Illuminating N-acylethanolamine biosynthesis with new chemical tools

Mock, E.D.

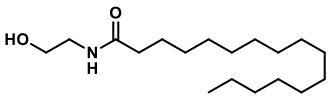
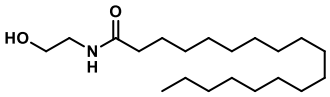
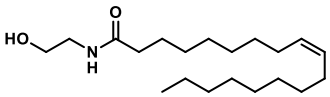
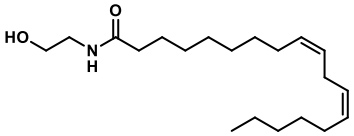
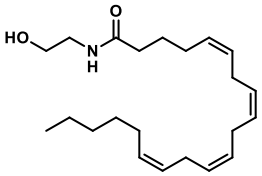
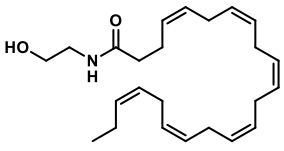
Chapter 1

Therapeutic opportunities of modulating the endogenous *N*-acylethanolamine tone

Over the past decades, lipids have emerged as important signaling molecules in health and disease. Lipid messengers come in a range of shapes and sizes and are classified in seven different categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides.[1] Signaling lipids often exert their bioactivities through activation of various proteins, including G protein-coupled receptors (GPCRs), ion channels and nuclear receptors. Within the class of fatty acyl lipids, the *N*-acylethanolamines (NAEs) have garnered attention as a family of bioactive fatty acid amides with diverse roles in inflammation, neurotransmission, appetite, fertility, stress and anxiety. The NAEs incorporate saturated, mono- or polyunsaturated fatty acyl groups in their structures, which determines their signaling function. The most frequently occurring NAEs are *N*-palmitoylethanolamine (PEA), *N*-stearoylethanolamine (SEA), *N*-oleoylethanolamine (OEA), *N*-linoleoylethanolamine (LEA), *N*-arachidonoylethanolamine (AEA) and *N*-docosahexaenoylethanolamine (DHEA) (Table 1). At present, many outstanding questions exist with regard to the biological actions of NAEs. In this chapter,

an overview is provided of NAE biosynthesis and degradation, current understanding of their physiological functions and potential therapeutic applications of modulating the NAE tone.

Table 1. *N*-acylethanolamine (NAE) family members and their reported biological activities.

Name	Structure	Receptor	Bioactivity
PEA (16:0)		PPAR-α[2] GPR55[3] GPR119[4]	Anti-inflammatory[5] Neuroprotective[6] Anti-epileptic[7] Analgesic[8] Anorectic[9]
SEA (18:0)		GPR119[4]	Anti-inflammatory[10] Anorectic[11]
OEA (18:1-ω9)		PPAR-α[12] GPR119[4]	Anti-inflammatory[13] Anorectic[9] Analgesic[14] Neuroprotective[15]
LEA (18:2-ω6)		PPAR-α[16] GPR119[17]	Anorectic[18] Neuroprotective[19]
AEA (20:4-ω6)		CB_1[20] CB_2[21] TRPV1[22]	Neurotransmission[23] Orexigenic[24] Analgesic[25] Anxiolytic[26] Memory formation[27] Neuroprotective[28] Fertility[29]
DHEA (22:6-ω3)		GPR110[30]	Neurogenesis[31] Anti-inflammatory[32]

Abbreviations: PEA = *N*-palmitoylethanolamine; SEA = *N*-stearoylethanolamine; OEA = *N*-oleoylethanolamine; LEA = *N*-linoleoylethanolamine; AEA = *N*-arachidonoylethanolamine; DHEA = *N*-docosahexaenoylethanolamine; PPAR-α = peroxisome proliferator-activated receptor α; GPR55, 110 or 119 = G-protein coupled receptor 55, 110 or 119; $CB_{1/2}$ = cannabinoid receptor 1 or 2; TRPV1 = transient receptor potential vanilloid 1.

1.1 NAE metabolism

In 1979, Schmid and co-workers reported the accumulation of NAEs in infarcted dog heart.[33] Shortly hereafter, the same lab showed that *N*-acylphosphatidylethanolamines (NAPEs), a previously unknown lipid class, were equally upregulated.[34] Due to the structural similarities of NAPEs and NAEs, a precursor-product relationship was

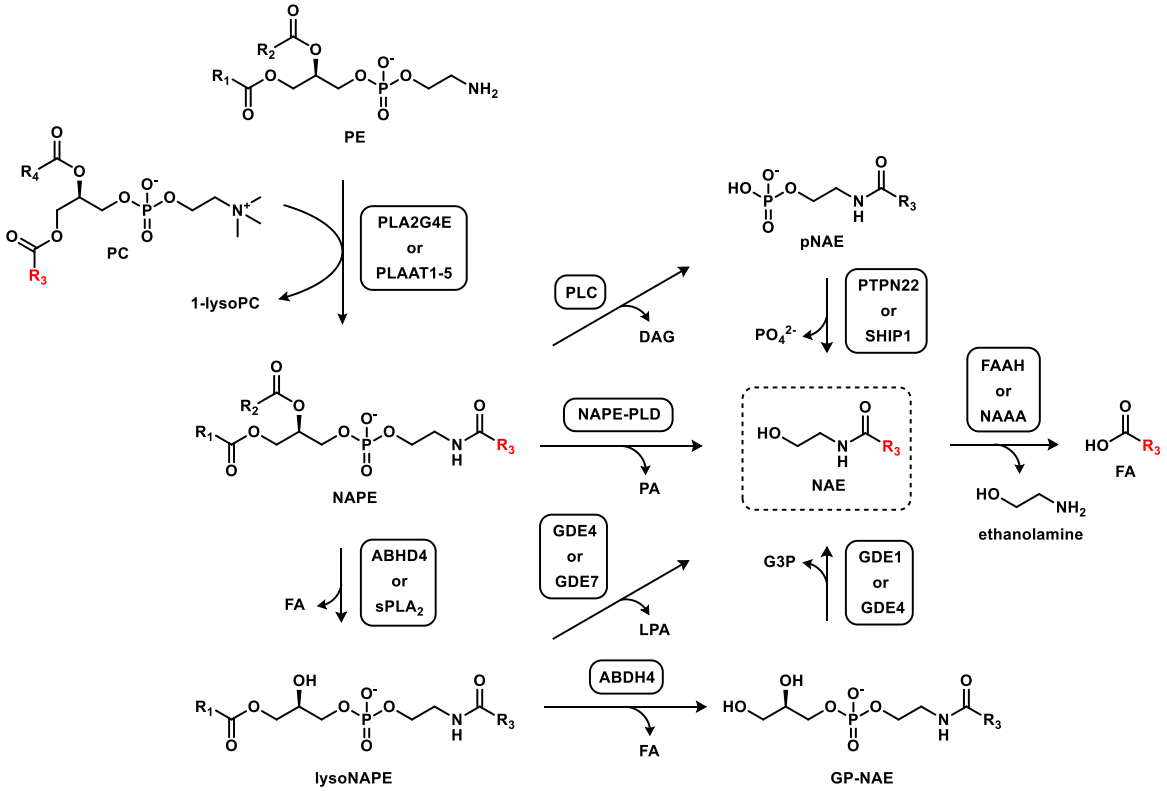


Figure 1. Biosynthetic pathways of *N*-acylethanolamines (NAEs). In total, four different enzymatic routes have been reported that can produce NAEs.[37] In the canonical pathway, *N*-acylphosphatidylethanolamine (NAPE) is formed from phosphatidylethanolamine (PE) and phosphatidylcholine (PC) catalyzed by phospholipase A_2 group IV E (PLA2G4E). This is followed by NAPE phospholipase D (NAPE-PLD)-mediated hydrolysis to NAE. Fatty acid amide hydrolase (FAAH) catabolizes NAEs into fatty acids (FAs) and ethanolamine. Abbreviations: PLAAT1-5 = phospholipase and acyltransferase 1-5; ABHD4 = α,β-hydrolase domain 4; GDE1, 4 or 7 = glycerophosphodiesterase 1, 4 or 7; PLC = phospholipase C; PTPN22 = protein tyrosine phosphatase non receptor type 22; SHIP1 = phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1; NAAA = *N*-acylethanolamine acid amidase; GP-NAE = glycerophospho-*N*-acylethanolamine; 1-LPC = 1-lysophosphatidylcholine; LPA = lysophosphatidic acid; PA = phosphatidic acid; DAG = diacylglycerol; G3P = glycerol-3-phosphate; pNAE = phospho-*N*-acylethanolamine.

proposed.[35] Ensuing studies revealed that NAPEs are produced by the transfer of the sn-1 acyl group of phosphatidylcholine (PC) to the amine of phosphatidylethanolamine (PE), forming NAPE and 1-lysoPC (Figure 1).[36] Next, the phosphodiester bond of NAPE is hydrolyzed to generate NAE and phosphatidic acid (PA). Finally, the NAE is degraded to fatty acid (FA) and ethanolamine.

1.1.1 NAPE biosynthesis

The canonical acyl transfer reaction that produces NAPEs, is carried out by a Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT). High Ca-NAT enzymatic activities were found in heart, brain and testis tissues.[35,38,39] Remaining elusive for more than two decades, the serine hydrolase phospholipase A2 group IV E (PLA2G4E) was recently identified as a NAPE-generating Ca-NAT in cells, matching the reported expression and activity profile.[40] Also plasmalogen-type PEs, which incorporates a vinyl ether at the sn-1 position, were found to be suitable substrates for PLA2G4E, thereby producing plasmalogen-NAPEs (pNAPEs).[41] pNAPEs are considered to be an important source of NAEs in the brain. In mouse brain, the total pNAPE amount was 4-fold higher than the NAPE content.[42] In contrast, NAPEs were almost exclusively observed in the mucosal layer of rat jejunum, while in the serosal layer both NAPE and pNAPE species were abundant.[43] Interestingly, in rat brain lysate, Ca-NAT activity preferably generated *N*-arachidonoyl-containing (p)NAPEs with polyunsaturated acyl groups at the sn-2 position.[44] This may indicate that the Ca^{2+}-dependent generation of AEA favors polyunsaturated (p)NAPEs as precursors.[44]

A second family of NATs was discovered that can produce NAPEs in a Ca^{2+}-independent manner, termed phospholipase and acyl transferase (PLAAT) 1-5.[45-50] These enzymes belong to the cysteine hydrolases and show expression in the central nervous system (CNS) as well in peripheral tissues. In particular, PLAAT2 showed high *N*-acyltransferase activity, comparable to PLA2G4E.[41,50] Also PLAAT2 accepted both PE and plasmalogen-type PE as substrates.[41] Expression of PLAAT2 was found to be high in the liver, kidney, small intestine, colon, testis and trachea.[47,51] This suggests that PLAAT2 may be involved in NAE biosynthesis in the gut. Notably, PLAAT2 expression was absent in rodents.[47] So far, no genetic or pharmacological tools have been described for the PLAAT family members. To what extend the Ca^{2+}-independent pathway contributes to NAPE and pNAPE biosynthesis *in vivo*, is therefore still unclear.[37]

1.1.2 NAE biosynthesis

In 2004, the enzyme that produces NAEs in a single step from NAPEs or pNAPEs was identified as *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) (Figure 1).[52] A crystal structure revealed that NAPE-PLD forms a membrane-bound homodimer with two Zn^{2+}-ions in its active site.[53] NAPE-PLD is classified as a metallo-β-lactamase and is distinct

from the PLD family.[52] Brain, kidney and testis tissues were found to abundantly express NAPE-PLD.[52] Interestingly, NAPE-PLD did not display any substrate preference *in vitro*.[54] Furthermore, PE increased the NAPE-PLD enzymatic activity, suggesting that the enzyme is constutively active.[55] *In vitro*, NAPE-PLD activity was found to be elevated by specific bile acids, as well as polyamines such as spermine and spermidine.[53,56,57] Multiple NAPE-PLD knockout (KO) studies described a significant reduction of saturated and unsaturated NAEs in the brains of mice.[42,58,59] In accordance, NAPE and pNAPE precursors were greatly enhanced.[42,58] However, levels of ω-6 and ω-3 polyunsaturated NAEs – AEA and DHEA, respectively – were not decreased in all KO strains.[58] It was therefore proposed that genetic deletion of NAPE-PLD stimulated compensatory mechanisms which counteract the reduction of AEA and DHEA content.[58] In peripheral organs such as heart, kidney, liver and jejunum, NAPE-PLD KO mice did not present decreased NAE levels, although NAPE concentrations were highly elevated, except for jejunum.[60] At present, the study of NAPE-PLD is hampered by a lack of *in vivo* active inhibitors which are needed to elucidate its role in NAE biosynthesis.

Three additional pathways have been discovered that can also produce NAEs (Figure 1). Firstly, two phospholipases were reported that can hydrolyze the fatty acyl esters of NAPEs. Three isoforms of secretory phospholipase A2 (sPLA$_2$-IB, IIA and V) were described to exclusively cleave the NAPE sn-2 ester to form lysoNAPE and a fatty acid.[61] The serine hydrolase α,β-hydrolase domain 4 (ABHD4) performed the same reaction, but did not show any specificity towards the sn-1 or sn-2 ester.[62] In addition, ABHD4 could hydrolyze the fatty acyl ester of lysoNAPE, generating glycerophospho-NAE (GP-NAE). This lipid species is converted by glycerophosphodiesterase 1 and 4 (GDE1/4) to afford NAE and glycerol-3-phosphate (G3P).[63,64] A second pathway involves cleavage of the lysoNAPE phosphodiester by GDE4 or GDE7 in a lysoPLD-type reaction, producing NAE and lysophosphatidic acid (LPA).[64,65] Expression of ABHD4 was found to be high in brain and testis, but not in heart.[62] ABHD4 KO mice displayed decreased levels of GP-NAE and lyso-(p)NAPE in the brain, however NAE content, including AEA, was not reduced.[66] The activity of GDE1 was stimulated by Mg^{2+}-ions and high protein expression levels were found in brain, testis, liver and kidney tissues.[63] Genetic deletion of GDE1 in mice also did not afford a significant decrease of brain NAE levels, therefore the physiological importance of this pathway for the formation of brain NAEs is still under debate.[67] The recently reported GDE4 and GDE7 enzymes, as well as the sPLA$_2$s have yet to be further characterized in KO models to establish their role in NAE biosynthesis *in vivo*.[37] It is interesting to note that the second product of the lysoPLD pathway is LPA, a bona fide signaling lipid in the CNS involved in cell proliferation and synaptic transmission.[68]

A third NAE biosynthetic pathway was described to be important in macrophages, where lipopolysaccharide (LPS) induced elevation of AEA in a NAPE-PLD-independent manner.[69,70] It was proposed that a yet unknown PLC-type enzyme hydrolyzes the phosphodiester of NAPE to produce phosphoNAE and diacylglycerol (DAG). Two phosphatases were identified, protein tyrosine phosphatase non-receptor type 22 (PTPN22) and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1 (SHIP1), that can catalyze the dephosphorylation of phosphoNAE to NAE and phosphate.[69,71] Both PTPN22 and SHIP1 were induced in macrophages upon LPS stimulation. Incubation of phosphoNAE with brain tissue from PTPN22 KO mice demonstrated reduced conversion to AEA compared to wild-type (WT), that could indicate a possible role *in vivo*.[69]

1.1.3 NAE degradation

The hydrolysis of NAE to fatty acid and ethanolamine can be performed by several enzymes (Figure 2).[72] Fatty acid amide hydrolase (FAAH) displays specificity towards AEA over saturated and mono-unsaturated NAEs and has high expression in human brain, but is absent in heart tissue.[73] Genetic or pharmacological blockade of FAAH resulted in a large increase of brain AEA levels in mice, as well as smaller but significant increases of PEA and OEA.[74-76] FAAH is therefore regarded as the primary AEA metabolizing enzyme in the brain. Surprisingly, in the liver, FAAH was found to catalyze the reverse reaction during liver regeneration which could be attributed to highly increased arachidonic acid levels, but not ethanolamine.[77] A second fatty acid amidase (FAAH-2) was identified that shares 20% sequence identity with FAAH.[78] FAAH-2 is specific for higher mammals including primates and marsupials and does not occur in rats or mice. It is expressed in peripheral organs such as heart and ovary. Whereas FAAH localizes to the endoplasmic reticulum in cells, FAAH-2 was reported to be enriched in lipid droplets.[79] Contrary to FAAH, FAAH-2 preferred primary fatty acid amides (*e.g.* oleamide) over NAEs as substrates.[78] A third NAE-hydrolyzing enzyme was described to be active in cells of the immune system.[80] *N*-acylethanolamine acid amidase (NAAA) is lysosomally located and preferentially hydrolyzes saturated NAE species.[81] NAAA is an *N*-terminal cysteine hydrolase and shares no homology with FAAH (a serine hydrolase). Pharmacological inhibition of NAAA in mice induced significant elevations of brain PEA and OEA, but not AEA levels.[82] The development of NAAA KO mice is necessary to confirm these findings.

Besides hydrolysis of the amide bond, polyunsaturated NAEs such as AEA and DHEA can undergo oxygenation of the double bonds which produces eicosanoid-type lipids (Figure 2). Each of these oxygenated products have reported lipid signaling functions of their own.[83,84] Cyclooxygenase (COX)-2 was described to convert AEA to various prostaglandin-ethanolamides (PG-EA), a lipid class designated as prostamides.[85,86] For

example, AEA cyclooxygenation by COX-2 followed by consecutive action of a PGF synthase produces $PGF_{2\alpha}$-EA. Both these enzymes occur in the CNS and it is suggested that $PGF_{2\alpha}$-EA is involved in inflammatory pain *in vivo*.[87,88] Also lipoxygenases (LOX) are able to use AEA as a substrate, generating for example 12-hydroxyeicosatetraenoic acid-ethanolamide (12-HETE-EA).[89,90] These oxygenated AEA derivatives inhibit FAAH and could therefore prolong NAE signaling.[91] Lastly, epoxidation of AEA by cytochrome P450 enzymes can produce different epoxides such as 5,6-epoxyeicosatrienoic acid-ethanolamide (5,6-EET-EA).[92,93] Since AEA is primarily hydrolyzed by FAAH and generally has low endogenous concentrations in most tissues, the biological importance of many of these oxygenated products is still unknown.[84]

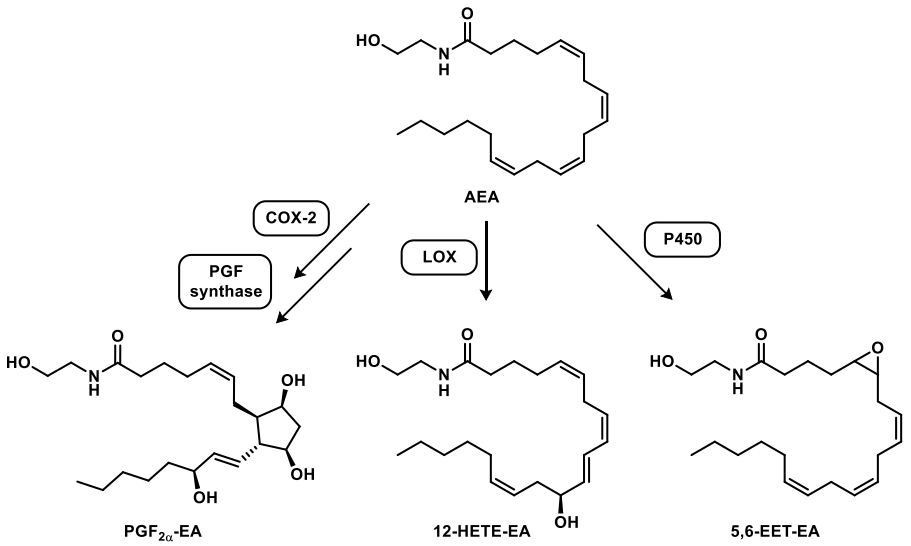


Figure 2. Oxidative degradation pathways of anandamide showing representative products. Cyclooxygenase 2 (COX-2) and prostaglandin synthases (*e.g.* PGF synthase) can convert AEA into prostamide-type lipids such as prostaglandin-$F_{2\alpha}$-ethanolamide ($PGF_{2\alpha}$-EA). Lipoxygenases (LOX) enzymes can hydroxylate AEA to form for example 12-hydroxyeicosatetraenoic acid-ethanolamide (12-HETE-EA). Cytochrome P450-type enzymes can produce various epoxygenated AEA derivatives such as 5,6-epoxyeicosatrienoic acid-ethanolamide (5,6-EET-EA).

1.2 Physiological functions of NAEs and (p)NAPEs

1.2.1 NAPE and pNAPE

NAPEs and pNAPEs are primarily considered to be precursors of NAEs. However, recent overviews have highlighted that (p)NAPEs may have biological functions of their own.[94,95] These include putative roles in neuroprotection, anti-inflammation and satiety. During

cellular injury, NAPEs accumulate in damaged tissue, presumably due to an influx of calcium ions.[96,97] This phenomenon has been observed in ischemia of the brain, heart and testis in various mammals such as mice, rats, dogs and humans.[34,98-103] Also in plants NAPEs increase under cellular stress.[94,104,105] Importantly, NAPE levels are higher than their corresponding NAE congeners in brain ischemia, which may suggest a neuroprotective function.[106] Conversion of PE to NAPE has a proposed membrane stabilizing role, possibly due to hydrogen bonding of the newly formed amide.[107,108] NAPE-enriched liposomes were found to be less prone to dye leakage.[109] Furthermore, NAPEs induced membrane fusion in the presence of Ca^{2+}-ions.[110,111] This effect was found to be NAPE-specific as other anionic phospholipids such as phosphatidylserine (PS) and phosphatidylglycerol (PG) did not stimulate membrane fusion.[110] The fusogenic properties of NAPE-liposomes have been exploited for drug delivery: liposomes incorporating the neuroprotective ganglioside GM1 were enriched in the brains of treated rats.[112] *N*-palmitoyl-PE-enriched liposomes decreased phagocytosis in mouse macrophages, thereby contributing to the termination of inflammation.[113] In the rat jejunum, NAPE levels, specifically *N*-oleoyl-PE, were increased after feeding.[43,114] NAPE has been described as a lipid hormone that can decrease food intake, while exogenous NAPE was able to induce weight loss in mice.[115] However, following reports have contested this claim and point towards NAE metabolites as the cause of the observed anorectic effect.[116,117] Collectively, these studies provide evidence for a putative biological role of NAPEs in neurodegeneration and inflammation. The molecular mechanisms through which NAPEs exert its bioactivities should therefore be addressed. Genetic or pharmacological tools that enable modulation of NAPE metabolic enzymes may help to answer these questions.

1.2.2 PEA

In the 1950s, PEA was the first member of the NAE family to be identified in egg yolk, soybean lecithin and later in mammalian tissues.[118,119] It was immediately noted that PEA possessed anti-allergic and anti-inflammatory properties in a guinea pig model of anaphylactic arthritis.[118] Following reports revealed that PEA also produces anti-epileptic, neuroprotective, analgesic and anorectic effects.[7,9,120-123] During acute brain ischemia in rats, PEA levels increased 30-fold specifically in damaged brain areas.[124] Exogenous administration of PEA showed to be neuroprotective in various disease models such as traumatic brain injury, Parkinson's and Alzheimer's disease.[122] Multiple biological targets have been identified for PEA that can explain its pharmacological effects.[123,125] The nuclear receptor peroxisome proliferator-activated receptor (PPAR)-α was found to mediate the anti-inflammatory and analgesic effects of PEA.[6,126] Furthermore, PEA displayed affinity for GPR119, a fat sensor in the gut, although OEA is regarded as a more potent agonist *in vivo*.[4,127] Another receptor through which PEA can exert its bioactive

effects is GPR55, however these findings have been questioned in later studies.[3,128] Today, PEA is marketed as a dietary supplement as well as a skin cream in many countries. Numerous clinical trials have been conducted with PEA for the treatment of pain, demonstrating that, overall, PEA produces few unwanted side effects and shows promise as an analgesic.[8]

1.2.3 SEA

Although SEA and PEA differ just two carbons in chain length, SEA has been studied far less extensively. This may be due to the fact that unlike PEA, SEA did not present affinity for PPAR-α.[2] Nevertheless, SEA showed affinity for GPR119 and shares several bioactivities with PEA.[4] SEA produced anti-inflammatory effects in a mouse cutaneous anaphylaxis model.[10] In rat brain, SEA levels were similar to PEA and showed a comparable 30-fold increase upon brain ischemia.[124] Furthermore, oral administration of SEA in mice produced an anorectic effect, presumably through increase of liver stearoyl-CoA desaturase-1 (SCD-1) mRNA expression.[11] These findings indicate that SEA may have therapeutic properties and the exclusion of this lipid species from NAE studies is unjustified. It is therefore recommended to include SEA in the standard NAE lipid panel to elucidate its biological role.

1.2.4 OEA

OEA is a well-studied member of the NAE family, especially in the gastrointestinal system. Upon oral administration in mice, OEA demonstrated anorectic effects that are mediated by peripheral PPAR-α.[12,129,130] Of the NAE members, OEA showed the highest potency for PPAR-α.[16] Endogenous OEA levels in the small intestine were markedly reduced in starved mice and significantly increased after refeeding compared to free-feeding mice.[9,43,131] As such, OEA is regarded as a satiety factor that is released upon food intake.[114,132] However, both short-term and chronic high fat diets were found to decrease levels of OEA in rat jejunum, but not in other tissues such as brain and liver.[16,133] It was proposed that reduction in OEA levels may cause the reduced satiety and hyperphagia as seen in obesity.[125,132] OEA also showed *in vitro* affinity for GPR119, a receptor that modulates feeding behavior.[4] Nevertheless, OEA produced anorectic effects in both GPR119 WT and KO mice, indicating that *in vivo*, this activity is not required for satiety.[134] Similar to PEA, administration of OEA in rodents was reported to generate anti-inflammatory, neuroprotective and analgesic effects.[13-15] These are likely mediated by activation of PPAR-α, although also PPAR-α-independent mechanisms have been described.[126,135,136] In rat brain, OEA concentrations were found to be roughly one-third of PEA and SEA levels and showed a comparable 30-fold increase upon cerebral ischemia.[124] A putative

neuroprotective role for the NAE family was therefore hypothesized, acting via multiple molecular mechanisms.[122,137]

1.2.5 LEA

LEA has received less attention compared to the other NAE family members, even though it possesses similar bioactivities as OEA and PEA. Importantly, endogenous levels of LEA in rat jejunum were found to be 4 to 6-fold higher than OEA, PEA and SEA upon fasting and refeeding.[131] Intraperitoneal (i.p.) administration of LEA in rats elicited a reduction of food intake, which was dependent on PPAR-α activation and was comparable to OEA and PEA.[9,16] Because of the high intestinal levels of LEA, it was proposed that the anorectic effect could also in part be mediated through GPR119, for which LEA shows equal activity as OEA.[17] This has yet to be confirmed in genetically deleted GPR119 rodents. In a rat stroke model, treatment with exogenous LEA demonstrated a neuroprotective effect.[19] Although endogenous LEA levels in rat brain accumulated 30-fold upon brain ischemia similar to PEA and SEA, the absolute concentrations were just 1% to 5% of saturated NAEs, suggesting only a minor role in the brain *in vivo*.[124]

1.2.6 AEA

AEA or anandamide has been studied most extensively of all the NAE family members. In most tissues, AEA levels are 10 to 100 times lower than PEA, SEA and OEA.[138,139] However, unlike other NAEs, AEA can activate the cannabinoid (CB_1)-receptor.[20] The CB_1 receptor is one of the most abundant GPCRs in the mammalian brain and is activated by (-)-Δ^9-tetrahydrocannabinol (THC), the psychoactive component of cannabis. As a result, anandamide and 2-arachidonoylglycerol (2-AG), a second endogenous CB_1 receptor agonist, are termed endogenous cannabinoids or endocannabinoids. AEA is regarded as a tonic neuromodulator – *i.e.*, it continuously signals in the basal state – which is released by neurons upon Ca^{2+}-stimulation and is quickly degraded by FAAH.[23,140-142] Although AEA was initially described as a retrograde neurotransmitter, NAPE-PLD is localized presynaptically and FAAH postsynaptically, suggesting that AEA may function as an anterograde signaling lipid.[143] AEA can also act as an intracellular messenger, formed upon an influx of Ca^{2+}-ions via activation of the G_q-pathway.[144]

The word '*ananda*' – meaning bliss in Sanskrit – was aptly chosen, as increased AEA signaling produces analgesic, anxiolytic and anti-depressant effects through CB_1 receptor signaling in the brain.[26,145,146] Conversely, acute and repeated stress exposure in rats afforded a decrease in AEA content in the amygdala, mediated by enhanced FAAH activity.[147] Stressed rats showed an inverse correlation between amygdalar AEA and plasma stress hormone levels (corticosterone).[148] Diminished brain AEA signaling upon repeated stress increased secretion of corticosterone.[149] In contrast, repeated stress

elevated amygdalar 2-AG levels, which attenuated hypothalamic-pituitary-adrenal (HPA) axis activation. AEA and 2-AG are therefore hypothesized to be the effectors of HPA-axis signaling in the brain, while having functionally distinct roles.[149,150] In addition to its roles in modulating fear and stress behavior, AEA was reported to promote neuroprotection, memory formation and food intake via brain CB_1 receptor activation.[27,28,151,152] Pharmacological studies in mice showed that exogenous AEA produces cannabimimetic responses, which are rapid in onset, but shorter and less potent than THC, presumably due to its fast metabolism.[153] Correspondingly, FAAH KO mice were supersensitive to AEA treatment.[74] Exogenous AEA administration in rats generated a central CB_1-receptor-dependent orexigenic (appetite-stimulating) effect similar to THC.[24]

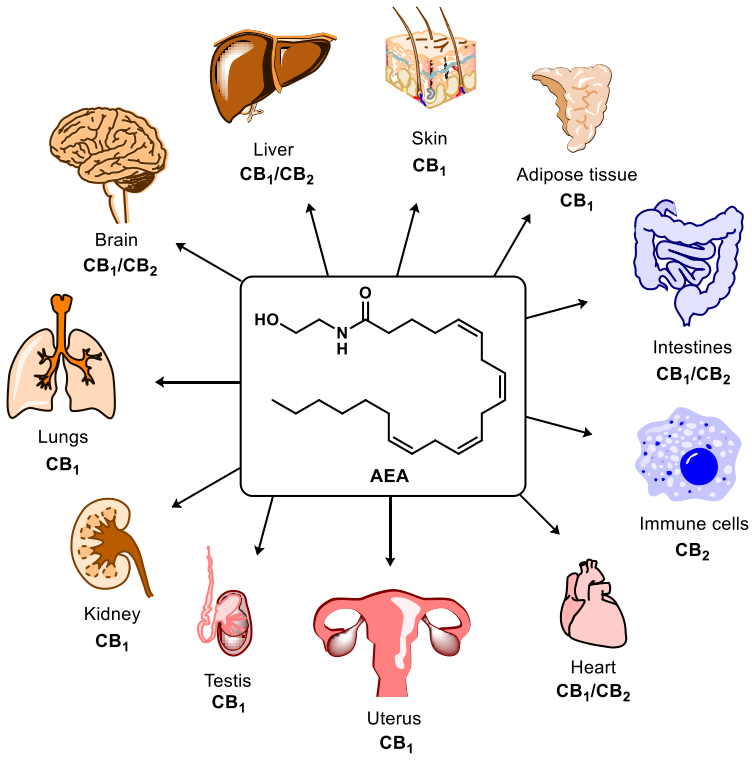


Figure 3. AEA is involved in cannabinoid receptor 1 and 2 (CB_1 and CB_2) signaling in both central and peripheral organs, of which several are depicted.[154]

Anandamide has also been linked to CB_1 receptor signaling in the periphery, for instance in adipocytes, the female reproductive system and skin tissue where it is involved in energy expenditure, implantation and epidermal differentiation, respectively (Figure 3).[154,155] Interestingly, peripheral CB_1 receptor activation is implicated in food intake as well, and intestinal AEA levels were found to be highly increased in starved mice.[156] Also

the analgesic effects of AEA were observed in the periphery, as peripheral blockade of FAAH produced antinociception via a CB_1 receptor-dependent mechanism.[157] Notably, the antinociceptive effect of AEA increased synergistically when combined with PEA in a mouse model of peripheral pain.[25]

AEA has additionally been described as a partial agonist for the CB_2 receptor, which is primarily expressed in the immune system and is involved in the inflammatory response.[21,158-160] Typically, AEA levels are 10- to 1000-fold lower compared to 2-AG in most tissues.[138] 2-AG has therefore been suggested to be the true endogenous CB_2 receptor ligand.[161] Nevertheless, AEA was reported to modulate inflammation via activation of the CB_2 receptor by reducing pro-inflammatory cytokines in cells.[154,162]

Besides the cannabinoid receptors, AEA also activates the transient receptor potential vanilloid 1 (TRPV1) ion channel.[22] AEA has therefore been termed an endovanilloid.[163,164] TRPV1, also known as the capsaicin receptor, is an important player in pain perception and is localized at peripheral sensory neurons.[165] Evidence is accumulating that TRPV1 is expressed in the CNS as well.[166,167] The activation of TPRV1 by AEA causes an cellular influx of Ca^{2+}-ions and has been linked to locomotor depression, hyperalgesia under inflammatory conditions, vasodilation and hypothermia.[144,165]

1.2.7 DHEA

Over the past ten years, DHEA has come into view as a member of the NAE family with unique properties in neuronal signaling.[31] As such, the name synaptamide was coined for its ability to induce neurogenesis.[168] Recently, DHEA was found to have nanomolar affinity for GPR110, an adhesion-type GPCR highly expressed in the hippocampus.[30] DHEA generated neurite outgrowth and synapse formation in neurons derived from WT mice, but not from GPR110 KO littermates. GPR110 KO mice showed reduced spatial memory and object recognition, but have yet to be profiled completely. DHEA has also been reported to have anti-inflammatory properties.[32,169] In LPS-treated microglia and macrophage cells, DHEA reduced pro-inflammatory cytokines or eicosanoids, respectively.[32,139] In addition, LPS-induced neuroinflammation in mice was significantly decreased after i.p. administration of DHEA. Brain DHEA levels are generally 2- to 10-fold higher than AEA, while the opposite is true for plasma.[58,59,138,170] Furthermore, brain DHEA concentrations are linked directly with brain content of docosahexaenoic acid (DHA, 22:6), an ω-3 polyunsaturated fatty acid.[171-173] DHA is preferably acquired from the diet, but can also be synthesized from the essential fatty acid α-linolenic acid (18:3-ω3).[174] The biosynthesis of DHEA is considered to follow the same route as other NAEs via formation of NAPE and hydrolysis by NAPE-PLD, which was confirmed in two NAPE-PLD KO mouse strains.[42,59] A third NAPE-PLD KO mouse strain did not show a reduction of brain DHEA and

displayed elevated levels of brain DHEA upon administration of a fish oil diet rich in DHA.[58,175] This suggests that alternative pathways are also involved in DHEA production in the brain.

Few studies have looked at the physiological role of DHEA in the periphery. It has been reported that under normal conditions, peripheral tissue levels of DHEA often exceed AEA, for example in the heart, kidney, jejunum and skin.[60,176] GPR110 was found to be expressed in various peripheral organs including kidney, prostate and lung, which points to a possible role of DHEA signaling in these tissues.[177]

1.3 Pharmacological modulation of NAE metabolism

As outlined in the prior section, NAEs possess desirable bioactivities that may be used for therapeutic intervention. Moreover, in certain pathological conditions NAE levels are disrupted, for example in cancer, obesity and neurodegenerative diseases and have been linked to disease progression and severity.[178-180] Modulating the NAE tone could therefore be a viable treatment strategy for these pathologies. However, due to the polypharmacology of NAEs acting on multiple receptors that can have opposing outcomes, it is not always clear whether NAE levels should be enhanced or reduced.[181] In the following section, a brief overview will be provided of the therapeutic potential of blockade of NAE degradation as well as its biosynthesis.

1.3.1 Inhibition of NAE degradation

After the discovery of the NAE-hydrolyzing enzyme FAAH in 1995, it became apparent that increasing NAE levels by genetic or pharmacological disruption of FAAH had profound effects on ECS signaling.[182] To date, multiple research groups and pharmaceutical companies have developed *in vivo* active and brain penetrant FAAH inhibitors (Figure 4A).[183] Upon administration in rats or mice, the irreversible FAAH inhibitors URB597, PF-3845 and PF-04457845 increased AEA levels with 3- to 7-fold in brain and plasma, while PEA and OEA were also enhanced with 8- to 20-fold in the same tissues.[26,75,184] Limited data is available of other NAE levels after FAAH inhibition, although one study reported that PF-3845 could similarly elevate SEA, LEA and DHEA levels with 5- to 20-fold in the brain, but in plasma only LEA and DHEA were increased.[185] Pre-clinical research in rodents revealed that inhibition of FAAH may be exploited for treatment of inflammatory or neuropathic pain, acting via central or peripheral CB_1 and CB_2 receptor activation.[75,157,186,187] Furthermore, pharmacological FAAH disruption has shown promise for treating anxiety[26], depression[145], post-traumatic stress disorder[188], Parkinson's

disease[189], nausea[190], skin inflammation[191], pruritus[192], inflammatory bowel disease[192], glaucoma[193], hypertension[194], traumatic brain injury[195], HIV-associated neurocognitive disorders[196] and multiple sclerosis-associated spasticity[197]. Several FAAH inhibitors have been tested in Phase I and II clinical trials with mixed success.[198,199] The selective inhibitor PF-04457845 was found to be well tolerated in healthy volunteers, completely blocked plasma FAAH activity and increased plasma AEA (10-fold), LEA (9-fold), OEA (6-fold) and PEA (3.5-fold) concentrations.[200] However, in a subsequent Phase II clinical trial for osteoarthritic pain of the knee, PF-04457845 did not produce analgesia.[201] Recently, PF-04457845 was reported to be efficacious for the treatment of cannabis withdrawal symptoms in a Phase II clinical study.[202]

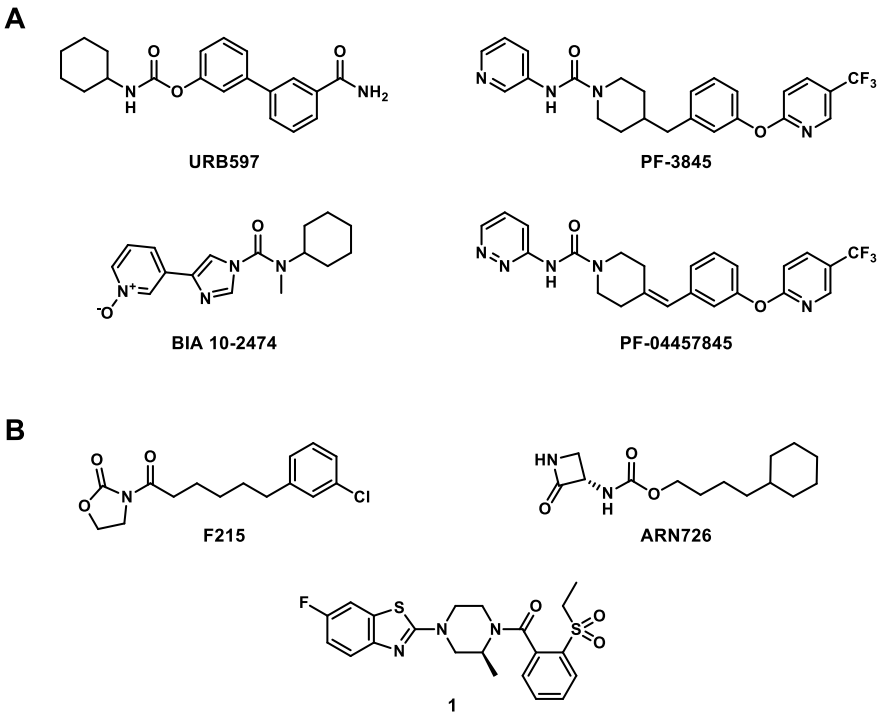


Figure 4. Structures of selected *in vivo* active inhibitors of **A**) fatty acid amide hydrolase (FAAH) or **B**) *N*-acylethanolamine acid amidase (NAAA).

In 2016, the covalent FAAH inhibitor BIA 10-2474 (Figure 4A) was tested in healthy volunteers in a Phase I clinical study, which led to the tragic death of one individual and mild-to-severe neurological symptoms in four others.[203] It was later revealed that BIA 10-2474 displayed off-target activities against multiple serine hydrolases in the CNS, whereas PF-04457845 was highly selective for FAAH and did not present adverse effects in

multiple clinical studies.[204] Accordingly, the observed neurotoxic side effects of BIA 10-2474 are presumed not to be caused by inhibition of FAAH.[199]

Due to the limited success of FAAH inhibitors in the clinic, in recent years, inhibitors of the other NAE-hydrolyzing enzyme NAAA have come to the foreground.[205] Several *in vivo* active NAAA inhibitors have been reported, showing encouraging results for the treatment of inflammatory and neuropathic pain, allergic dermatitis and multiple sclerosis.[82,206-208] Considerable evidence point towards a PPAR-α-mediated mechanism.[198,207,209] First generation irreversible NAAA inhibitors ARN276 and F215 (Figure 4B) were able to increase PEA and OEA concentrations 2- to 4-fold in lungs of mice after an inflammatory stimulus, but not in naïve mice.[208,210] It is possible that these compounds elicit an inflammation-specific effect, although their low plasma stability and fast clearance could also explain the observed results.[183] Importantly, the increase of OEA illustrates the difference between *in vivo* and *in vitro* NAAA activity, since in the latter case NAAA showed high preference towards hydrolysis of PEA.[81] A second generation reversible NAAA inhibitor (**1**, Figure 4B) presented improved drug-like properties and was able to elevate brain PEA and OEA levels (2-fold) of healthy mice, but not AEA.[82] It is anticipated that the newly reported crystal structure of NAAA will aide future inhibitor design.[211] In addition, the therapeutic exploitation of NAAA blockade will require KO mice to confirm the effects observed with pharmacological inhibitors.

1.3.2 Inhibition of NAE biosynthesis

Blocking NAE biosynthesis by pharmacological agents is an underdeveloped strategy in endocannabinoid research and so far no selective and *in vivo* active inhibitors have been described.[212] Nevertheless, there is substantial evidence that reducing the NAE tone could be beneficial in pathological conditions such as obesity, metabolic syndrome, cancer and liver cirrhosis.[181] The potential net effect of inhibiting NAE production would be indirect antagonism of the respective NAE receptors. Because the cannabinoid receptors, PPAR-α, TRPV1, GPR55, GPR110 and GPR119 have additional endogenous agonists besides the NAEs, this will likely lead to only partial receptor deactivation.[199] Here, different conditions are outlined where decreasing NAE levels could be of therapeutic value.

Obesity and metabolic syndrome

The endocannabinoid system is a key player in energy balance and food intake, both in the CNS and the periphery.[213] The centrally active CB_1 receptor antagonist rimonabant (Acomplia®, Figure 5) was clinically approved for treatment of obesity and metabolic syndrome, as it induced significant weight-loss, decreased food intake and improved insulin resistance.[214-216] Unfortunately, patients treated with rimonabant suffered from depression-like side effects leading to its withdrawal from the market.[217,218] Peripherally

restricted CB_1 receptor antagonists have shown comparable pre-clinical efficacy and are currently being pursued as potential anti-obesity drugs without psychiatric side effects.[219,220] Alternatively, inhibiting NAE biosynthesis could be a possible therapeutic strategy. It has become increasingly clear from human studies and animal models that endocannabinoid and NAE signaling is disrupted during diet-induced obesity and metabolic disease.[180] Mice receiving a high fat diet for 18 weeks showed sustained elevation of plasma NAE levels including AEA, as well as increased expression of the NAE biosynthetic enzyme NAPE-PLD in brown adipose tissue.[221] In adipocytes, CB_1 receptor activation is associated with energy storage by increasing fatty acid uptake and lipogenesis and decreasing mitochondrial biogenesis, resulting in attenuated browning of white adipose tissue.[213,222] In the liver, mice fed a high fat diet for 3 weeks developed steatosis and showed greatly increased hepatic AEA levels, but not 2-AG.[223] This was credited to reduced FAAH activity, although NAPE-PLD activity was not determined. In the small intestine of rodents administered a high fat diet for 1 week, normal OEA mobilization after feeding was disrupted, possibly explaining the diminished satiety and hyperphagia observed in diet-induced obesity.[16,125,133] Sham feeding of a lipid-based meal to rats for 5 days resulted in an increase of jejunal AEA and 2-AG levels, which was dependent on signaling of the vagus nerve.[224] Enhanced NAPE-PLD and reduced FAAH activities in the jejunum were reported, yet interestingly, OEA levels were not affected. Peripheral CB_1 receptor blockade (URB447, Figure 5) attenuated fat sham feeding, which supports the hypothesis that endocannabinoids are released upon high fat food consumption and drive a positive feedback loop via CB_1 receptor signaling.[224] In pancreatic islets, AEA content and NAPE-PLD gene expression was enhanced in fatty diabetic versus lean rats.[225] It was shown that AEA induced apoptosis of insulin producing beta cells via peripheral CB_1 receptor activation, thereby enabling the progression of type II diabetes. Accordingly, chronic treatment with the peripherally restricted CB_1 receptor antagonist

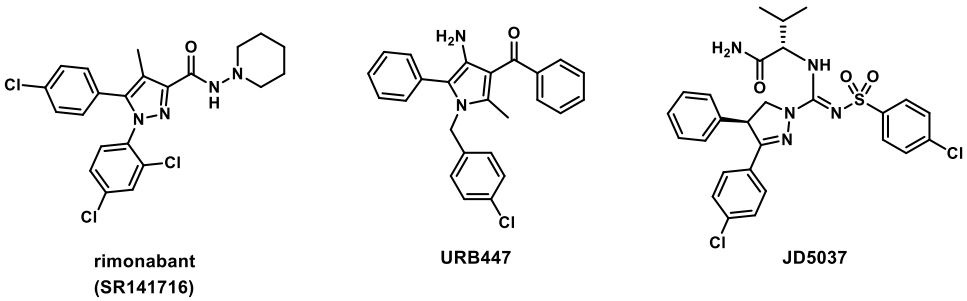


Figure 5. Structures of selected central (rimonabant) or peripherally restricted (URB447, JD5037) CB_1 receptor antagonists.

JD5037 (Figure 5) reversed islet elevation of AEA levels and NAPE-PLD expression and restored blood glucose levels to normal in overweight diabetic rats, although they remained insulin resistant.[225]

In humans, an analogous relationship between NAEs and obesity has been described. In a small human study (24 subjects), circulating AEA levels, but not 2-AG, peaked before a meal and significantly decreased postprandially in lean, but not in obese individuals.[226] A larger human study (328 subjects) revealed that obesity is associated with an increased AEA tone in plasma, as well as altered circulatory PEA/AEA and OEA/AEA ratios, indicative of enhanced appetite and diminished satiety.[227] In the same cohort, plasma 2-AG levels were not found to be upregulated in obese individuals.[228] In another large human trial (997 subjects), circulating AEA concentrations were also associated with BMI.[229] Furthermore, AEA correlated with non-alcoholic steatohepatitis (NASH) disease severity and was therefore proposed as a biomarker.[229] These combined clinical and pre-clinical data suggest that lowering plasma AEA concentrations may offer a therapeutic opportunity for treatment of obesity, metabolic syndrome, type II diabetes and liver steatosis. At the same time, it is not yet known which organs contribute to circulatory NAEs, which needs to be addressed.[230]

Several studies have looked at the role of NAPE-PLD in energy metabolism. In a large human cohort, a common NAPE-PLD haplotype was described to be protective against severe obesity.[231] Mice with a genetic deletion of NAPE-PLD presented a reduced food intake and overall leaner phenotype than their WT littermates.[175] Of note, these effects were not observed in a different NAPE-PLD KO strain.[232] On the other hand, FAAH ablation in mice increased energy storage, body weight and adipose tissue and promoted the appetite-stimulating effect of AEA, rather than the OEA-induced satiety.[233] These studies suggest that inhibition of NAPE-PLD may constitute as a potential treatment for metabolic syndrome. However, mice with a specific deletion of NAPE-PLD in adipose tissue had a predisposition for obesity while receiving a normal diet.[234] When administered a high fat diet for 8 weeks, adipocyte NAPE-PLD KO mice showed increased body weight gain compared to WT. Notably, in both diets levels of the anorectic OEA, PEA and SEA were decreased in NAPE-PLD KO adipose tissue, but not of orexigenic AEA. A similar NAE profile was observed in WT mice receiving a high fat versus a control diet.[234] Conditional KO of intestinal NAPE-PLD in mice induced hyperphagia upon initial high fat diet administration and exacerbated fat mass accumulation compared to WT mice.[235] When receiving a normal diet, intestinal NAPE-PLD KO mice displayed reduced intestinal levels of AEA, OEA, PEA and SEA. In contrast, after 16 weeks of high fat diet, jejunal NAE concentrations in WT and intestinal NAPE-PLD KO mice did not significantly differ.[235] Collectively, these data

indicate that NAPE-PLD functioning in the gut and adipose tissue is altered during obese conditions. It remains to be determined what the effect of global or peripheral pharmacological NAPE-PLD blockade will be on energy balance and food intake in metabolic syndrome and obesity.

Cancer

Multiple studies have reported disrupted NAE levels in cancer and associations between NAE receptors and tumor proliferation. Hepatic CB_1 receptor and NAPE-PLD expression as well as AEA concentrations were found to be elevated in hepatocellular carcinoma (HCC) both in humans and mice.[236] Treatment with the peripherally restricted CB_1 receptor antagonist JD5037 or CB_1 receptor KO mice demonstrated suppressed tumor growth. These findings were underscored in a second study, showing that AEA acts as a tumor promotor in HCC via the CB_1 receptor.[237] Accordingly, FAAH KO mice displayed a worsened tumor progression. In addition, human hepatic tumor tissue exhibited reduced FAAH expression.[237] In chronic lymphocytic leukemia (CLL) patients, plasma levels of OEA were upregulated and correlated with the number of circulating tumor cells.[238] After treatment with the chemotherapy drug lenalidomide, patients in clinical remission presented significantly reduced plasma OEA. Patient derived CLL cells expressed NAPE-PLD and a role for overproduction of OEA by these cells was proposed.[238] Importantly, PPAR-α expression was found to be elevated in CLL patients and associated with an advanced disease stage.[239] Furthermore, a PPAR-α antagonist was able to reduce tumor burden in a mouse model of CLL.[240] Taken together, these studies suggest that targeting NAE biosynthetic enzymes, in particular NAPE-PLD, could have beneficial therapeutic effects in leukemia or hepatic cancer.

Chronic liver disease

Besides hepatic cancer and steatosis, also cirrhosis has been implicated in aberrant NAE signaling.[241-243] Liver cirrhosis is most often caused by alcohol abuse, hepatitis or steatosis and has a high mortality rate. In monocytes derived from humans and rats with cirrhotic liver, AEA levels were found to be elevated.[244,245] Similar findings were observed in another study, reporting increased circulatory AEA, OEA and PEA levels in cirrhotic patients, which correlated with advanced disease stage.[246] Hypertension of the portal vein is a major complication of advanced cirrhosis as a result of intrahepatic vascular resistance due to excessive scarring (fibrosis) and vasodilation in mesenteric arteries.[243] AEA induced vasodilation in mesenteric vessels from cirrhotic rats, whereas control samples were less sensitive to AEA.[247] Antagonists for the CB_1 receptor (rimonabant) or TRPV1 (capsazepine) blocked this effect.[247] Accordingly, administration of rimonabant in cirrhotic rats decreased mesenteric blood flow and portal hypertension.[245] CB_1 receptor expression is

low in healthy human liver, but it was upregulated in fibrotic and cirrhotic samples.[248] Genetic deletion or pharmacological blockade of CB_1 receptors (rimonabant) reduced hepatic fibrogenesis in three different fibrotic rat models.[248] This was extended to advanced cirrhotic rats, where treatment with rimonabant for two weeks reversed fibrosis.[249]

The relevant biosynthetic pathway of circulatory AEA in cirrhosis is still unknown. It is well established that cirrhotic patients have elevated plasma levels of endotoxins and increased hepatic macrophages.[250-252] LPS was reported to induce AEA production in mouse macrophages, which was dependent on the PLC/phosphatase biosynthetic pathway.[69,71] In addition, pro-inflammatory stimuli such as LPS were found to downregulate NAPE-PLD expression in mouse macrophages, thereby reducing anti-inflammatory PEA concentrations.[70] To summarize, the described studies point towards pathological signaling of AEA in hepatic fibrosis and cirrhosis and suggest that blocking CB_1 receptor activation or AEA biosynthesis, possibly via the PLC/phosphatase pathway, could be of potential therapeutic benefit.

Reducing NAE levels in the brain?

In neurodegenerative diseases, for example multiple sclerosis and Parkinson's disease, AEA levels were found to be elevated in human cerebrospinal fluid.[253,254] It is proposed that the AEA increase does not induce disease progression, but rather provides neuroprotection via CB_1 or CB_2 receptor activation as a result of the neuroinflammatory component of these diseases.[179,255] Substantial evidence has been collected for the beneficial effects of CB_1 and CB_2 receptor signaling in CNS injury, however, several studies also point to a positive effect of CB_1 receptor inhibition.[255] For example, CB_1 receptor blockade with rimonabant was neuroprotective in various rodent models of brain injury and enhanced AEA levels were harmful.[124,256,257] OEA and PEA, which are more abundant in the brain, have neuroprotective or anti-inflammatory effects acting in part via PPAR-α.[6,126] In a mouse model of cerebral ischemia, activation of brain PPAR-α by OEA reduced infarct volume.[258] Currently, different strategies are being investigated that activate the cannabinoid and PPAR receptors by enhancing the NAE tone (*e.g.* FAAH inhibition) or by using CB_1/CB_2 agonists as therapeutic treatment for neurological conditions.[199] Recently, a frameshift variant of NAPE-PLD in several dog breeds was reported to be a risk factor for leukoencephalomyelopathy, a myelination disorder.[259] The impact of this NAPE-PLD variant on the enzymatic activity or brain NAE concentrations, has yet to be determined.

NAE signaling in the brain is involved in numerous physiological processes, such as memory formation, stress and anxiety.[150] At present, the potential benefits of reducing NAE levels in the CNS are unclear.[260] The depressive side effects associated with brain CB_1

receptor antagonism, suggest that depletion of tonic AEA signaling could have a similar negative outcome. Recently, two reports looked at selective overexpression of the AEA degrading enzyme FAAH in specific brain regions using a viral vector. In the hippocampus, this afforded an elevation of anxiety-like behavior and a deficit in object recognition memory and in extinction of aversive memory.[261] Interestingly, reduced NAE levels were observed for AEA and PEA, but not OEA. In contrast, FAAH overexpression in the amygdala produced an anxiolytic effect and decreased conditioned fear responses.[262] These studies indicate that depleting the brain NAE tone can have brain region-specific outcomes. The neurophysiological behavior of mice with a genetic deletion in one of the NAE-producing enzymes such as NAPE-PLD, ABHD4 and GDE1 have not yet been profiled, since brain AEA concentrations were not unambiguously reduced.[37] This highlights the need for centrally active NAE biosynthesis inhibitors, to expose the primary pathway of NAE and AEA generation, and to establish the effect of decreased NAE signaling.

1.4 Aim and outline of this thesis:

Selective and *in vivo* active pharmacological tools that modulate NAE biosynthetic enzymes are necessary to elucidate the importance of these pathways. Furthermore, reducing the NAE tone may hold promise for the treatment of several pathological conditions. So far, no inhibitors have been described that can decrease NAE levels in cells or live animals. The aim of this thesis work is the discovery and application of new chemical tools for two NAE-generating enzymes: NAPE-PLD and PLAAT2.

To obtain new molecules that can inhibit NAPE-PLD, in **Chapter 2**, a fluorescence-based activity assay for NAPE-PLD was optimized to enable high-throughput screening for hit identification. A library of ~350,000 compounds was screened. After multiple deselection rounds, five hit compounds were obtained with (sub)micromolar potency and reasonable physicochemical properties. Resynthesis and testing of the most promising hit – a pyrimidine-4-carboxamide – confirmed its activity for NAPE-PLD and provided a suitable starting point for the development of *in vivo* active NAPE-PLD inhibitors.

In **Chapter 3**, a library of pyrimidine-4-carboxamides was generated to increase the potency of the HTS-hit compound for NAPE-PLD and to improve its physicochemical properties. By modifying different substituents one at a time, a structure-activity relationship map was created. This afforded the optimized NAPE-PLD inhibitor **LEI-401** with nanomolar potency and favorable physicochemical features.

In drug discovery and development, establishing target engagement of a drug candidate and its intended protein target is an essential step for success in pre-clinical and clinical research. To assess whether **LEI-401** can bind to NAPE-PLD in live cells, in **Chapter 4**, a photoaffinity labeling approach was investigated. First, photoaffinity probes were synthesized that allowed visualization of NAPE-PLD using gel-based fluorescent labeling or chemical proteomics. Finally, cellular target engagement of **LEI-401** with NAPE-PLD was confirmed by performing competition experiments in the photoaffinity assay.

In **Chapter 5**, the NAPE-PLD inhibitor **LEI-401** was profiled in cellular and *in vivo* models to characterize its effect on NAE biosynthesis. In neuronal cells, **LEI-401** produced a marked reduction of multiple NAEs, including AEA, among a broad lipid panel. This effect was dependent on NAPE-PLD protein expression. Intraperitoneal administration in mice showed that **LEI-401** exhibited a good pharmacokinetic profile and passed the blood-brain barrier. A significant time- and dose-dependent decrease of AEA was observed in the brain, but not of other NAEs. Behavioral profiling in mice indicated that **LEI-401** produced hypomotility, antinociception and hypothermia. Also in a mouse model of inflammatory pain **LEI-401** elicited an analgesic effect. In short, **LEI-401** was identified as an *in vivo* active NAPE-PLD inhibitor, capable of decreasing brain AEA levels.

PLAAT2 is a Ca^{2+}-independent *N*-acyltransferase that was reported to produce high levels of NAEs in cells. In **Chapter 6**, α-ketoamide inhibitors were identified as PLAAT2 inhibitors through library screening with an activity-based probe. A structure-activity relationship analysis was performed, which yielded **LEI-301** as a nanomolar potent PLAAT2 inhibitor. **LEI-301** was able to significantly reduce NAE levels including AEA after PLAAT2 overexpression in cells.

Chapter 7 summarizes the work described in this thesis and provides new avenues for future research.

References

1. Fahy, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R.H., Shimizu, T., Spener, F., van Meer, G., Wakelam, M.J.O. & Dennis, E.A. Update of the LIPID MAPS comprehensive classification system for lipids. *Journal of Lipid Research* **50**, S9-S14 (2009).
2. Lo Verme, J., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A. & Piomelli, D. The nuclear receptor peroxisome proliferator-activated receptor-α mediates the anti-inflammatory actions of palmitoylethanolamide. *Molecular Pharmacology* **67**, 15-19 (2005).
3. Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N.-O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T. & Greasley, P.J. The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology* **152**, 1092-1101 (2007).
4. Overton, H.A., Babbs, A.J., Doel, S.M., Fyfe, M.C.T., Gardner, L.S., Griffin, G., Jackson, H.C., Procter, M.J., Rasamison, C.M., Tang-Christensen, M., Widdowson, P.S., Williams, G.M. & Reynet, C. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metabolism* **3**, 167-175 (2006).
5. Didier, M.L., Severine, V., Kent-Olov, J. & Christopher, J.F. The palmitoylethanolamide family: a new class of anti-inflammatory agents? *Current Medicinal Chemistry* **9**, 663-674 (2002).
6. Hansen, H.S. Palmitoylethanolamide and other anandamide congeners. Proposed role in the diseased brain. *Experimental Neurology* **224**, 48-55 (2010).
7. Lambert, D.M., Vandevoorde, S., Diependaele, G., Govaerts, S.J. & Robert, A.R. Anticonvulsant activity of *N*-palmitoylethanolamide, a putative endocannabinoid, in mice. *Epilepsia* **42**, 321-327 (2001).
8. Gabrielsson, L., Mattsson, S. & Fowler, C.J. Palmitoylethanolamide for the treatment of pain: pharmacokinetics, safety and efficacy. *British Journal of Clinical Pharmacology* **82**, 932-942 (2016).
9. Rodríguez de Fonseca, F., Navarro, M., Gómez, R., Escuredo, L., Nava, F., Fu, J., Murillo-Rodríguez, E., Giuffrida, A., LoVerme, J., Gaetani, S., Kathuria, S., Gall, C. & Piomelli, D. An anorexic lipid mediator regulated by feeding. *Nature* **414**, 209-212 (2001).
10. Dalle Carbonare, M., Del Giudice, E., Stecca, A., Colavito, D., Fabris, M., D'Arrigo, A., Bernardini, D., Dam, M. & Leon, A. A saturated *N*-acylethanolamine other than *N*-palmitoyl ethanolamine with anti-inflammatory properties: a neglected story…. *Journal of Neuroendocrinology* **20**, 26-34 (2008).
11. Terrazzino, S., Berto, F., Carbonare, M.D., Fabris, M., Guiotto, A., Bernardini, D. & Leon, A. Stearoylethanolamide exerts anorexic effects in mice via down-regulation of liver stearoyl-coenzyme A desaturase-1 mRNA expression. *The FASEB Journal* **18**, 1580-1582 (2004).
12. Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodríguez de Fonseca, F., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G. & Piomelli, D. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. *Nature* **425**, 90 (2003).
13. Rodríguez de Fonseca, F., Leza, J.C., Sayd, A., Antón, M., García-Bueno, B., Alén, F., Pavón, J., Caso, J.R. & Orio, L. Systemic administration of oleoylethanolamide protects from neuroinflammation and anhedonia induced by LPS in rats. *International Journal of Neuropsychopharmacology* **18** (2015).
14. Suardíaz, M., Estivill-Torrús, G., Goicoechea, C., Bilbao, A. & Rodríguez de Fonseca, F. Analgesic properties of oleoylethanolamide (OEA) in visceral and inflammatory pain. *PAIN* **133**, 99-110 (2007).
15. Gonzalez-Aparicio, R., Blanco, E., Serrano, A., Pavon, F.J., Parsons, L.H., Maldonado, R., Robledo, P., Fernandez-Espejo, E. & de Fonseca, F.R. The systemic administration of oleoylethanolamide exerts neuroprotection of the nigrostriatal system in experimental Parkinsonism. *International Journal of Neuropsychopharmacology* **17**, 455-468 (2014).
16. Artmann, A., Petersen, G., Hellgren, L.I., Boberg, J., Skonberg, C., Nellemann, C., Hansen, S.H. & Hansen, H.S. Influence of dietary fatty acids on endocannabinoid and *N*-acylethanolamine levels in rat brain, liver and small intestine. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1781**, 200-212 (2008).

17. Syed, S.K., Bui, H.H., Beavers, L.S., Farb, T.B., Ficorilli, J., Chesterfield, A.K., Kuo, M.-S., Bokvist, K., Barrett, D.G. & Efanov, A.M. Regulation of GPR119 receptor activity with endocannabinoid-like lipids. *American Journal of Physiology-Endocrinology and Metabolism* **303**, E1469-E1478 (2012).
18. Diep, T.A., Madsen, A.N., Holst, B., Kristiansen, M.M., Wellner, N., Hansen, S.H. & Hansen, H.S. Dietary fat decreases intestinal levels of the anorectic lipids through a fat sensor. *The FASEB Journal* **25**, 765-774 (2011).
19. Garg, P., Duncan, R.S., Kaja, S., Zabaneh, A., Chapman, K.D. & Koulen, P. Lauroylethanolamide and linoleoylethanolamide improve functional outcome in a rodent model for stroke. *Neuroscience Letters* **492**, 134-138 (2011).
20. Devane, W., Hanus, L., Breuer, A., Pertwee, R., Stevenson, L., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. & Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946-1949 (1992).
21. Soethoudt, M., Grether, U., Fingerle, J., Grim, T.W., Fezza, F., de Petrocellis, L., Ullmer, C., Rothenhäusler, B., Perret, C., van Gils, N., Finlay, D., MacDonald, C., Chicca, A., Gens, M.D., Stuart, J., de Vries, H., Mastrangelo, N., Xia, L., Alachouzos, G., Baggelaar, M.P., Martella, A., Mock, E.D., Deng, H., Heitman, L.H., Connor, M., Di Marzo, V., Gertsch, J., Lichtman, A.H., Maccarrone, M., Pacher, P., Glass, M. & van der Stelt, M. Cannabinoid CB_2 receptor ligand profiling reveals biased signalling and off-target activity. *Nature Communications* **8**, 13958 (2017).
22. Zygmunt, P.M., Petersson, J., Andersson, D.A., Chuang, H.-h., Sørgård, M., Di Marzo, V., Julius, D. & Högestätt, E.D. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**, 452-457 (1999).
23. Katona, I. & Freund, T.F. Multiple functions of endocannabinoid signaling in the brain. *Annual Review of Neuroscience* **35**, 529-558 (2012).
24. Williams, C.M. & Kirkham, T.C. Anandamide induces overeating: mediation by central cannabinoid (CB_1) receptors. *Psychopharmacology* **143**, 315-317 (1999).
25. Calignano, A., Rana, G.L., Giuffrida, A. & Piomelli, D. Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277-281 (1998).
26. Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Rana, G.L., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V. & Piomelli, D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nature Medicine* **9**, 76 (2002).
27. Morena, M., Roozendaal, B., Trezza, V., Ratano, P., Peloso, A., Hauer, D., Atsak, P., Trabace, L., Cuomo, V., McGaugh, J.L., Schelling, G. & Campolongo, P. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. *Proceedings of the National Academy of Sciences* **111**, 18333-18338 (2014).
28. Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., López-Rodríguez, M.L., Casanova, E., Schütz, G., Zieglgänsberger, W., Di Marzo, V., Behl, C. & Lutz, B. CB_1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* **302**, 84-88 (2003).
29. Fernando, C., Manuel, L.W., Paula, V., Julieta, A. & Ana María, F. Endocannabinoid system and pregnancy. *Reproduction* **152**, R191-R200 (2016).
30. Lee, J.-W., Huang, B.X., Kwon, H., Rashid, M.A., Kharebava, G., Desai, A., Patnaik, S., Marugan, J. & Kim, H.-Y. Orphan GPR110 (ADGRF1) targeted by *N*-docosahexaenoylethanolamine in development of neurons and cognitive function. *Nature Communications* **7**, 13123 (2016).
31. Kim, H.-Y. & Spector, A.A. *N*-Docosahexaenoylethanolamine: A neurotrophic and neuroprotective metabolite of docosahexaenoic acid. *Molecular Aspects of Medicine* **64**, 34-44 (2018).
32. Park, T., Chen, H., Kevala, K., Lee, J.-W. & Kim, H.-Y. *N*-Docosahexaenoylethanolamine ameliorates LPS-induced neuroinflammation via cAMP/PKA-dependent signaling. *Journal of Neuroinflammation* **13**, 284 (2016).

33. Epps, D.E., Schmid, P.C., Natarajan, V. & Schmid, H.H.O. *N*-acylethanolamine accumulation in infarcted myocardium. *Biochemical and Biophysical Research Communications* **90**, 628-633 (1979).
34. Epps, D.E., Natarajan, V., Schmid, P.C. & Schmid, H.H.O. Accumulation of *N*-acylethanolamine glycerophospholipids in infarcted myocardium. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **618**, 420-430 (1980).
35. Schmid, H.H.O., Schmid, P.C. & Natarajan, V. *N*-Acylated glycerophospholipids and their derivatives. *Progress in Lipid Research* **29**, 1-43 (1990).
36. Schmid, H.H.O. Pathways and mechanisms of *N*-acylethanolamine biosynthesis: can anandamide be generated selectively? *Chemistry and Physics of Lipids* **108**, 71-87 (2000).
37. Hussain, Z., Uyama, T., Tsuboi, K. & Ueda, N. Mammalian enzymes responsible for the biosynthesis of *N*-acylethanolamines. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1862**, 1546-1561 (2017).
38. Natarajan, V., Schmid, P.C., Reddy, P.V., Zuzarte-Augustin, M.L. & Schmid, H.H.O. Biosynthesis of *N*-acylethanolamine phospholipids by dog brain preparations. *Journal of Neurochemistry* **41**, 1303-1312 (1983).
39. Reddy, P.V., Natarajan, V., Schmid, P.C. & Schmid, H.H.O. *N*-acylation of dog heart ethanolamine phospholipids by transacylase activity. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **750**, 472-480 (1983).
40. Ogura, Y., Parsons, W.H., Kamat, S.S. & Cravatt, B.F. A calcium-dependent acyltransferase that produces *N*-acyl phosphatidylethanolamines. *Nature Chemical Biology* **12**, 669 (2016).
41. Hussain, Z., Uyama, T., Kawai, K., Binte Mustafiz, S.S., Tsuboi, K., Araki, N. & Ueda, N. Phosphatidylserine-stimulated production of *N*-acyl-phosphatidylethanolamines by Ca^{2+}-dependent *N*-acyltransferase. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1863**, 493-502 (2018).
42. Tsuboi, K., Okamoto, Y., Ikematsu, N., Inoue, M., Shimizu, Y., Uyama, T., Wang, J., Deutsch, D.G., Burns, M.P., Ulloa, N.M., Tokumura, A. & Ueda, N. Enzymatic formation of *N*-acylethanolamines from *N*-acylethanolamine plasmalogen through *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1811**, 565-577 (2011).
43. Fu, J., Astarita, G., Gaetani, S., Kim, J., Cravatt, B.F., Mackie, K. & Piomelli, D. Food intake regulates oleoylethanolamide formation and degradation in the proximal small intestine. *Journal of Biological Chemistry* **282**, 1518-1528 (2007).
44. Astarita, G., Ahmed, F. & Piomelli, D. Identification of biosynthetic precursors for the endocannabinoid anandamide in the rat brain. *Journal of Lipid Research* **49**, 48-57 (2008).
45. Jin, X.-H., Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Discovery and characterization of a Ca^{2+}-independent phosphatidylethanolamine *N*-acyltransferase generating the anandamide precursor and its congeners. *Journal of Biological Chemistry* **282**, 3614-3623 (2007).
46. Jin, X.-H., Uyama, T., Wang, J., Okamoto, Y., Tonai, T. & Ueda, N. cDNA cloning and characterization of human and mouse Ca^{2+}-independent phosphatidylethanolamine *N*-acyltransferases. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1791**, 32-38 (2009).
47. Uyama, T., Jin, X.-H., Tsuboi, K., Tonai, T. & Ueda, N. Characterization of the human tumor suppressors TIG3 and HRASLS2 as phospholipid-metabolizing enzymes. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1791**, 1114-1124 (2009).
48. Ueda, N., Tsuboi, K. & Uyama, T. Enzymological studies on the biosynthesis of *N*-acylethanolamines. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1801**, 1274-1285 (2010).
49. Shinohara, N., Uyama, T., Jin, X.-H., Tsuboi, K., Tonai, T., Houchi, H. & Ueda, N. Enzymological analysis of the tumor suppressor A-C1 reveals a novel group of phospholipid-metabolizing enzymes. *Journal of Lipid Research* **52**, 1927-1935 (2011).

50. Uyama, T., Ikematsu, N., Inoue, M., Shinohara, N., Jin, X.-H., Tsuboi, K., Tonai, T., Tokumura, A. & Ueda, N. Generation of *N*-acylphosphatidylethanolamine by members of the phospholipase A/acyltransferase (PLA/AT) family. *Journal of Biological Chemistry* **287**, 31905-31919 (2012).
51. Shyu, R.-Y., Hsieh, Y.-C., Tsai, F.-M., Wu, C.-C. & Jiang, S.-Y. Cloning and functional characterization of the HRASLS2 gene. *Amino Acids* **35**, 129-137 (2008).
52. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Molecular characterization of a phospholipase D generating anandamide and its congeners. *Journal of Biological Chemistry* **279**, 5298-5305 (2004).
53. Magotti, P., Bauer, I., Igarashi, M., Babagoli, M., Marotta, R., Piomelli, D. & Garau, G. Structure of human *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D: regulation of fatty acid ethanolamide biosynthesis by bile acids. *Structure* **23**, 598-604 (2015).
54. Wang, J., Okamoto, Y., Morishita, J., Tsuboi, K., Miyatake, A. & Ueda, N. Functional analysis of the purified anandamide-generating phospholipase D as a member of the metallo-β-lactamase family. *Journal of Biological Chemistry* **281**, 12325-12335 (2006).
55. Wang, J., Okamoto, Y., Tsuboi, K. & Ueda, N. The stimulatory effect of phosphatidylethanolamine on *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD). *Neuropharmacology* **54**, 8-15 (2008).
56. Margheritis, E., Castellani, B., Magotti, P., Peruzzi, S., Romeo, E., Natali, F., Mostarda, S., Gioiello, A., Piomelli, D. & Garau, G. Bile acid recognition by NAPE-PLD. *ACS Chemical Biology* **11**, 2908-2914 (2016).
57. Liu, Q., Tonai, T. & Ueda, N. Activation of *N*-acylethanolamine-releasing phospholipase D by polyamines. *Chemistry and Physics of Lipids* **115**, 77-84 (2002).
58. Leung, D., Saghatelian, A., Simon, G.M. & Cravatt, B.F. Inactivation of *N*-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* **45**, 4720-4726 (2006).
59. Leishman, E., Mackie, K., Luquet, S. & Bradshaw, H.B. Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1861**, 491-500 (2016).
60. Inoue, M., Tanaka, T., Okamoto, Y., Tokumura, A., Tsuboi, K., Ueda, N., Uyama, T., Hidaka, M. & Tsutsumi, T. Peripheral tissue levels and molecular species compositions of *N*-acyl-phosphatidylethanolamine and its metabolites in mice lacking *N*-acyl-phosphatidylethanolamine-specific phospholipase D. *The Journal of Biochemistry* **162**, 449-458 (2017).
61. Sun, Y.X., Tsuboi, K., Okamoto, Y., Tonai, T., Murakami, M., Kudo, I. & Ueda, N. Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A(2) and lysophospholipase D. *Biochemical Journal* **380**, 749-756 (2004).
62. Simon, G.M. & Cravatt, B.F. Endocannabinoid biosynthesis proceeding through glycerophospho-*N*-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway. *Journal of Biological Chemistry* **281**, 26465-26472 (2006).
63. Simon, G.M. & Cravatt, B.F. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-*N*-acyl ethanolamine precursors in mouse brain. *Journal of Biological Chemistry* **283**, 9341-9349 (2008).
64. Tsuboi, K., Okamoto, Y., Rahman, I.A.S., Uyama, T., Inoue, T., Tokumura, A. & Ueda, N. Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: a possible involvement in bioactive *N*-acylethanolamine biosynthesis. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1851**, 537-548 (2015).
65. Rahman, I.A.S., Tsuboi, K., Hussain, Z., Yamashita, R., Okamoto, Y., Uyama, T., Yamazaki, N., Tanaka, T., Tokumura, A. & Ueda, N. Calcium-dependent generation of *N*-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1861**, 1881-1892 (2016).

66. Lee, H.-C., Simon, G.M. & Cravatt, B.F. ABHD4 regulates multiple classes of *N*-acyl phospholipids in the mammalian central nervous system. *Biochemistry* **54**, 2539-2549 (2015).
67. Simon, G.M. & Cravatt, B.F. Characterization of mice lacking candidate *N*-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production in vivo. *Molecular BioSystems* **6**, 1411-1418 (2010).
68. Yung, Yun C., Stoddard, Nicole C., Mirendil, H. & Chun, J. Lysophosphatidic acid signaling in the nervous system. *Neuron* **85**, 669-682 (2015).
69. 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. A biosynthetic pathway for anandamide. *Proceedings of the National Academy of Sciences* **103**, 13345-13350 (2006).
70. Zhu, C., Solorzano, C., Sahar, S., Realini, N., Fung, E., Sassone-Corsi, P. & Piomelli, D. Proinflammatory stimuli control *N*-acylphosphatidylethanolamine-specific phospholipase D expression in macrophages. *Molecular Pharmacology* **79**, 786-792 (2011).
71. 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. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**, 1-7 (2008).
72. Ahn, K., McKinney, M.K. & Cravatt, B.F. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chemical Reviews* **108**, 1687-1707 (2008).
73. Giang, D.K. & Cravatt, B.F. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proceedings of the National Academy of Sciences* **94**, 2238-2242 (1997).
74. Cravatt, B.F., Demarest, K., Patricelli, M.P., Bracey, M.H., Giang, D.K., Martin, B.R. & Lichtman, A.H. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proceedings of the National Academy of Sciences* **98**, 9371-9376 (2001).
75. Ahn, K., Johnson, D.S., Mileni, M., Beidler, D., Long, J.Z., McKinney, M.K., Weerapana, E., Sadagopan, N., Liimatta, M., Smith, S.E., Lazerwith, S., Stiff, C., Kamtekar, S., Bhattacharya, K., Zhang, Y., Swaney, S., Van Becelaere, K., Stevens, R.C. & Cravatt, B.F. Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chemistry & Biology* **16**, 411-420 (2009).
76. Saghatelian, A., Trauger, S.A., Want, E.J., Hawkins, E.G., Siuzdak, G. & Cravatt, B.F. Assignment of endogenous substrates to enzymes by global metabolite profiling. *Biochemistry* **43**, 14332-14339 (2004).
77. Mukhopadhyay, B., Cinar, R., Yin, S., Liu, J., Tam, J., Godlewski, G., Harvey-White, J., Mordi, I., Cravatt, B.F., Lotersztajn, S., Gao, B., Yuan, Q., Schuebel, K., Goldman, D. & Kunos, G. Hyperactivation of anandamide synthesis and regulation of cell-cycle progression via cannabinoid type 1 (CB_1) receptors in the regenerating liver. *Proceedings of the National Academy of Sciences* **108**, 6323-6328 (2011).
78. Wei, B.Q., Mikkelsen, T.S., McKinney, M.K., Lander, E.S. & Cravatt, B.F. A second fatty acid amide hydrolase with variable distribution among placental mammals. *Journal of Biological Chemistry* **281**, 36569-36578 (2006).
79. Kaczocha, M., Glaser, S.T., Chae, J., Brown, D.A. & Deutsch, D.G. Lipid droplets are novel sites of *N*-acylethanolamine inactivation by fatty acid amide hydrolase-2. *Journal of Biological Chemistry* **285**, 2796-2806 (2010).
80. Ueda, N., Yamanaka, K. & Yamamoto, S. Purification and characterization of an acid amidase selective for *N*-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *Journal of Biological Chemistry* **276**, 35552-35557 (2001).
81. Tsuboi, K., Sun, Y.-X., Okamoto, Y., Araki, N., Tonai, T. & Ueda, N. Molecular characterization of *N*-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *Journal of Biological Chemistry* **280**, 11082-11092 (2005).
82. Migliore, M., Pontis, S., Fuentes de Arriba, A.L., Realini, N., Torrente, E., Armirotti, A., Romeo, E., Di Martino, S., Russo, D., Pizzirani, D., Summa, M., Lanfranco, M., Ottonello, G., Busquet, P., Jung, K.-M.,

Garcia-Guzman, M., Heim, R., Scarpelli, R. & Piomelli, D. Second-generation non-covalent NAAA inhibitors are protective in a model of multiple sclerosis. *Angewandte Chemie International Edition* **55**, 11193-11197 (2016).

83. Rouzer, C.A. & Marnett, L.J. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chemical Reviews* **111**, 5899-5921 (2011).
84. Urquhart, P., Nicolaou, A. & Woodward, D.F. Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1851**, 366-376 (2015).
85. Yu, M., Ives, D. & Ramesha, C.S. Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *Journal of Biological Chemistry* **272**, 21181-21186 (1997).
86. Kozak, K.R., Crews, B.C., Morrow, J.D., Wang, L.-H., Ma, Y.H., Weinander, R., Jakobsson, P.-J. & Marnett, L.J. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *Journal of Biological Chemistry* **277**, 44877-44885 (2002).
87. Moriuchi, H., Koda, N., Okuda-Ashitaka, E., Daiyasu, H., Ogasawara, K., Toh, H., Ito, S., Woodward, D.F. & Watanabe, K. Molecular characterization of a novel type of prostamide/prostaglandin F synthase, belonging to the thioredoxin-like superfamily. *Journal of Biological Chemistry* **283**, 792-801 (2008).
88. Gatta, L., Piscitelli, F., Giordano, C., Boccella, S., Lichtman, A., Maione, S. & Di Marzo, V. Discovery of prostamide F2α and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLOS ONE* **7**, e31111 (2012).
89. Hampson, A.J., Hill, W.A.G., Zan-Phillips, M., Makriyannis, A., Leung, E., Eglen, R.M. & Bornheim, L.M. Anandamide hydroxylation by brain lipoxygenase:metabolite structures and potencies at the cannabinoid receptor. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **1259**, 173-179 (1995).
90. Ueda, N., Yamamoto, K., Yamamoto, S., Tokunaga, T., Shirakawa, E., Shinkai, H., Ogawa, M., Sato, T., Kudo, I., Inoue, K., Takizawa, H., Nagano, T., Hirobe, M., Matsuki, N. & Saito, H. Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **1254**, 127-134 (1995).
91. van der Stelt, M., van Kuik, J.A., Bari, M., van Zadelhoff, G., Leeflang, B.R., Veldink, G.A., Finazzi-Agrò, A., Vliegenthart, J.F.G. & Maccarrone, M. Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. *Journal of Medicinal Chemistry* **45**, 3709-3720 (2002).
92. Bornheim, L.M., Kim, K.Y., Chen, B.L. & Correia, M.A. The effect of cannabidiol on mouse hepatic microsomal cytochrome P450-dependent anandamide metabolism. *Biochemical and Biophysical Research Communications* **197**, 740-746 (1993).
93. Snider, N.T., Nast, J.A., Tesmer, L.A. & Hollenberg, P.F. A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist. *Molecular Pharmacology* **75**, 965-972 (2009).
94. Coulon, D., Faure, L., Salmon, M., Wattelet, V. & Bessoule, J.-J. Occurrence, biosynthesis and functions of *N*-acylphosphatidylethanolamines (NAPE): Not just precursors of *N*-acylethanolamines (NAE). *Biochimie* **94**, 75-85 (2012).
95. Wellner, N., Diep, T.A., Janfelt, C. & Hansen, H.S. *N*-Acylation of phosphatidylethanolamine and its biological functions in mammals. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1831**, 652-662 (2013).
96. Cadas, H., Gaillet, S., Beltramo, M., Venance, L. & Piomelli, D. Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *The Journal of Neuroscience* **16**, 3934-3942 (1996).

97. Hansen, H.S., Lauritzen, L., Strand, A.M., Vinggaard, A.M., Frandsen, A. & Schousboe, A. Characterization of glutamate-induced formation of *N*-acylphosphatidylethanolamine and *N*-acylethanolamine in cultured neocortical neurons. *Journal of Neurochemistry* **69**, 753-761 (1997).
98. Natarajan, V., Schmid, P.C. & Schmid, H.H.O. *N*-Acylethanolamine phospholipid metabolism in normal and ischemic rat brain. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **878**, 32-41 (1986).
99. Moesgaard, B., Petersen, G., Jaroszewski, J.W. & Hansen, H.S. Age dependent accumulation of *N*-acyl-ethanolamine phospholipids in ischemic rat brain: a 31P NMR and enzyme activity study. *Journal of Lipid Research* **41**, 985-990 (2000).
100. Hansen, H.H., Ikonomidou, C., Bittigau, P., Hansen, S.H. & Hansen, H.S. Accumulation of the anandamide precursor and other *N*-acylethanolamine phospholipids in infant rat models of in vivo necrotic and apoptotic neuronal death. *Journal of Neurochemistry* **76**, 39-46 (2001).
101. Moesgaard, B., Hansen, H.H., Hansen, S.L., Hansen, S.H., Petersen, G. & Hansen, H.S. Brain levels of *N*-acylethanolamine phospholipids in mice during pentylenetetrazol-induced seizure. *Lipids* **38**, 387-390 (2003).
102. Janfelt, C., Wellner, N., Leger, P.-L., Kokesch-Himmelreich, J., Hansen, S.H., Charriaut-Marlangue, C. & Hansen, H.S. Visualization by mass spectrometry of 2-dimensional changes in rat brain lipids, including *N*-acylphosphatidylethanolamines, during neonatal brain ischemia. *The FASEB Journal* **26**, 2667-2673 (2012).
103. Basit, A., Pontis, S., Piomelli, D. & Armirotti, A. Ion mobility mass spectrometry enhances low-abundance species detection in untargeted lipidomics. *Metabolomics* **12**, 50 (2016).
104. Rawyler, A.J. & Braendle, R.A. *N*-Acylphosphatidylethanolamine accumulation in potato cells upon energy shortage caused by anoxia or respiratory inhibitors. *Plant Physiology* **127**, 240-251 (2001).
105. Gao, Y., Vasilyev, D.V., Goncalves, M.B., Howell, F.V., Hobbs, C., Reisenberg, M., Shen, R., Zhang, M.-Y., Strassle, B.W., Lu, P., Mark, L., Piesla, M.J., Deng, K., Kouranova, E.V., Ring, R.H., Whiteside, G.T., Bates, B., Walsh, F.S., Williams, G., Pangalos, M.N., Samad, T.A. & Doherty, P. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *The Journal of Neuroscience* **30**, 2017-2024 (2010).
106. Hansen, H.H., Schmid, P.C., Bittigau, P., Lastres-Becker, I., Berrendero, F., Manzanares, J., Ikonomidou, C., Schmid, H.H.O., Fernández-Ruiz, J.J. & Hansen, H.S. Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. *Journal of Neurochemistry* **78**, 1415-1427 (2001).
107. Domingo, J.C., Mora, M. & Africa de Madariaga, M. Role of headgroup structure in the phase behaviour of *N*-acylethanolamine phospholipids: hydrogen-bonding ability and headgroup size. *Chemistry and Physics of Lipids* **69**, 229-240 (1994).
108. Swamy, M.J., Tarafdar, P.K. & Kamlekar, R.K. Structure, phase behaviour and membrane interactions of *N*-acylethanolamines and *N*-acylphosphatidylethanolamines. *Chemistry and Physics of Lipids* **163**, 266-279 (2010).
109. Domingo, J.C., Mora, M. & Africa de Madariaga, M. Incorporation of *N*-acylethanolamine phospholipids into egg phosphatidylcholine vesicles: characterization and permeability properties of the binary systems. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1148**, 308-316 (1993).
110. Shangguan, T., Pak, C.C., Ali, S., Janoff, A.S. & Meers, P. Cation-dependent fusogenicity of an *N*-acyl phosphatidylethanolamine. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1368**, 171-183 (1998).
111. Mora, M., Mir, F., de Madariaga, M.A. & Sagrista, M.L. Aggregation and fusion of vesicles composed of *N*-palmitoyl derivatives of membrane phospholipids. *Lipids* **35**, 513-524 (2000).
112. Mora, M., Sagristá, M.-L., Trombetta, D., Bonina, F.P., De Pasquale, A. & Saija, A. Design and characterization of liposomes containing long-chain *N*-acylPEs for brain delivery: penetration of liposomes incorporating GM1 into the rat brain. *Pharmaceutical Research* **19**, 1430-1438 (2002).

113. Ichiki, M., Sugimoto, N., Ueda, N., Okamoto, Y., Takuwa, Y., Nakanishi, Y. & Shiratsuchi, A. Inhibitory effect of *N*-palmitoylphosphatidylethanolamine on macrophage phagocytosis through inhibition of Rac1 and Cdc42. *The Journal of Biochemistry* **145**, 43-50 (2008).
114. Piomelli, D. A fatty gut feeling. *Trends in Endocrinology & Metabolism* **24**, 332-341 (2013).
115. Gillum, M.P., Zhang, D., Zhang, X.-M., Erion, D.M., Jamison, R.A., Choi, C., Dong, J., Shanabrough, M., Duenas, H.R., Frederick, D.W., Hsiao, J.J., Horvath, T.L., Lo, C.M., Tso, P., Cline, G.W. & Shulman, G.I. *N*-Acylphosphatidylethanolamine, a gut- derived circulating factor induced by fat ingestion, inhibits food intake. *Cell* **135**, 813-824 (2008).
116. Wellner, N., Tsuboi, K., Madsen, A.N., Holst, B., Diep, T.A., Nakao, M., Tokumura, A., Burns, M.P., Deutsch, D.G., Ueda, N. & Hansen, H.S. Studies on the anorectic effect of *N*-acylphosphatidylethanolamine and phosphatidylethanolamine in mice. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1811**, 508-512 (2011).
117. Chen, Z., Zhang, Y., Guo, L., Dosoky, N., de Ferra, L., Peters, S., Niswender, K.D. & Davies, S.S. Leptogenic effects of NAPE require activity of NAPE-hydrolyzing phospholipase D. *Journal of Lipid Research* **58**, 1624-1635 (2017).
118. Kuehl, F.A., Jacob, T.A., Ganley, O.H., Ormond, R.E. & Meisinger, M.A.P. The identification of N-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. *Journal of the American Chemical Society* **79**, 5577-5578 (1957).
119. Bachur, N.R., Masek, K., Melmon, K.L. & Udenfriend, S. Fatty acid amides of ethanolamine in mammalian tissues. *Journal of Biological Chemistry* **240**, 1019-1024 (1965).
120. Skaper, S.D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L. & Leon, A. The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proceedings of the National Academy of Sciences* **93**, 3984-3989 (1996).
121. Calignano, A., La Rana, G. & Piomelli, D. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *European Journal of Pharmacology* **419**, 191-198 (2001).
122. Esposito, E., Cordaro, M. & Cuzzocrea, S. Roles of fatty acid ethanolamides (FAE) in traumatic and ischemic brain injury. *Pharmacological Research* **86**, 26-31 (2014).
123. Petrosino, S. & Di Marzo, V. The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. *British Journal of Pharmacology* **174**, 1349-1365 (2017).
124. Berger, C., Schmid, P.C., Schabitz, W.-R., Wolf, M., Schwab, S. & Schmid, H.H.O. Massive accumulation of *N*-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? *Journal of Neurochemistry* **88**, 1159-1167 (2004).
125. Hansen, H.S. & Vana, V. Non-endocannabinoid *N*-acylethanolamines and 2-monoacylglycerols in the intestine. *British Journal of Pharmacology* **176**, 1443-1454 (2019).
126. O'Sullivan, S.E. An update on PPAR activation by cannabinoids. *British Journal of Pharmacology* **173**, 1899-1910 (2016).
127. Hansen, H.S., Rosenkilde, M.M., Holst, J.J. & Schwartz, T.W. GPR119 as a fat sensor. *Trends in Pharmacological Sciences* **33**, 374-381 (2012).
128. Lauckner, J.E., Jensen, J.B., Chen, H.-Y., Lu, H.-C., Hille, B. & Mackie, K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proceedings of the National Academy of Sciences* **105**, 2699-2704 (2008).
129. Nielsen, M.J., Petersen, G., Astrup, A. & Hansen, H.S. Food intake is inhibited by oral oleoylethanolamide. *Journal of Lipid Research* **45**, 1027-1029 (2004).
130. Oveisi, F., Gaetani, S., Eng, K.T.-P. & Piomelli, D. Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. *Pharmacological Research* **49**, 461-466 (2004).
131. Petersen, G., Sørensen, C., Schmid, P.C., Artmann, A., Tang-Christensen, M., Hansen, S.H., Larsen, P.J., Schmid, H.H.O. & Hansen, H.S. Intestinal levels of anandamide and oleoylethanolamide in food-

deprived rats are regulated through their precursors. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1761**, 143-150 (2006).

132. Bowen, K.J., Kris-Etherton, P.M., Shearer, G.C., West, S.G., Reddivari, L. & Jones, P.J.H. Oleic acid-derived oleoylethanolamide: A nutritional science perspective. *Progress in Lipid Research* **67**, 1-15 (2017).
133. Igarashi, M., DiPatrizio, N.V., Narayanaswami, V. & Piomelli, D. Feeding-induced oleoylethanolamide mobilization is disrupted in the gut of diet-induced obese rodents. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1851**, 1218-1226 (2015).
134. Hong, L., Galya, V., Aaron, C., Li, L., Hana, B., Andrei, G., Susan, J.A., Weiwen, H., Shijun, Y., Yun, N., Robert, A.D.V., Frederique, P., Maureen, L., Eric, L.G., Joseph, A.H. & Timothy, J.K. GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis. *Journal of Endocrinology* **201**, 219-230 (2009).
135. Iannotti, F.A., Di Marzo, V. & Petrosino, S. Endocannabinoids and endocannabinoid-related mediators: Targets, metabolism and role in neurological disorders. *Progress in Lipid Research* **62**, 107-128 (2016).
136. Orio, L., Alen, F., Pavón, F.J., Serrano, A. & García-Bueno, B. Oleoylethanolamide, neuroinflammation, and alcohol abuse. *Frontiers in Molecular Neuroscience* **11** (2019).
137. Hansen, H.S., Moesgaard, B., Petersen, G. & Hansen, H.H. Putative neuroprotective actions of *N*-acyl-ethanolamines. *Pharmacology & Therapeutics* **95**, 119-126 (2002).
138. Gouveia-Figueira, S. & Nording, M.L. Validation of a tandem mass spectrometry method using combined extraction of 37 oxylipins and 14 endocannabinoid-related compounds including prostamides from biological matrices. *Prostaglandins & Other Lipid Mediators* **121**, 110-121 (2015).
139. Hansen, H.S. & Diep, T.A. *N*-Acylethanolamines, anandamide and food intake. *Biochemical Pharmacology* **78**, 553-560 (2009).
140. Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.-C. & Piomelli, D. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**, 686 (1994).
141. Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A. & Piomelli, D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094-1097 (1997).
142. Wilson, R.I. & Nicoll, R.A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**, 588-592 (2001).
143. Nyilas, R., Dudok, B., Urbán, G.M., Mackie, K., Watanabe, M., Cravatt, B.F., Freund, T.F. & Katona, I. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. *The Journal of Neuroscience* **28**, 1058-1063 (2008).
144. van der Stelt, M., Trevisani, M., Vellani, V., De Petrocellis, L., Schiano Moriello, A., Campi, B., McNaughton, P., Geppetti, P. & Di Marzo, V. Anandamide acts as an intracellular messenger amplifying Ca^{2+} influx via TRPV1 channels. *The EMBO Journal* **24**, 3026-3037 (2005).
145. Gobbi, G., Bambico, F.R., Mangieri, R., Bortolato, M., Campolongo, P., Solinas, M., Cassano, T., Morgese, M.G., Debonnel, G., Duranti, A., Tontini, A., Tarzia, G., Mor, M., Trezza, V., Goldberg, S.R., Cuomo, V. & Piomelli, D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 18620-18625 (2005).
146. Hohmann, A.G., Suplita, R.L., Bolton, N.M., Neely, M.H., Fegley, D., Mangieri, R., Krey, J.F., Michael Walker, J., Holmes, P.V., Crystal, J.D., Duranti, A., Tontini, A., Mor, M., Tarzia, G. & Piomelli, D. An endocannabinoid mechanism for stress-induced analgesia. *Nature* **435**, 1108-1112 (2005).
147. Morena, M., Patel, S., Bains, J.S. & Hill, M.N. Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology* **41**, 80 (2015).
148. Hill, M.N., McLaughlin, R.J., Morrish, A.C., Viau, V., Floresco, S.B., Hillard, C.J. & Gorzalka, B.B. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic–pituitary–adrenal axis. *Neuropsychopharmacology* **34**, 2733 (2009).

149. Hill, M.N., McLaughlin, R.J., Bingham, B., Shrestha, L., Lee, T.T.Y., Gray, J.M., Hillard, C.J., Gorzalka, B.B. & Viau, V. Endogenous cannabinoid signaling is essential for stress adaptation. *Proceedings of the National Academy of Sciences* **107**, 9406-9411 (2010).
150. Lutz, B., Marsicano, G., Maldonado, R. & Hillard, C.J. The endocannabinoid system in guarding against fear, anxiety and stress. *Nature Reviews Neuroscience* **16**, 705 (2015).
151. Jamshidi, N. & Taylor, D.A. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *British Journal of Pharmacology* **134**, 1151-1154 (2001).
152. van der Stelt, M., Veldhuis, W.B., van Haaften, G.W., Fezza, F., Bisogno, T., Bär, P.R., Veldink, G.A., Vliegenthart, J.F.G., Di Marzo, V. & Nicolay, K. Exogenous anandamide protects rat brain against acute neuronal injury *in vivo*. *The Journal of Neuroscience* **21**, 8765-8771 (2001).
153. Smith, P.B., Compton, D.R., Welch, S.P., Razdan, R.K., Mechoulam, R. & Martin, B.R. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *Journal of Pharmacology and Experimental Therapeutics* **270**, 219-227 (1994).
154. Maccarrone, M., Bab, I., Bíró, T., Cabral, G.A., Dey, S.K., Di Marzo, V., Konje, J.C., Kunos, G., Mechoulam, R., Pacher, P., Sharkey, K.A. & Zimmer, A. Endocannabinoid signaling at the periphery: 50 years after THC. *Trends in Pharmacological Sciences* **36**, 277-296 (2015).
155. van Eenige, R., van der Stelt, M., Rensen, P.C.N. & Kooijman, S. Regulation of adipose rissue metabolism by the endocannabinoid system. *Trends in Endocrinology & Metabolism* **29**, 326-337 (2018).
156. Gómez, R., Navarro, M., Ferrer, B., Trigo, J.M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D. & Rodríguez de Fonseca, F. A peripheral mechanism for CB_1 cannabinoid receptor-dependent modulation of feeding. *The Journal of Neuroscience* **22**, 9612-9617 (2002).
157. Clapper, J.R., Moreno-Sanz, G., Russo, R., Guijarro, A., Vacondio, F., Duranti, A., Tontini, A., Sanchini, S., Sciolino, N.R., Spradley, J.M., Hohmann, A.G., Calignano, A., Mor, M., Tarzia, G. & Piomelli, D. Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nature Neuroscience* **13**, 1265 (2010).
158. Munro, S., Thomas, K.L. & Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61-65 (1993).
159. Pertwee, R.G., Howlett, A.C., Abood, M.E., Alexander, S.P.H., Di Marzo, V., Elphick, M.R., Greasley, P.J., Hansen, H.S., Kunos, G., Mackie, K., Mechoulam, R. & Ross, R.A. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. *Pharmacological Reviews* **62**, 588-631 (2010).
160. Turcotte, C., Blanchet, M.-R., Laviolette, M. & Flamand, N. The CB_2 receptor and its role as a regulator of inflammation. *Cellular and Molecular Life Sciences* **73**, 4449-4470 (2016).
161. Di Marzo, V. & De Petrocellis, L. Why do cannabinoid receptors have more than one endogenous ligand? *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 3216-3228 (2012).
162. Turcotte, C., Chouinard, F., Lefebvre Julie, S. & Flamand, N. Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites. *Journal of Leukocyte Biology* **97**, 1049-1070 (2015).
163. Stelt, M.v.d. & Marzo, V.D. Endovanilloids. *European Journal of Biochemistry* **271**, 1827-1834 (2004).
164. Di Marzo, V. & De Petrocellis, L. Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. *Current Medicinal Chemistry* **17**, 1430-1449 (2010).
165. Tóth, A., Blumberg, P.M. & Boczán, J. Anandamide and the vanilloid receptor (TRPV1), in *Vitamins & Hormones*, Vol. 81 389-419 (Academic Press, 2009).
166. Martins, D., Tavares, I. & Morgado, C. "Hotheaded": The role of TRPV1 in brain functions. *Neuropharmacology* **85**, 151-157 (2014).
167. Marrone, M.C., Morabito, A., Giustizieri, M., Chiurchiù, V., Leuti, A., Mattioli, M., Marinelli, S., Riganti, L., Lombardi, M., Murana, E., Totaro, A., Piomelli, D., Ragozzino, D., Oddi, S., Maccarrone, M., Verderio,

C. & Marinelli, S. TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. *Nature Communications* **8**, 15292 (2017).

168. Kim, H.-Y., Spector, A.A. & Xiong, Z.-M. A synaptogenic amide *N*-docosahexaenoylethanolamide promotes hippocampal development. *Prostaglandins & Other Lipid Mediators* **96**, 114-120 (2011).
169. Meijerink, J., Poland, M., Balvers, M.G.J., Plastina, P., Lute, C., Dwarkasing, J., van Norren, K. & Witkamp, R.F. Inhibition of COX-2-mediated eicosanoid production plays a major role in the anti-inflammatory effects of the endocannabinoid *N*-docosahexaenoylethanolamine (DHEA) in macrophages. *British Journal of Pharmacology* **172**, 24-37 (2015).
170. Kim, J., Carlson, M.E., Kuchel, G.A., Newman, J.W. & Watkins, B.A. Dietary DHA reduces downstream endocannabinoid and inflammatory gene expression and epididymal fat mass while improving aspects of glucose use in muscle in C57BL/6J mice. *International Journal Of Obesity* **40**, 129 (2015).
171. Berger, A., Crozier, G., Bisogno, T., Cavaliere, P., Innis, S. & Di Marzo, V. Anandamide and diet: Inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding *N*-acylethanolamines in piglets. *Proceedings of the National Academy of Sciences* **98**, 6402-6406 (2001).
172. Wood, J.T., Williams, J.S., Pandarinathan, L., Janero, D.R., Lammi-Keefe, C.J. & Makriyannis, A. Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. *Journal of Lipid Research* **51**, 1416-1423 (2010).
173. Kim, H.-Y., Moon, H.-S., Cao, D., Lee, J., Kevala, K., Jun, Sang B., Lovinger, David M., Akbar, M. & Huang, Bill X. *N*-Docosahexaenoylethanolamide promotes development of hippocampal neurons. *Biochemical Journal* **435**, 327-336 (2011).
174. Lacombe, R.J.S., Chouinard-Watkins, R. & Bazinet, R.P. Brain docosahexaenoic acid uptake and metabolism. *Molecular Aspects of Medicine* **64**, 109-134 (2018).
175. Lin, L., Metherel, A.H., Kitson, A.P., Alashmali, S.M., Hopperton, K.E., Trépanier, M.-O., Jones, P.J. & Bazinet, R.P. Dietary fatty acids augment tissue levels of *N*-acylethanolamines in *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) knockout mice. *The Journal of Nutritional Biochemistry* **62**, 134-142 (2018).
176. Kendall, A.C., Pilkington, S.M., Massey, K.A., Sassano, G., Rhodes, L.E. & Nicolaou, A. Distribution of bioactive lipid mediators in human skin. *Journal of Investigative Dermatology* **135**, 1510-1520 (2015).
177. Lum, A.M., Wang, B.B., Beck-Engeser, G.B., Li, L., Channa, N. & Wabl, M. Orphan receptor GPR110, an oncogene overexpressed in lung and prostate cancer. *BMC Cancer* **10**, 40 (2010).
178. Pisanti, S., Picardi, P., D'Alessandro, A., Laezza, C. & Bifulco, M. The endocannabinoid signaling system in cancer. *Trends in Pharmacological Sciences* **34**, 273-282 (2013).
179. Chiurchiù, V., van der Stelt, M., Centonze, D. & Maccarrone, M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases. *Progress in Neurobiology* **160**, 82-100 (2018).
180. Mazier, W., Saucisse, N., Gatta-Cherifi, B. & Cota, D. The endocannabinoid system: pivotal orchestrator of obesity and metabolic disease. *Trends in Endocrinology & Metabolism* **26**, 524-537 (2015).
181. Di Marzo, V. Targeting the endocannabinoid system: to enhance or reduce? *Nature Reviews Drug Discovery* **7**, 438 (2008).
182. Blankman, J.L. & Cravatt, B.F. Chemical probes of endocannabinoid metabolism. *Pharmacological Reviews* **65**, 849-871 (2013).
183. Tuo, W., Leleu-Chavain, N., Spencer, J., Sansook, S., Millet, R. & Chavatte, P. Therapeutic potential of fatty acid amide hydrolase, monoacylglycerol lipase, and *N*-acylethanolamine acid amidase inhibitors. *Journal of Medicinal Chemistry* **60**, 4-46 (2017).
184. Ahn, K., Smith, S.E., Liimatta, M.B., Beidler, D., Sadagopan, N., Dudley, D.T., Young, T., Wren, P., Zhang, Y., Swaney, S., Van Becelaere, K., Blankman, J.L., Nomura, D.K., Bhattachar, S.N., Stiff, C., Nomanbhoy, T.K., Weerapana, E., Johnson, D.S. & Cravatt, B.F. Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces

inflammatory and noninflammatory pain. *Journal of Pharmacology and Experimental Therapeutics* **338**, 114-124 (2011).

185. Long, J.Z., LaCava, M., Jin, X. & Cravatt, B.F. An anatomical and temporal portrait of physiological substrates for fatty acid amide hydrolase. *Journal of Lipid Research* **52**, 337-344 (2011).
186. Donvito, G., Nass, S.R., Wilkerson, J.L., Curry, Z.A., Schurman, L.D., Kinsey, S.G. & Lichtman, A.H. The endogenous cannabinoid system: a budding source of targets for treating inflammatory and neuropathic pain. *Neuropsychopharmacology* **43**, 52 (2017).
187. Booker, L., Kinsey, S.G., Abdullah, R.A., Blankman, J.L., Long, J.Z., Ezzili, C., Boger, D.L., Cravatt, B.F. & Lichtman, A.H. The fatty acid amide hydrolase (FAAH) inhibitor PF-3845 acts in the nervous system to reverse LPS-induced tactile allodynia in mice. *British Journal of Pharmacology* **165**, 2485-2496 (2012).
188. Hill, M.N., Campolongo, P., Yehuda, R. & Patel, S. Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. *Neuropsychopharmacology* **43**, 80 (2017).
189. Celorrio, M., Fernández-Suárez, D., Rojo-Bustamante, E., Echeverry-Alzate, V., Ramírez, M.J., Hillard, C.J., López-Moreno, J.A., Maldonado, R., Oyarzábal, J., Franco, R. & Aymerich, M.S. Fatty acid amide hydrolase inhibition for the symptomatic relief of Parkinson's disease. *Brain, Behavior, and Immunity* **57**, 94-105 (2016).
190. Rock, E.M., Limebeer, C.L., Mechoulam, R., Piomelli, D. & Parker, L.A. The effect of cannabidiol and URB597 on conditioned gaping (a model of nausea) elicited by a lithium-paired context in the rat. *Psychopharmacology* **196**, 389-395 (2008).
191. Oláh, A., Ambrus, L., Nicolussi, S., Gertsch, J., Tubak, V., Kemény, L., Soeberdt, M., Abels, C. & Bíró, T. Inhibition of fatty acid amide hydrolase exerts cutaneous anti-inflammatory effects both in vitro and in vivo. *Experimental Dermatology* **25**, 328-330 (2016).
192. Schlosburg, J.E., Boger, D.L., Cravatt, B.F. & Lichtman, A.H. Endocannabinoid modulation of scratching response in an acute allergenic model: a new prospective neural therapeutic target for pruritus. *Journal of Pharmacology and Experimental Therapeutics* **329**, 314-323 (2009).
193. Nucci, C., Gasperi, V., Tartaglione, R., Cerulli, A., Terrinoni, A., Bari, M., De Simone, C., Agrò, A.F., Morrone, L.A., Corasaniti, M.T., Bagetta, G. & Maccarrone, M. Involvement of the endocannabinoid system in retinal damage after high intraocular pressure–induced ischemia in rats. *Investigative Ophthalmology & Visual Science* **48**, 2997-3004 (2007).
194. Bátkai, S., Pacher, P., Osei-Hyiaman, D., Radaeva, S., Liu, J., Harvey-White, J., Offertáler, L., Mackie, K., Rudd, M.A., Bukoski, R.D. & Kunos, G. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* **110**, 1996-2002 (2004).
195. Tchantchou, F., Tucker, L.B., Fu, A.H., Bluett, R.J., McCabe, J.T., Patel, S. & Zhang, Y. The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury. *Neuropharmacology* **85**, 427-439 (2014).
196. Hermes, D.J., Xu, C., Poklis, J.L., Niphakis, M.J., Cravatt, B.F., Mackie, K., Lichtman, A.H., Ignatowska-Jankowska, B.M. & Fitting, S. Neuroprotective effects of fatty acid amide hydrolase catabolic enzyme inhibition in a HIV-1 Tat model of neuroAIDS. *Neuropharmacology* **141**, 55-65 (2018).
197. Pryce, G., Cabranes, A., Fernández-Ruiz, J., Bisogno, T., Di Marzo, V., Long, J., Cravatt, B., Giovannoni, G. & Baker, D. Control of experimental spasticity by targeting the degradation of endocannabinoids using selective fatty acid amide hydrolase inhibitors. *Multiple Sclerosis Journal* **19**, 1896-1904 (2013).
198. Toczek, M. & Malinowska, B. Enhanced endocannabinoid tone as a potential target of pharmacotherapy. *Life Sciences* **204**, 20-45 (2018).
199. Di Marzo, V. New approaches and challenges to targeting the endocannabinoid system. *Nature Reviews Drug Discovery* **17**, 623 (2018).
200. Li, G.L., Winter, H., Arends, R., Jay, G.W., Le, V., Young, T. & Huggins, J.P. Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *British Journal of Clinical Pharmacology* **73**, 706-716 (2012).

201. Huggins, J.P., Smart, T.S., Langman, S., Taylor, L. & Young, T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *PAIN* **153**, 1837-1846 (2012).
202. D'Souza, D.C., Cortes-Briones, J., Creatura, G., Bluez, G., Thurnauer, H., Deaso, E., Bielen, K., Surti, T., Radhakrishnan, R., Gupta, A., Gupta, S., Cahill, J., Sherif, M.A., Makriyannis, A., Morgan, P.T., Ranganathan, M. & Skosnik, P.D. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *The Lancet Psychiatry* **6**, 35-45 (2019).
203. Kerbrat, A., Ferré, J.-C., Fillatre, P., Ronzière, T., Vannier, S., Carsin-Nicol, B., Lavoué, S., Vérin, M., Gauvrit, J.-Y., Le Tulzo, Y. & Edan, G. Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. *New England Journal of Medicine* **375**, 1717-1725 (2016).
204. van Esbroeck, A.C.M., Janssen, A.P.A., Cognetta, A.B., Ogasawara, D., Shpak, G., van der Kroeg, M., Kantae, V., Baggelaar, M.P., de Vrij, F.M.S., Deng, H., Allarà, M., Fezza, F., Lin, Z., van der Wel, T., Soethoudt, M., Mock, E.D., den Dulk, H., Baak, I.L., Florea, B.I., Hendriks, G., De Petrocellis, L., Overkleeft, H.S., Hankemeier, T., De Zeeuw, C.I., Di Marzo, V., Maccarrone, M., Cravatt, B.F., Kushner, S.A. & van der Stelt, M. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science* **356**, 1084-1087 (2017).
205. Bottemanne, P., Muccioli, G.G. & Alhouayek, M. *N*-Acylethanolamine hydrolyzing acid amidase inhibition: tools and potential therapeutic opportunities. *Drug Discovery Today* **23**, 1520-1529 (2018).
206. Sasso, O., Moreno-Sanz, G., Martucci, C., Realini, N., Dionisi, M., Mengatto, L., Duranti, A., Tarozzo, G., Tarzia, G., Mor, M., Bertorelli, R., Reggiani, A. & Piomelli, D. Antinociceptive effects of the *N*-acylethanolamine acid amidase inhibitor ARN077 in rodent pain models. *PAIN* **154**, 350-360 (2013).
207. Bonezzi, F.T., Sasso, O., Pontis, S., Realini, N., Romeo, E., Ponzano, S., Nuzzi, A., Fiasella, A., Bertozzi, F. & Piomelli, D. An important role for *N*-acylethanolamine acid amidase in the complete Freund's adjuvant rat model of arthritis. *Journal of Pharmacology and Experimental Therapeutics* **356**, 656-663 (2016).
208. Li, Y., Zhou, P., Chen, H., Chen, Q., Kuang, X., Lu, C., Ren, J. & Qiu, Y. Inflammation-restricted anti-inflammatory activities of a *N*-acylethanolamine acid amidase (NAAA) inhibitor F215. *Pharmacological Research* **132**, 7-14 (2018).
209. Solorzano, C., Zhu, C., Battista, N., Astarita, G., Lodola, A., Rivara, S., Mor, M., Russo, R., Maccarrone, M., Antonietti, F., Duranti, A., Tontini, A., Cuzzocrea, S., Tarzia, G. & Piomelli, D. Selective *N*-acylethanolamine-hydrolyzing acid amidase inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. *Proceedings of the National Academy of Sciences* **106**, 20966-20971 (2009).
210. Ribeiro, A., Pontis, S., Mengatto, L., Armirotti, A., Chiurchiù, V., Capurro, V., Fiasella, A., Nuzzi, A., Romeo, E., Moreno-Sanz, G., Maccarrone, M., Reggiani, A., Tarzia, G., Mor, M., Bertozzi, F., Bandiera, T. & Piomelli, D. A potent systemically active *N*-acylethanolamine acid amidase inhibitor that suppresses inflammation and human macrophage activation. *ACS Chemical Biology* **10**, 1838-1846 (2015).
211. Gorelik, A., Gebai, A., Illes, K., Piomelli, D. & Nagar, B. Molecular mechanism of activation of the immunoregulatory amidase NAAA. *Proceedings of the National Academy of Sciences* **115**, E10032-E10040 (2018).
212. Maccarrone, M. Metabolism of the endocannabinoid anandamide: open questions after 25 years. *Frontiers in Molecular Neuroscience* **10** (2017).
213. Piazza, P.V., Cota, D. & Marsicano, G. The CB_1 receptor as the cornerstone of exostasis. *Neuron* **93**, 1252-1274 (2017).
214. Després, J.-P., Golay, A. & Sjöström, L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *New England Journal of Medicine* **353**, 2121-2134 (2005).

215. Van Gaal, L.F., Rissanen, A.M., Scheen, A.J., Ziegler, O. & Rössner, S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *The Lancet* **365**, 1389-1397 (2005).
216. Pi-Sunyer, F.X., Aronne, L.J., Heshmati, H.M., Devin, J., Rosenstock, J. & RIO-North America Study Group, f.t. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patientsrio-north america: a randomized controlled trial. *JAMA* **295**, 761-775 (2006).
217. Christensen, R., Kristensen, P.K., Bartels, E.M., Bliddal, H. & Astrup, A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *The Lancet* **370**, 1706-1713 (2007).
218. Topol, E.J., Bousser, M.-G., Fox, K.A.A., Creager, M.A., Despres, J.-P., Easton, J.D., Hamm, C.W., Montalescot, G., Steg, P.G., Pearson, T.A., Cohen, E., Gaudin, C., Job, B., Murphy, J.H. & Bhatt, D.L. Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. *The Lancet* **376**, 517-523 (2010).
219. Shrestha, N., Cuffe, J.S.M., Hutchinson, D.S., Headrick, J.P., Perkins, A.V., McAinch, A.J. & Hryciw, D.H. Peripheral modulation of the endocannabinoid system in metabolic disease. *Drug Discovery Today* **23**, 592-604 (2018).
220. Tam, J., Hinden, L., Drori, A., Udi, S., Azar, S. & Baraghithy, S. The therapeutic potential of targeting the peripheral endocannabinoid/CB_1 receptor system. *European Journal of Internal Medicine* **49**, 23-29 (2018).
221. Kuipers, E.N., Kantae, V., Maarse, B.C.E., van den Berg, S.M., van Eenige, R., Nahon, K.J., Reifel-Miller, A., Coskun, T., de Winther, M.P.J., Lutgens, E., Kooijman, S., Harms, A.C., Hankemeier, T., van der Stelt, M., Rensen, P.C.N. & Boon, M.R. High fat diet increases circulating endocannabinoids accompanied by increased synthesis enzymes in adipose tissue. *Frontiers in Physiology* **9** (2019).
222. Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C.E., Pasquali, R., Lutz, B., Stalla, G.K. & Pagotto, U. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *The Journal of Clinical Investigation* **112**, 423-431 (2003).
223. Osei-Hyiaman, D., DePetrillo, M., Pacher, P., Liu, J., Radaeva, S., Bátkai, S., Harvey-White, J., Mackie, K., Offertáler, L., Wang, L. & Kunos, G. Endocannabinoid activation at hepatic CB_1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *The Journal of Clinical Investigation* **115**, 1298-1305 (2005).
224. DiPatrizio, N.V., Astarita, G., Schwartz, G., Li, X. & Piomelli, D. Endocannabinoid signal in the gut controls dietary fat intake. *Proceedings of the National Academy of Sciences* **108**, 12904-12908 (2011).
225. Jourdan, T., Godlewski, G., Cinar, R., Bertola, A., Szanda, G., Liu, J., Tam, J., Han, T., Mukhopadhyay, B., Skarulis, M.C., Ju, C., Aouadi, M., Czech, M.P. & Kunos, G. Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates beta cell loss in type 2 diabetes. *Nature Medicine* **19**, 1132 (2013).
226. Gatta-Cherifi, B., Matias, I., Vallée, M., Tabarin, A., Marsicano, G., Piazza, P.V. & Cota, D. Simultaneous postprandial deregulation of the orexigenic endocannabinoid anandamide and the anorexigenic peptide YY in obesity. *International Journal Of Obesity* **36**, 880 (2011).
227. Fanelli, F., Mezzullo, M., Repaci, A., Belluomo, I., Ibarra Gasparini, D., Di Dalmazi, G., Mastroroberto, M., Vicennati, V., Gambineri, A., Morselli-Labate, A.M., Pasquali, R. & Pagotto, U. Profiling plasma *N*-acylethanolamine levels and their ratios as a biomarker of obesity and dysmetabolism. *Molecular Metabolism* **14**, 82-94 (2018).
228. Fanelli, F., Mezzullo, M., Belluomo, I., Di Lallo, V.D., Baccini, M., Ibarra Gasparini, D., Casadio, E., Mastroroberto, M., Vicennati, V., Gambineri, A., Morselli-Labate, A.M., Pasquali, R. & Pagotto, U. Plasma 2-arachidonoylglycerol is a biomarker of age and menopause related insulin resistance and dyslipidemia in lean but not in obese men and women. *Molecular Metabolism* **6**, 406-415 (2017).

229. Kimberly, W.T., O'Sullivan, J.F., Nath, A.K., Keyes, M., Shi, X., Larson, M.G., Yang, Q., Long, M.T., Vasan, R., Peterson, R.T., Wang, T.J., Corey, K.E. & Gerszten, R.E. Metabolite profiling identifies anandamide as a biomarker of nonalcoholic steatohepatitis. *JCI Insight* **2** (2017).
230. Hillard, C.J. Circulating endocannabinoids: from whence do they come and where are they going? *Neuropsychopharmacology* **43**, 155 (2017).
231. Wangensteen, T., Akselsen, H., Holmen, J., Undlien, D. & Retterstøl, L. A common haplotype in NAPEPLD is associated with severe obesity in a Norwegian population-based cohort (the HUNT study). *Obesity* **19**, 612-617 (2011).
232. Powell, D.R., Gay, J.P., Wilganowski, N., Doree, D., Savelieva, K.V., Lanthorn, T.H., Read, R., Vogel, P., Hansen, G.M., Brommage, R., Ding, Z.-M., Desai, U. & Zambrowicz, B. Diacylglycerol lipase α knockout mice demonstrate metabolic and behavioral phenotypes similar to those of cannabinoid receptor 1 knockout mice. *Frontiers in Endocrinology* **6** (2015).
233. Touriño, C., Oveisi, F., Lockney, J., Piomelli, D. & Maldonado, R. FAAH deficiency promotes energy storage and enhances the motivation for food. *International Journal Of Obesity* **34**, 557 (2009).
234. Geurts, L., Everard, A., Van Hul, M., Essaghir, A., Duparc, T., Matamoros, S., Plovier, H., Castel, J., Denis, R.G.P., Bergiers, M., Druart, C., Alhouayek, M., Delzenne, N.M., Muccioli, G.G., Demoulin, J.-B., Luquet, S. & Cani, P.D. Adipose tissue NAPE-PLD controls fat mass development by altering the browning process and gut microbiota. *Nature Communications* **6**, 6495 (2015).
235. Everard, A., Plovier, H., Rastelli, M., Van Hul, M., de Wouters d'Oplinter, A., Geurts, L., Druart, C., Robine, S., Delzenne, N.M., Muccioli, G.G., de Vos, W.M., Luquet, S., Flamand, N., Di Marzo, V. & Cani, P.D. Intestinal epithelial *N*-acylphosphatidylethanolamine phospholipase D links dietary fat to metabolic adaptations in obesity and steatosis. *Nature Communications* **10**, 457 (2019).
236. Mukhopadhyay, B., Schuebel, K., Mukhopadhyay, P., Cinar, R., Godlewski, G., Xiong, K., Mackie, K., Lizak, M., Yuan, Q., Goldman, D. & Kunos, G. Cannabinoid receptor 1 promotes hepatocellular carcinoma initiation and progression through multiple mechanisms. *Hepatology* **61**, 1615-1626 (2015).
237. Suk, K.-T., Mederacke, I., Gwak, G.-Y., Cho, S.W., Adeyemi, A., Friedman, R. & Schwabe, R.F. Opposite roles of cannabinoid receptors 1 and 2 in hepatocarcinogenesis. *Gut* **65**, 1721-1732 (2016).
238. Masoodi, M., Lee, E., Eiden, M., Bahlo, A., Shi, Y., Ceddia, R.B., Baccei, C., Prasit, P. & Spaner, D.E. A role for oleoylethanolamide in chronic lymphocytic leukemia. *Leukemia* **28**, 1381 (2014).
239. Spaner, D.E., Lee, E., Shi, Y., Wen, F., Li, Y., Tung, S., McCaw, L., Wong, K., Gary-Gouy, H., Dalloul, A., Ceddia, R. & Gorzcynski, R. PPAR-alpha is a therapeutic target for chronic lymphocytic leukemia. *Leukemia* **27**, 1090 (2012).
240. Messmer, D., Lorrain, K., Stebbins, K., Bravo, Y., Stock, N., Cabrera, G., Correa, L., Chen, A., Jacintho, J., Chiorazzi, N., Yan, X.J., Spaner, D., Prasit, P. & Lorrain, D. A selective novel peroxisome proliferator-activated receptor (PPAR)-α antagonist induces apoptosis and inhibits proliferation of CLL cells *in vitro* and *in vivo*. *Molecular Medicine* **21**, 410-419 (2015).
241. Moezi, L., Gaskari, S.A. & Lee, S.S. Endocannabinoids and liver disease. V. Endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **295**, G649-G653 (2008).
242. Ying-Ying, Y. & Lin, H.-C. Endocannabinoids and non-endocannabinoid fatty acid amides in cirrhosis. *Liver International* **30**, 780-781 (2010).
243. Baldassarre, M., Giannone, F.A., Napoli, L., Tovoli, A., Ricci, C.S., Tufoni, M. & Caraceni, P. The endocannabinoid system in advanced liver cirrhosis: pathophysiological implication and future perspectives. *Liver International* **33**, 1298-1308 (2013).
244. Ros, J., Clària, J., To–Figueras, J., Planagumà, A., Cejudo–Martín, P., Fernández–Varo, G., Martín–Ruiz, R., Arroyo, V., Rivera, F., Rodés, J. & Jiménez, W. Endogenous cannabinoids: A new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* **122**, 85-93 (2002).

245. Bátkai, S., Járai, Z., Wagner, J.A., Goparaju, S.K., Varga, K., Liu, J., Wang, L., Mirshahi, F., Khanolkar, A.D., Makriyannis, A., Urbaschek, R., Garcia Jr, N., Sanyal, A.J. & Kunos, G. Endocannabinoids acting at vascular CB_1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nature Medicine* **7**, 827 (2001).
246. Caraceni, P., Viola, A., Piscitelli, F., Giannone, F., Berzigotti, A., Cescon, M., Domenicali, M., Petrosino, S., Giampalma, E., Riili, A., Grazi, G., Golfieri, R., Zoli, M., Bernardi, M. & Di Marzo, V. Circulating and hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. *Liver International* **30**, 816-825 (2010).
247. Domenicali, M., Ros, J., Fernández-Varo, G., Cejudo-Martín, P., Crespo, M., Morales-Ruiz, M., Briones, A.M., Campistol, J.-M., Arroyo, V., Vila, E., Rodés, J. & Jiménez, W. Increased anandamide induced relaxation in mesenteric arteries of cirrhotic rats: role of cannabinoid and vanilloid receptors. *Gut* **54**, 522-527 (2005).
248. Teixeira-Clerc, F., Julien, B., Grenard, P., Van Nhieu, J.T., Deveaux, V., Li, L., Serriere-Lanneau, V., Ledent, C., Mallat, A. & Lotersztajn, S. CB_1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nature Medicine* **12**, 671 (2006).
249. Giannone, F.A., Baldassarre, M., Domenicali, M., Zaccherini, G., Trevisani, F., Bernardi, M. & Caraceni, P. Reversal of liver fibrosis by the antagonism of endocannabinoid CB_1 receptor in a rat model of CCl_4-induced advanced cirrhosis. *Laboratory Investigation* **92**, 384 (2011).
250. Lumsden, A.B., Henderson, J.M. & Kutner, M.H. Endotoxin levels measured by a chromogenic assay in portal, hepatic and peripheral venous blood in patients with cirrhosis. *Hepatology* **8**, 232-236 (1988).
251. Lin, R.-S., Lee, F.-Y., Lee, S.-D., Tsai, Y.-T., Lin, H.C., Rei-Hwa, L., Wan-Ching, H., Cheng-Chun, H., Sun-Sang, W. & Kwang-Juei, L. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophaegeal varices, and hyperdynamic circulation. *Journal of Hepatology* **22**, 165-172 (1995).
252. Ju, C. & Tacke, F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. *Cellular And Molecular Immunology* **13**, 316 (2016).
253. Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., De Chiara, V., Battistini, L., Bernardi, G., Bernardini, S., Martino, G. & Maccarrone, M. The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* **130**, 2543-2553 (2007).
254. Pisani, V., Madeo, G., Tassone, A., Sciamanna, G., Maccarrone, M., Stanzione, P. & Pisani, A. Homeostatic changes of the endocannabinoid system in Parkinson's disease. *Movement Disorders* **26**, 216-222 (2011).
255. Gowran, A., Noonan, J. & Campbell, V.A. The multiplicity of action of cannabinoids: implications for treating neurodegeneration. *CNS Neuroscience & Therapeutics* **17**, 637-644 (2011).
256. Hansen, H.H., Azcoitia, I., Pons, S., Romero, J., García-Segura, L.M., Ramos, J.A., Hansen, H.S. & Fernández-Ruiz, J. Blockade of cannabinoid CB_1 receptor function protects against *in vivo* disseminating brain damage following NMDA-induced excitotoxicity. *Journal of Neurochemistry* **82**, 154-158 (2002).
257. Muthian, S., Rademacher, D.J., Roelke, C.T., Gross, G.J. & Hillard, C.J. Anandamide content is increased and CB_1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. *Neuroscience* **129**, 743-750 (2004).
258. Sun, Y., Alexander, S.P.H., Garle, M.J., Gibson, C.L., Hewitt, K., Murphy, S.P., Kendall, D.A. & Bennett, A.J. Cannabinoid activation of PPARα; a novel neuroprotective mechanism. *British Journal of Pharmacology* **152**, 734-743 (2007).
259. Minor, K.M., Letko, A., Becker, D., Drögemüller, M., Mandigers, P.J.J., Bellekom, S.R., Leegwater, P.A.J., Stassen, Q.E.M., Putschbach, K., Fischer, A., Flegel, T., Matiasek, K., Ekenstedt, K.J., Furrow, E., Patterson, E.E., Platt, S.R., Kelly, P.A., Cassidy, J.P., Shelton, G.D., Lucot, K., Bannasch, D.L., Martineau, H., Muir, C.F., Priestnall, S.L., Henke, D., Oevermann, A., Jagannathan, V., Mickelson, J.R. &

Drögemüller, C. Canine NAPEPLD-associated models of human myelin disorders. *Scientific Reports* **8**, 5818 (2018).

260. Morena, M. & Hill, M.N. Buzzkill: the consequences of depleting anandamide in the hippocampus. *Neuropsychopharmacology* (2019).
261. Zimmermann, T., Bartsch, J.C., Beer, A., Lomazzo, E., Guggenhuber, S., Lange, M.D., Bindila, L., Pape, H.-C. & Lutz, B. Impaired anandamide/palmitoylethanolamide signaling in hippocampal glutamatergic neurons alters synaptic plasticity, learning, and emotional responses. *Neuropsychopharmacology* (2018).
262. Morena, M., Aukema, R.J., Leitl, K.D., Rashid, A.J., Vecchiarelli, H.A., Josselyn, S.A. & Hill, M.N. Upregulation of anandamide hydrolysis in the basolateral complex of amygdala reduces fear memory expression and indices of stress and anxiety. *The Journal of Neuroscience* **39**, 1275-1292 (2019).