related *N*-acyl derivatives constitute true signaling molecules or reflect metabolic artifacts is yet to be established.

Conclusions. Work conducted over the past 20 years has provided compelling evidence that fatty acid amides serve as a new and additional class of endogenous signaling molecules. To date, these fall largely into the two classes of ethanolamides and the primary amides. In spite of their simple structures and their apparent lack of distinguishing features, they exhibit surprisingly selective physiological activity that has been directly related to their selective activation (agonist) of receptor-mediated events. Because of their rapid inactivation largely by enzymatic hydrolysis, they are synthesized, released, and inactivated proximal to their sites of action providing a temporal and spatial pharmacological control of their effects. To date, these have impacted some of the most fundamental processes including pain perception (analgesia), inflammation, sleep, and feeding behavior suggesting they may be among the first and most fundamental of our present day classes of signaling molecules. Devoted efforts to characterizing their synthesis,

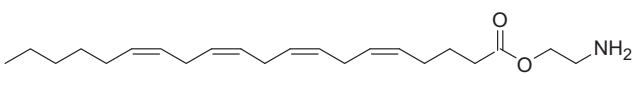

Figure 15. Structure of virodhamine.

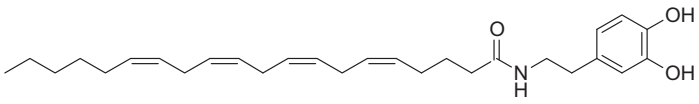

Figure 16. Structure of arachidonyl dopamine.

storage, and release as well as continued efforts to identify their site(s) of action are sure to not only reveal new biology not yet appreciated, but to provide new approaches and targets for therapeutic intervention in some of our most fundamental clinical disorders.

Acknowledgments

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References and notes

1. Bachur, N. R.; Masek, K.; Melmon, K. L.; Udenfriend, S. *J. Biol. Chem.* **1965**, *240*, 1019.
2. (a) Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605; (b) Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561; (c) Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
3. Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
4. Okamoto, Y.; Wang, J.; Morishita, J.; Ueda, N. *Chem. Biodiv.* **2007**, *4*, 1842.
5. (a) Natarajan, V.; Reddy, P. V.; Schmid, P. C.; Schmid, H. H. *Biochim. Biophys. Acta* **1982**, *712*, 342; (b) Schmid, P. C.; Reddy, P. V.; Natarajan, V.; Schmid, H. H. *J. Biol. Chem.* **1983**, *258*, 9302; (c) Ahn, K.; McKinney, M. K.; Cravatt, B. F. *Chem. Rev.* **2008**, *108*, 1687.
6. Di Marzo, V.; Fontana, A.; Cadas, H.; Schinelli, S.; Cimino, G.; Schwartz, J.-C.; Piomelli, D. *Nature* **1994**, *372*, 686.
7. Cadas, H.; Gaillet, S.; Beltramo, M.; Venance, L.; Piomelli, D. *J. Neurosci.* **1996**, *16*, 3934.
8. Cadas, H.; Di Tomaso, E.; Piomelli, D. *J. Neurosci.* **1997**, *17*, 1226.
9. Leung, D.; Saghatelian, A.; Simon, G. M.; Cravatt, B. F. *Biochemistry* **2006**, *45*, 4720.
10. Natarajan, V.; Schmid, P. C.; Reddy, P. V.; Schmid, H. H. *J. Neurochem.* **1984**, *42*, 1613.
11. (a) Simon, G. M.; Cravatt, B. F. *J. Biol. Chem.* **2006**, *281*, 26465; (b) Simon, G. M.; Cravatt, B. F. *J. Biol. Chem.* **2008**, *281*, 9341.
12. (a) Liu, J.; Wang, L.; Harvey-White, J.; Osei-Hyiaman, D.; Razdan, R.; Gong, Q.; Chan, A. C.; Zhou, Z.; Huang, B. X.; Kim, H. Y.; Kunos, G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13345; (b) Liu, J.; Wang, L.; Harvey-White, J.; Huang, B. X.; Kim, H. Y.; Luquet, S.; Palmiter, R. D.; Krystal, G.; Rai, R.; Mahadevan, A.; Razdan, R. K.; Kunos, G. *Neuropharmacology* **2008**, *54*, 1.
13. (a) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83; (b) Boger, D. L.; Fecik, R. A.; Patterson, J. E.; Miyauchi, H.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2613; (c) Fowler, C. J.; Jonsson, K. D.; Tiger, G. *Biochem. Pharmacol.* **2001**, *62*, 517; (d) Kaczocha, M.; Hermann, A.; Glaser, S. T.; Bojesen, I. N.; Deutsch, D. G. *J. Biol. Chem.* **2006**, *281*, 9066; (e) Day, T. A.; Rakhshan, F.; Deutsch, D. G.; Barker, E. L. *Mol. Pharmacol.* **2001**, *59*, 1369.
14. Giang, D. K.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2238.
15. Snider, N. T.; Kornilov, A. M.; Kent, U. M.; Hollenberg, P. F. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 590.
16. (a) Axelrod, J.; Felder, C. C. *Neurochem. Res.* **1998**, *23*, 575; (b) Di Marzo, V.; De Petrocellis, L.; Bisogno, T.; Melck, D. *Lipids* **1999**, *34*, S319; (c) Di Marzo, V. *Biochim. Biophys. Acta* **1998**, *1392*, 153; (d) Mechoulam, R.; Fride, E.; Di Marzo, V. *Eur. J. Pharmacol.* **1998**, *359*, 1; (e) Di Marzo, V.; Bisogno, T.; De Petrocellis, L.; Melck, D.; Martin, B. R. *Curr. Med. Chem.* **1999**, *6*, 721; (f) Martin, B. R.; Mechoulam, R.; Razdan, R. K. *Life Sci.* **1999**, *65*, 573.
17. Adams, I. B.; Ryan, W.; Singer, M.; Thomas, B. F.; Compton, D. R.; Razdan, R. K.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1172.
18. Showalter, V. M.; Compton, D. R.; Martin, B. R.; Abood, M. R. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 989.
19. Pertwee, R. G. *Curr. Med. Chem.* **1999**, *6*, 635.
20. Review: (a) Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327; For one class of potent FAAH inhibitors, see: (b) Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Fecik, R. A.; Miyauchi, H.; Wilkie, G. D.; Austin, B. J.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5044; (c) Boger, D. L.; Miyauchi, H.; Hedrick, M. P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1517; (d) Boger, D. L.; Miyauchi, H.; Du, W.; Hardouin, C.; Fecik, R. A.; Cheng, H.; Hwang, I.; Hedrick, M. P.; Leung, D.; Aceredo, O.; Guimaraes, C. R. W.; Jorgensen, W. L.; Cravatt, B. F. *J. Med. Chem.* **2005**, *48*, 1849; (e) Leung, D.; Du, W.; Hardouin, C.; Cheng, H.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1423; (f) Romero, F. A.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 14004; (g) Romero, F. A.; Du, W.; Hwang, I.; Rayl, T. J.; Kimball, F. S.; Leung, D.; Hoover, H. S.; Apodaca, R. L.; Breitenbucher, J. G.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2007**, *50*, 1058; (h) Hardouin, C.; Kelso, M. J.; Romero, F. A.; Rayl, T. J.; Leung, D.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2007**, *50*, 3359; (i) Kimball, F. S.; Romero, F. A.; Ezzili, C.; Garfunkle, J.; Rayl, T. J.; Hochstatter, D. G.; Hwang, I.; Boger, D. L. *J. Med. Chem.* **2008**, *51*, 937; (j) Garfunkle, J.; Ezzili, C.; Hochstatter, D. G.; Rayl, T. J.; Hwang, I.; Boger, D. L. *J. Med. Chem.* **2008**, *51*, 4392; (k) DeMartino, J. K.; Garfunkle, J.; Hochstatter, D. G.; Cravatt, B. F.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5842; (l) Mileni, M.; Garfunkle, J.; DeMartino, J. K.; Cravatt, B. F.; Boger, D. L.; Stevens, R. C. *J. Am. Chem. Soc.* **2009**, *131*, 10497; (m) Mileni, M.; Garfunkle, J.; Ezzili, C.; Kimball, F. S.; Cravatt, B. F.; Stevens, R. C.; Boger, D. L. *J. Med. Chem.* **2010**, *53*, 230.
21. (a) Brown, A. J.; Wise, A. Patent WO0186305, 2001.; (b) Drmota, E.; Greasley, P.; Groblewski, T. Patent WO2004074844, 2004.; (c) Brown, A. J.; Hiley, C. R. *Vit. Hormones* **2009**, *81*, 111.
22. (a) Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E. D. *Nature* **1999**, *400*, 452; (b) Smart, D.; Gunthorpe, M. J.; Jerman, J. C.; Nasir, S.; Gray, J.; Muir, A. I.; Chambers, J. K.; Randall, A. D.; Davis, J. B. *Br. J. Pharmacol.* **2000**, *129*, 227; (c) Smart, D.; Jerman, J. C. *Trends Pharmacol. Sci.* **2000**, *21*, 134; (d) Olah, Z.; Karai, L.; Iadarola, M. J. *J. Biol. Chem.* **2001**, *276*, 31163.
23. (a) Calignano, A.; La Rana, G.; Giuffrida, A.; Piomelli, D. *Nature* **1998**, *394*, 277; (b) Walker, J. M.; Huang, S. M.; Strangman, N. M.; Tsou, K.; Sanudo-Pena, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12198.
24. Gaetani, S.; Cuomo, V.; Piomelli, D. *Trends Mol. Med.* **2004**, *9*, 474.
25. (a) Cravatt, B. F.; Saghatelian, A.; Hawkins, E. G.; Clement, A. B.; Bracey, M. H.; Lichtman, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10821; (b) Massa, F.; Marsicano, G.; Hermann, H.; Cannich, A.; Monory, K.; Cravatt, B. F.; Ferri, G. L.; Sibaev, A.; Storr, M.; Lutz, B. *J. Clin. Invest.* **2004**, *113*, 1202; (c) Lambert, D. M.; Vandevoorde, S.; Jonsson, K. O.; Fowler, C. J. *Curr. Med. Chem.* **2002**, *9*, 663.
26. Rodriguez de Fonseca, F.; Navarro, M.; Gomez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodriguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Gall, C.; Piomelli, D. *Nature* **2001**, *414*, 209.
27. (a) Lichtman, A. H.; Varvel, S. A.; Martin, B. R. *Prostaglandins Leukot. Essent. Fatty Acids* **2002**, *66*, 269; (b) Marsicano, G.; Wotjak, C. T.; Azad, S. C.; Bisogno, T.; Rammes, G.; Cascio, M. G.; Hermann, H.; Tang, J.; Hofmann, C.; Zieglgansberger, W.; Di Marzo, V.; Lutz, B. *Nature* **2002**, *418*, 530.
28. De Petrocellis, L.; Melck, D.; Palmisano, A.; Bisogno, T.; Laezza, C.; Bifulco, M.; Di Marzo, V. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8375.
29. Meisinger, M. A. P.; Ormond, R. E.; Ganley, O. H.; Jacob, T. A.; Kuehl, F. A., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 5577.
30. Okamoto, Y.; Morishita, J.; Tsuboi, K.; Tonai, T.; Ueda, N. *J. Biol. Chem.* **2004**, *279*, 5298.
31. Ueda, N.; Yamanaka, K.; Yamamoto, S. *J. Biol. Chem.* **2001**, *276*, 35552.
32. Vandevoorde, S.; Tsuboi, K.; Ueda, N.; Jonsson, K. O.; Fowler, C. J.; Lambert, D. M. *J. Med. Chem.* **2003**, *46*, 4373.
33. Perlik, F.; Raskova, H.; Ellis, J. *Acta Physiol. Acad. Sci. Hung.* **1971**, *39*, 395.
34. Lambert, D. M.; Vandervoorde, S.; Jonsson, K. O.; Fowler, C. J. *Curr. Med. Chem.* **2002**, *9*, 663.
35. Kahlich, R.; Klima, J.; Cihla, F.; Frankova, V.; Masek, K.; Rosicky, M.; Matousek, F.; Bruthans, J. *J. Hyg. Epidemiol. Microbiol. Immunol.* **1979**, *23*, 11.
36. Mazzari, S.; Canella, R.; Petrelli, L.; Manolongo, G.; Leon, A. *Eur. J. Pharmacol.* **1996**, *300*, 227.
37. Berdyshev, E.; Boichot, E.; Corbel, M.; Germain, N.; Lagente, V. *Life Sci.* **1998**, *63*, PL125.
38. Aloe, L.; Leon, A.; Levi-Montalcini, R. *Agents Actions* **1993**, *39*, C145.
39. Lambert, D. M.; Vandevoorde, S.; Diependaele, G.; Govaerts, S. J.; Robert, A. R. *Epilepsia* **2001**, *42*, 321.
40. Jaggar, S. I.; Hasnie, F. S.; Sellaturay, S.; Rice, A. S. *Pain* **1998**, *76*, 189.
41. Ross, R. A.; Brochie, H. C.; Pertwee, R. G. *Eur. J. Pharmacol.* **2000**, *401*, 121.
42. Farquhar-Smith, W. P.; Rice, A. S. *Anesthesiology* **2003**, *99*, 1391.
43. Costa, B.; Conti, S.; Giagnoni, G.; Colleoni, M. *Br. J. Pharmacol.* **2002**, *137*, 413.
44. Lo Verme, J.; Fu, J.; Astarita, G.; La Rana, G.; Russo, R.; Calignano, A.; Piomelli, D. *Mol. Pharmacol.* **2005**, *67*, 15.
45. Hoareau, L.; Buyse, M.; Festy, F.; Ravanan, P.; Gonthier, M. P.; Matias, I.; Petrosino, S.; Tallet, F.; Lefebvre d'Hellencourt, C.; Cesari1, M.; Di Marzo, V.; Roche, R. *Obesity* **2009**, *17*, 431.
46. Lambert, D. M.; DiPaolo, F.; Sonveaux, P.; Kanyonyo, M.; Govaerts, S. J.; Hermans, E.; Bueb, J. L.; Delzenne, N. M. *Biochem. Biophys. Acta* **1999**, *1440*, 266.
47. Del Carmen García, M.; Adler-Graschinsky, A.; Celuch, S. M. *Eur. J. Pharmacol.* **2009**, *610*, 75.
48. Ho, W. S. V.; Barrett, D. A.; Randall, M. D. *Br. J. Pharmacol.* **2008**, *155*, 837.
49. Bisogno, T.; Martire, A.; Petrosino, S.; Popoli, P.; Di Marzo, V. *Neurochem. Intern.* **2008**, *52*, 307.
50. Schuel, H.; Burkman, L. J.; Lippes, J.; Crickard, K.; Forester, E.; Piomelli, D.; Giuffrida, A. *Chem. Phys. Lipids* **2002**, *121*, 211.
51. Fu, J.; Gaetani, S.; Oveisi, F.; Lo Verme, J.; Serrano, A.; Rodriguez de Fonseca, F. *Nature* **2003**, *425*, 90.
52. Ahern, G. P. *J. Biol. Chem.* **2003**, *278*, 30429.
53. Wang, X.; Miyares, R. L.; Ahern, G. P. *J. Physiol.* **2005**, *564*, 541.
54. Lo Verme, J.; Russo, R.; La Rana, G.; Fu, J.; Farthing, J.; Mattace-Raso, G.; Meli, R.; Hohmann, A.; Calignano, A.; Piomelli, D. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1051.
55. Suardíaz, M.; Estivill-Torrús, G.; Goicoechea, C.; Bilbao, A.; Rodríguez de Fonseca, F. *Pain* **2007**, *133*, 99.
56. Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C. T.; Gardner, L. S.; Griffin, G.; Jackson, H. C.; Proctor, M. J.; Rasamison, C. M.; Tang-Christensen, M.; Widdowson, P. S.; Williams, G. M.; Reynet, C. *Cell Metab.* **2006**, *3*, 167.
57. (a) Soga, T.; Ohishi, T.; Matsui, T.; Saito, T.; Matsumoto, M.; Takasaki, J.; Matsumoto, S.; Kamohara, M.; Hiyama, H.; Yoshida, S.; Momose, K.; Ueda, Y.; Matsushime, H.; Kobori, M.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2005**,

326, 744; (b) Chu, A.-L.; Jones, R. M.; He, H.; Carroll, C.; Gutierrez, V.; Lucman, A.; Moloney, M.; Gao, H.; Mondala, H.; Bagnol, D.; Unett, D.; Liang, Y.; Demarest, K.; Semple, G.; Behan, D. P.; Leonard, J. *Endocrinology* **2007**, *148*, 2601; (c) Chu, Z. L.; Carroll, C.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Pedraza, M.; Mondala, H.; Gao, H.; Bagnol, D.; Chen, R.; Jones, R. M.; Behan, D. P.; Leonard, J. *Endocrinology* **2008**, *149*, 2038.
58. Lan, H.; Vassileva, G.; Corona, A.; Liu, L.; Baker, H.; Golovko, A. *Diabetes: Molecular Genetics, Signalling Pathways and Integrated Physiology* **2007**, Abstract 253.
59. Cano, C.; Pavon, J.; Serrano, A.; Goya, P.; Paez, J. A.; Rodriguez de Fonseca, F.; Macias-Gonzalez, M. *J. Med. Chem.* **2007**, *50*, 389.
60. Schmid, P. C.; Krebsbach, R. J.; Perry, S. R.; Dettmer, T. M.; Maasson, J. L.; Schmid, H. H. O. *FEBS Lett.* **1995**, *375*, 117.
61. Sugiura, T.; Kondo, S.; Kodaka, T.; Tonegawa, T.; Nakane, S.; Yamashita, A.; Ishima, Y.; Waku, K. *Biochem. Mol. Biol. Int.* **1996**, *40*, 931.
62. Di Marzo, V.; De Petrocellis, L.; Sepe, N.; Buono, A. *Biochem. J.* **1996**, *316*, 977.
63. Bigosno, T.; Maurelli, S.; Melck, D. *J. Biol. Chem.* **1997**, *272*, 3315.
64. Berdyshev, E. V.; Schmid, P. C.; Krebsbach, R. J.; Hillard, C. J.; Huang, C.; Chen, N.; Dong, Z.; Schmid, H. O. *Biochem. J.* **2001**, *360*, 67.
65. Carbonare, M. D.; Giudice, E. D.; Stecca, A.; Colavito, D.; Fabris, M.; D'Arrigo, A.; Bernardini, D.; Dam, M.; Leon, A. *J. Neuroendoc.* **2008**, *20*, 26.
66. Hanus, L.; Gopher, A.; Almog, S.; Mechoulam, R. *J. Med. Chem.* **1993**, *36*, 3032.
67. Bisogno, T.; Venriglia, M.; Milone, A.; Mosca, M.; Cimino, G.; Di Marzo, V. *Biochim. Biophys. Acta* **1997**, *1345*, 338.
68. Watanabe, K.; Matsunaga, T.; Nakamura, S.; Kimura, T.; Ho, I. K.; Yoshimura, H.; Yamamoto, I. *Biol. Pharm. Bull.* **1999**, *22*, 366.
69. Pertwee, R.; Griffin, G.; Hanus, L.; Mechoulam, R. *Eur. J. Pharmacol.* **1994**, *259*, 115.
70. Lin, S.; Khanolkar, A. D.; Fan, P.; Goutopoulos, A.; Qin, C.; Papahadjis, D.; Makriyannis, A. *J. Med. Chem.* **1998**, *41*, 5353.
71. Hansen, H. S.; Diep, T. A. *Biochem. Pharmacol.* **2009**, *78*, 553.
72. Boger, D. L.; Henriksen, S. J.; Cravatt, B. F. *Curr. Pharm. Des.* **1998**, *4*, 303.
73. Lerner, R. A.; Suizdak, G.; Prospero-Garcia, O.; Hendriksen, S. J.; Boger, D. L.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9505.
74. Cravatt, B. F.; Prospero-Garcia, O.; Szuidak, S.; Gilula, N. B.; Henriksen, S. J.; Boger, D. L.; Lerner, R. A. *Science* **1995**, *268*, 1506.
75. Cravatt, B. F.; Lerner, R. A.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 580.
76. (a) Patterson, J. E.; Ollmann, I. R.; Cravatt, B. F.; Boger, D. L.; Wong, C. H.; Lerner, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 5938; (b) Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 265.
77. (a) Huitron-Resendiz, S.; Gombart, L.; Cravatt, B. F.; Henriksen, S. *J. Exp. Neurol.* **2001**, *172*, 235; (b) Huitron-Resendiz, S.; Sanchez-Alavez, M.; Will, D. N.; Cravatt, B. F.; Henriksen, S. J. *Sleep* **2004**, *27*, 857.
78. Cheer, J. F.; Cadogan, A. K.; Marsden, C. A.; Fone, K. C. F.; Kendall, D. A. *Neuropharmacology* **1999**, *38*, 533.
79. Leggett, J. D.; Aspley, S.; Beckett, S. R. G.; D'Antona, A. M.; Kendall, D. A. *Br. J. Pharmacol.* **2004**, *141*, 253.
80. Bisogno, T.; Sepe, N.; De Petrocellis, L.; Mechoulam, R.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 473.
81. Driscoll, W. J.; Mueller, S. A.; Eipper, B. A.; Mueller, G. P. *Mol. Pharmacol.* **1999**, *55*, 1067.
82. Wilcox, B. J.; Ritenour-Rogers, K. J.; Asser, A. S.; Baumgart, L. E.; Baumgart, M. A.; Boger, D. L.; DeBlessio, J. L.; deLong, M. A.; Glufke, U.; Henz, M. E.; King, L., III; Merkler, K. A.; Patterson, J. E.; Robleski, J. J.; Vederas, J. C.; Merkler, D. J. *Biochemistry* **1999**, *38*, 3235.
83. Merkler, D. J.; Chew, G. H.; Gee, A. J.; Merkler, K. A.; Sorondo, J. P. O.; Johnson, M. E. *Biochemistry* **2004**, *43*, 12667.
84. Ritenour-Rogers, K. J.; Driscoll, W. J.; Merkler, K. A.; Merkler, D. J.; Mueller, G. P. *Biochem. Biophys. Res. Commun.* **2000**, *267*, 521.
85. Mechoulam, R.; Fride, E.; Hanus, L.; Sheskin, T.; Bisogno, T.; Di Marzo, V.; Bayewitch, M.; Vogel, Z. *Nature* **1997**, *389*, 25.
86. Federova, I.; Hahsimoto, A.; Fecik, R. A.; Hedrick, M. P.; Hanus, L. O.; Boger, D. L.; Rice, K. C.; Basile, A. S. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 332.
87. Murillo-Rodriguez, E. *Prog. NeuroPsycopharmacol. Biol. Psychiatry* **2008**, *32*, 1420.
88. Basile, A. S.; Hanus, L.; Mendelson, W. B. *NeuroReport* **1999**, *10*, 947.
89. Lichtman, A. H.; Hawkins, E. G.; Griffin, G.; Cravatt, B. F. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 73.
90. Lambert, D. M.; Di Marzo, V. *Curr. Med. Chem.* **1999**, *6*, 757.
91. Laposky, A. D.; Homanics, G. E.; Basile, A.; Mendelson, W. B. *NeuroReport* **2001**, *12*, 4134.
92. Verdon, B.; Zheng, J.; Nicholson, R. A.; Ganellin, C. R.; Less, G. *Br. J. Pharmacol.* **2000**, *129*, 283.
93. Less, G.; Edwards, M. D.; Hassoni, A. A.; Ganellin, C. R.; Galanakis, D. *Br. J. Pharmacol.* **1998**, *124*, 873.
94. Coyne, L.; Lees, G.; Nicholson, R. A.; Zheng, J.; Neufield, K. D. *Br. J. Pharmacol.* **2002**, *135*, 1977.
95. Yost, C. S.; Hampson, A. J.; Leonoudakis, D.; Koblin, D. D.; Bornheim, L. M.; Gray, A. T. *Anesth. Analg.* **1998**, *86*, 1294.
96. Boger, D. L.; Patterson, J. E.; Jin, Q. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4102.
97. Huidobro-Toro, J. P.; Harris, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8078.
98. Thomas, E. A.; Carson, M. J.; Neal, M. J.; Sutcliffe, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14115.
99. Thomas, E. A.; Cravatt, B. F.; Sutcliffe, J. G. *J. Neurochem.* **1999**, *21*, 2370.
100. Hedlund, P. B.; Carson, M. J.; Sutcliffe, J. G.; Thomas, E. A. *Biochem. Pharmacol.* **1999**, *58*, 1807.
101. Boger, D. L.; Sato, H.; Lerner, R. A.; Guan, X.; Gilula, N. B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1151.
102. Guan, X.; Cravatt, B. F.; Ehring, G. R.; Hall, J. E.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *J. Cell Biol.* **1997**, *139*, 1785.
103. Boger, D. L.; Patterson, J. E.; Guan, X.; Cravatt, B. F.; Lerner, R. A.; Gilula, N. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4810.
104. Arafat, E. S.; Trimble, J. W.; Andersen, R. N.; Dass, C.; Desiderio, D. M. *Life Sci.* **1989**, *45*, 1679.
105. Huang, J. K.; Jan, C. R. *Life Sci.* **2001**, *68*, 997.
106. (a) Wakamatsu, K.; Masaki, T.; Itoh, F.; Kondo, K.; Sudo, K. *Biochem. Biophys. Res. Commun.* **1990**, *168*, 423; (b) Hamberger, A.; Stenhagen, G. *Neurochem. Res.* **2003**, *28*, 177.
107. Nichols, K. K.; Ham, B. M.; Nichols, J. J.; Ziegler, C.; Green-Church, K. B. *Invest. Ophtalmol. Vis. Sci.* **2007**, *48*, 34.
108. Burstein, S. H.; Rossetti, R. G.; Yagen, B.; Zurier, R. B. *Prostaglandins Other Lipid Mediat.* **2000**, *61*, 29.
109. 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. *J. Biol. Chem.* **2001**, *276*, 42639.
110. Sheskin, T.; Hanus, L.; Slager, J.; Vogel, Z.; Mechoulam, R. *J. Med. Chem.* **1997**, *40*, 659.
111. Bradshaw, H. H.; Rimmerman, N.; Shu-Jung Hu, S.; Benton, V. M.; Stuart, J. M.; Masuda, K.; Cravatt, B. F.; O'Dell, D. K.; Walker, J. M. *BMC Biochem.* **2009**, *10*, 1.
112. (a) Succar, R.; Mitchell, V. A.; Vaughan, C. W. *Mol. Pain* **2007**, *3*, 1; (b) Vuong, L. A. Q.; Mitchell, V. A.; Vaughan, C. W. *Neuropharmacology* **2008**, *54*, 189.
113. (a) Kohno, M.; Hasegawa, H.; Inoue, A.; Muraoka, M.; Miyazaki, T.; Oka, K.; Yasukawa, M. *Biochem. Biophys. Res. Commun.* **2006**, *347*, 827; (b) McHugh, D.; Hu, S. S.-J.; Rimmerman, N.; Juknat, A.; Vogel, Z.; Walker, J. M.; Bradshaw, H. B. *BMC Neurosci.* **2010**, *11*, 44.
114. Oh, D. Y.; Yoon, J. M.; Moon, M. J.; Hwang, J.-L.; Choe, H.; Lee, J. Y.; Kim, J.; Kim, S.; Rhim, H.; O'Dell, D. K.; Walker, J. M.; Na, H. S.; Lee, M. G.; Kwon, H. B.; Kim, K.; Seong, J. Y. *J. Biol. Chem.* **2008**, *283*, 21054.
115. Wiles, A. L.; Pearlman, R.-J.; Rosvall, M.; Aubrey, K. R.; Vandenberg, R. J. *J. Neurochem.* **2006**, *99*, 781.
116. Burstein, S. H.; Huang, S. M.; Petros, T. J.; Rossetti, R. G.; Walker, J. M.; Zurier, R. B. *Biochem. Pharmacol.* **2002**, *64*, 1147.
117. Prusakiewicz, J. J.; Kingsley, P. J.; Kozak, K. R.; Marnett, L. J. *Biochem. Biophys. Res. Commun.* **2002**, *296*, 612.
118. Burstein, S. H.; Adams, J. K.; Bradshaw, H. B.; Fraioli, C.; Rossetti, R. G.; Salmonson, R. A.; Shaw, J. W.; Walker, J. M.; Zipkin, R. E.; Zurier, R. B. *Bioorg. Med. Chem.* **2007**, *15*, 3345.
119. Rimmerman, N.; Bradshaw, H. B.; Hughes, H. V.; Chen, J. S.-H.; Hu, S. S.-J.; McHugh, D.; Vefring, E.; Jahnsen, J. A.; Thompson, E. L.; Masuda, K.; Cravatt, B. F.; Burstein, S.; Vasko, M. R.; Prieto, A. L.; O'Dell, D. K.; Walker, M. *Mol. Pharmacol.* **2008**, *74*, 213.
120. Bradshaw, H. B.; Rimmerman, N.; Hu, S. S.-J.; Burstein, S.; Walker, J. M. *Vit. Hormones* **2009**, *81*, 191.
121. Mueller, G. P.; Driscoll, W. J. *J. Biol. Chem.* **2007**, *282*, 22364.
122. Merkler, D. J.; Chew, G. H.; Gee, A. J.; Merkler, K. A.; Sorondo, J.-P.; Johnson, M. E. *Biochemistry* **2004**, *43*, 12667.
123. Chaturvedi, S.; Driscoll, W. J.; Elliot, B. M.; Faraday, M. M.; Grunberg, N. E.; Mueller, G. P. *Prostaglandins Other Lipid Mediat.* **2006**, *81*, 136.
124. Burstein, S. H.; Adams, J. K.; Bradshaw, H. B.; Fraioli, C.; Rossetti, R. G.; Salmonsen, R. A.; Shaw, J. W.; Walker, J. M.; Zipkin, R. E.; Zurier, R. B. *Bioorg. Med. Chem.* **2007**, *15*, 3345.
125. Saghatelian, A.; Trauger, S. A.; Want, E. J.; Hawkins, E. G.; Siuzdak, G.; Cravatt, B. F. *Biochemistry* **2004**, *43*, 14332.
126. Saghetelian, A.; Cravatt, B. F. *Life Sci.* **2005**, *77*, 1759.
127. Saghatelian, A.; McKinney, M. K.; Bandell, M.; Smith, C. A.; Patapoutian, A.; Cravatt, B. F. *Biochemistry* **2006**, *45*, 9007.
128. Hay, D. W.; Carey, M. C. *Hepatology* **1990**, *12*, 6S.
129. Falany, C. N.; Fortinberry, H.; Leitner, E. H.; Barnes, S. *J. Lipid Res.* **1997**, *38*, 1139.
130. O'Byrne, J.; Hunt, M. C.; Rai, D. K.; Saeki, M.; Alexson, S. E. *J. Biol. Chem.* **2003**, *278*, 34237.
131. McKinney, M. K.; Cravatt, B. F. *Biochemistry* **2006**, *45*, 9016.
132. 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. *Biochem. Pharmacol.* **1995**, *50*, 83.
133. Stella, N.; Schweitzer, P.; Piomelli, D. *Science* **1997**, *388*, 773.
134. Kondo, S.; Kondo, H.; Nakane, S.; Kodaka, T.; Tokumara, A.; Waku, K.; Sugiura, T.; Waku, K.; Sugiura, T. *FEBS Lett.* **1998**, *429*, 152.
135. Blankman, J. L.; Simon, G. M.; Cravatt, B. F. *Chem. Biol.* **2007**, *14*, 1347.
136. (a) Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89; (b) Sugiura, T.; Kodaka, T.; Nakane, S.; Miyashita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. *J. Biol. Chem.* **1999**, *274*, 2794.
137. Savinainen, J. R.; Jarvinen, T.; Laine, K.; Laitinen, J. T. *Br. J. Pharmacol.* **2001**, *134*, 664.
138. Vadivel, S. K.; Vardarajan, S.; Duclos, R. I., Jr.; Wood, J. T.; Guo, J.; Makriyannis, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5959.
139. Hanus, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662.

140. Fezza, F.; Bisogno, T.; Minassi, A.; Appendino, G.; Mechoulam, R.; Di Marzo, V. *FEBS Lett.* **2002**, *513*, 294.
141. Oka, S.; Tsuchie, A.; Tokumura, A.; Muramatsu, M.; Suhara, Y.; Takayama, H.; Waku, K.; Sugiura, T. *J. Neurochem.* **2003**, *85*, 1374.
142. Richardson, D.; Ortori, C. A.; Chapman, V.; Kendall, D. A.; Barrett, D. A. *Anal. Biochem.* **2007**, *360*, 216.
143. Shoemaker, J. L.; Joseph, B. K.; Ruckle, M. B.; Mayeux, P. R.; Prather, P. L. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 868.
144. Duncan, M.; Millns, P.; Smart, D.; Wright, J. E.; Kendall, D. A.; Ralevic, V. *Br. J. Pharmacol.* **2004**, *142*, 509.
145. Venderova, K.; Brown, T. M.; Brotchie, J. M. *Exp. Neurol.* **2005**, *194*, 284.
146. Sun, Y.; Alexander, S. P. H.; Garle, M. J.; Hewitt, K.; Murphy, S. P.; Kendall, D. A.; Bennett, A. J. *Br. J. Pharmacol.* **2007**, *152*, 734.
147. Laine, K.; Jarvinen, K.; Mechoulam, R.; Breuer, A.; Jarvinen, T. *Invest. Ophtalmol. Vis. Sci.* **2002**, *43*, 3216.
148. Parkkari, T.; Salo, O. M.; Huttunen, K. M.; Savinainen, J. R.; Laitinen, J. T.; Poso, A.; Nevalainen, T.; Järvinen, T. *Bioorg. Med. Chem.* **2006**, *14*, 2850.
149. Juntunen, J.; Vepsalainen, J.; Niemi, R.; Laine, K.; Jarvinen, T. *J. Med. Chem.* **2003**, *46*, 5083.
150. Appendino, G.; Ligresti, A.; Minassi, A.; Daddario, N.; Bisogno, T.; Di Marzo, V. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 43.
151. Porter, A. C.; Sauer, J. M.; Knierman, M. D.; Becker, G. W.; Berna, M. J.; Bao, J.; Nomikos, G. G.; Carter, P.; Bymaster, F. P.; Leese, A. B.; Felder, C. C. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1020.
152. Markey, S. P.; Dudding, T.; Wang, T. C. *J. Lipid Res.* **2000**, *41*, 657.
153. Patricelli, M. P.; Cravatt, B. F. *Biochemistry* **1999**, *38*, 14125.
154. Kozlowska, H.; Baranowska, M.; Schlicker, E.; Kozlowski, M.; Laudanski, J.; Malinowska, B. *Br. J. Pharmacol.* **2008**, *115*, 1034.
155. Pertwee, R. G. *Br. J. Pharmacol.* **2007**, *152*, 984.
156. Bisogno, T.; Melck, D.; Bobrov, M. Y. U.; Gretskaya, N. M.; Berzuglov, V. V.; De Petrocellis, L.; Di Marzo, V. *Biochem. J.* **2000**, *351*, 817.
157. Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezzo, F.; Tognotto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Walker, J. M.; Di Marzo, V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8400.
158. Chu, C. J.; Huang, S. M.; De Petrocellis, L.; Bisogno, T.; Ewig, S. A.; Miller, J. D.; Zipkin, R. E.; Daddario, T.; Appendino, G.; Di Marzo, V.; Walker, J. M. *J. Biol. Chem.* **2003**, *278*, 13633.
159. De Petrocellis, L.; Chu, C. J.; Moriello, A. S.; Kellner, J. C.; Walker, J. M.; Di Marzo, V. *Br. J. Pharmacol.* **2004**, *143*, 251.