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Commentary

N-acylethanolamines, anandamide and food intake

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ABSTRACT

Anandamide and the other *N*-acylethanolamines, *e.g.* oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and linoleoylethanolamide (LEA), may be formed by several enzymatic pathways from their precursors, which are the *N*-acylated ethanolamine phospholipids. The exact enzymatic pathways involved in their biosynthesis in specific tissues are not clarified. It has been suggested that endogenous anandamide could stimulate food intake by activation of cannabinoid receptors in the brain and/or in the intestinal tissue. On the other hand, endogenous OEA and PEA have been suggested to inhibit food intake by acting on receptors in the intestine. At present, there is no clear role for endogenous anandamide in controlling food intake via cannabinoid receptors, neither centrally nor in the gastrointestinal tract.

However, OEA, PEA and perhaps also LEA may be involved in regulation of food intake by selective prolongation of feeding latency and post-meal interval. These *N*-acylethanolamines seem to be formed locally in the intestine, where they can activate PPARα located in close proximity to their site of synthesis. The rapid onset of OEA response and its reliance on an intact vagus nerve suggests that activation of PPARα does not result in formation of a transcription-dependent signal but must rely on an unidentified non-genomic signal that translates to activation of vagal afferents. Whether GPR119, TRPV1 and/or intestinal ceramide levels also contribute to the anorectic and weight-reducing effect of exogenous OEA is less clear.

Prolonged intake of dietary fat (45 energy%) may promote over-consumption of food by decreasing the endogenous levels of OEA, PEA and LEA in the intestine.

1. Introduction

N-acylethanolamines (NAE) are a group of fatty acid derivatives (Fig. 1) that can be found in small concentrations in nearly all living organisms including yeast, molluscs, insects, plants, fish and mammals [1–5]. The saturated *N*-acylethanolamines are also used by industry and may be found in cosmetics, detergent powders, detergent liquids, fabric softeners, corrosion inhibitors, textile auxiliaries and foodstuffs [6] and they may thus represent a potential source of contamination, when measuring tissue levels of these compounds by mass spectrometry. The same contamination phenomenon may also be true for oleamide that can be found in plastics used in the laboratory [7,8].

Besides anandamide (*N*-arachidonoylethanolamine or arachidonoylethanolamide) that can function as a partial agonist for cannabinoid receptors [9], anandamide can also activate several other receptors and ion channels [10]. Other *N*-acylethanolamines also have biological activity, *e.g.* inhibition of food intake [11,12]. This biological activity has been suggested to be mediated by activation of vanilloid receptor [13], GPR119 [14], PPARα [15–17] and PPARγ [16].

The present review will summarize our knowledge of the biological role of these lipid signalling molecules in the regulation of food intake, acting either centrally or in the intestine. Biological roles of 2-arachidonoyl glycerol are beyond the scope of this review.

2. Synthesis and degradation of *N*-acylethanolamines

At present there are three known principal enzymatic pathways, *pathways A–C*, (Fig. 2) for mammalian biosynthesis of NAEs where the unusual phospholipids, *N*-acylated ethanolamine phospholipids (NAPEs) serve as the precursor in all three pathways. A fourth pathway (Fig. 2, *pathway D*) has previously been suggested to occur, involving an anandamide synthase later

Abbreviations: Acyl-S-CoA, acylated coenzyme A (thioester); CB1, cannabinoid receptor-1; FAAH, fatty acid amide hydrolase; FAAH-2, fatty acid amide hydrolase-2; FATP1, fatty acid transport protein 1; GDE1, glycerophospho diester phosphodiesterase-1; GP-NAE, glycerophospho-*N*-acyl ethanolamine; HF, high fat; HS-CoA, coenzyme A (free form); LEA, linoleoylethanolamide; NAAA, NAE-hydrolysing acid amidase; NAE, *N*-acylethanolamine; NAPE, *N*-acylated ethanolamine phospholipid; NAPE-PLD, NAPE-hydrolysing phospholipase D; *N*-arachidonoyl-PE, *N*-arachidonoyl ethanolamine phospholipids; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPARα, peroxisome proliferators activated receptor α; PPARγ, peroxisome proliferators activated receptor γ; PPY, polypeptide Y; PTPN22, a non-receptor tyrosine phosphatase; SEA, stearoylethanolamide; TRPV1, transient receptor potential vanilloid receptor; WT, wild type.

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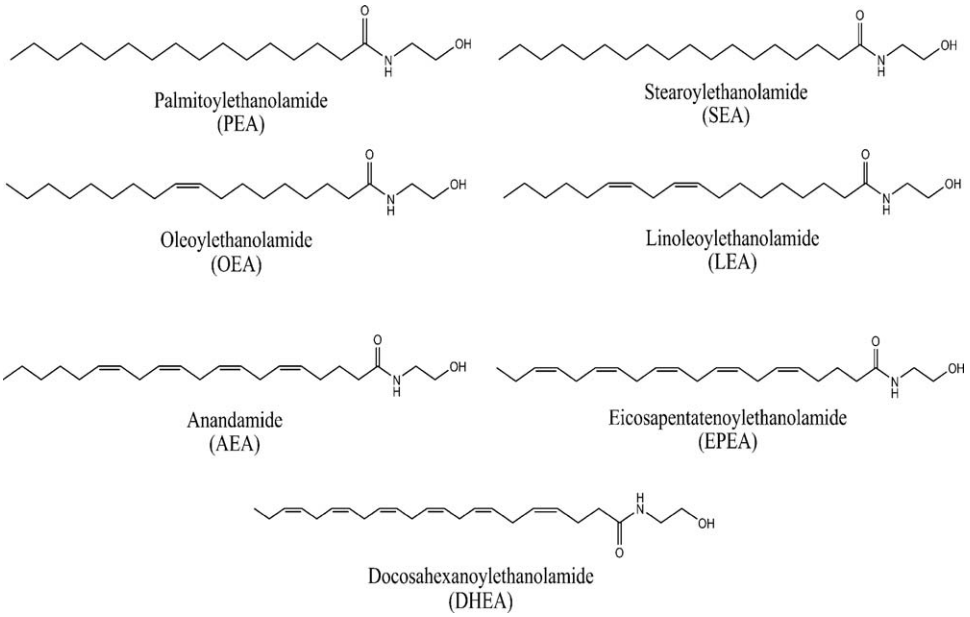

Fig. 1. Structure of *N*-acylethanolamine.

identified to be fatty acid amide hydrolase (FAAH), an enzyme that generally is assumed mainly to hydrolyse NAEs *in vivo* [18]. At high concentrations of ethanolamine and fatty acids, FAAH can instead generate NAE [19] but this seems to occur in *in vitro* settings only. A fifth pathway (Fig. 2, *pathway E*) represents non-enzymatic aminolysis of fatty acyl-S-CoA [20] that may accumulate post-mortem in some tissues, *e.g.* the brain [21]. This latter pathway may be responsible for an artificially increased level of specifically anandamide that can accumulate during prolonged tissue sampling [22], and therefore of no relevance for *in vivo* levels.

NAPE, is formed by transferring the *sn*-1 fatty acid from a donor phospholipid to phosphatidylethanolamine or plasmalogen, a reaction catalysed by a ubiquitous but poorly characterized calcium-activated *N*-acyltransferase [23]. It seems to have high activity especially in the brain of young rodents and it appears to decrease with age [24,25]. The subcellular localization of this enzyme is not clear but it has been found both in the intracellular and plasma membranes [23]. The enzyme is not considered to be fatty acid specific, *i.e.* it will transfer any acyl group found in the *sn*-1 position of the donor phospholipid [23,26,27]. A calcium-independent *N*-acyltransferase found mainly in the testis has recently been cloned and partially characterized [28].

Pathway A for NAE formation is catalysed by NAPE-hydrolysing phospholipase D (NAPE-PLD) that has been cloned [29]. No inhibitors of this enzyme are known but it can be activated by phosphatidylethanolamine *in vitro* [29,30]. This enzyme can generate all NAEs, including anandamide with apparently little or no selectivity for different acyl groups [31]. It has very limited activity *in vitro* towards lyso-NAPEs or glycerophospho-NAEs (GP-NAEs) as substrates [31] and it does not perform transphosphatidylation as do most other enzymes of the phospholipase D type [32]. NAPE-PLD is widely expressed in many tissues, including the intestine [33] and the brain [34]. Its expression in the heart seems to vary considerably between species [35] and it is not clear whether this also is true for the intestine and the brain. One immunocytochemical study of the brain seems to identify the location of NAPE-PLD to specific populations of neurons, *e.g.* in dentate gyrus, primarily the axonal part [36], whereas another study of the rat cerebellum found NAPE-PLD primarily located to the neuronal somata, *e.g.* in Purkinje cells [37,38]. A NAPE-PLD knockout mouse has been generated and surprisingly the levels of polyunsaturated NAEs including anandamide in the brain of these mice were not decreased [38]. This latter study suggests that NAPE-PLD *in vivo* may mainly be responsible for the formation of saturated NAEs and other pathways could be responsible for the formation of anandamide and other polyunsaturated NAEs. The monounsaturated NAEs such as oleoylethanolamide (OEA) may be formed by several pathways. However, as described below further studies are needed to clarify the biosynthesis of NAEs. In rat intestine, transient overexpression of NAPE-PLD resulted in increased levels of OEA and palmitoylethanolamide (PEA) [39]. Anandamide levels were also increased but this was not statistically significant [39] thereby supporting the suggestion that NAPE-PLD preferentially hydrolyses polyunsaturated *N*-acyl groups of NAPE *in vivo*.

Pathway B (Fig. 2) was discovered following the knockout of NAPE-PLD in mice [38,40,41]. A previously uncharacterized serine hydrolase, α/β-hydrolase-4 (Abh4) was identified as a NAPE-selective hydrolase that cleaved off the two fatty acids in the *sn*-1/*sn*-2-position of NAPE [40,41]. The GP-NAE thus generated could be hydrolysed by a glycerophosphodiesterase-1 (GDE1) [40]. The enzymes of *pathway B* are found in the brain and other tissues but no data for the small intestine is present [40,41]. There was no selectivity for generating NAEs with saturated, monounsaturated or polyunsaturated acyl groups. A large fraction of the ethanolamine phospholipids in both brain and intestine are ether-linked (alkyl and alkenyl) in the *sn*-1 position [42] and thus a large fraction of the NAPE molecules in both brain and intestine are ether-linked [33,43]. No data has been reported whether GDE1 can use lyso-NAPE as substrate but if that is not the case, it excludes GDE1 from taking part in hydrolysing a major pool of NAPE molecules in both brain and intestine. Interestingly, ether-linked NAPE is not very abundant in the luminal layer of rat jejunum as opposed to the serosal layer of the same tissue [33]. Whether this has any significance for NAE biosynthetic pathways in the two types of layers is not known.

The group of N. Ueda had previously reported that anandamide and PEA could be formed *in vitro* by the sequential reactions of a secreted phospholipase A_2, mainly found in gastrointestinal tissues and testis and a lysophospholipase D, which is widely distributed in many rat tissues including brain and small intestine [44]. It is not clear whether this lysophospholipase D is the same as GDE1 but

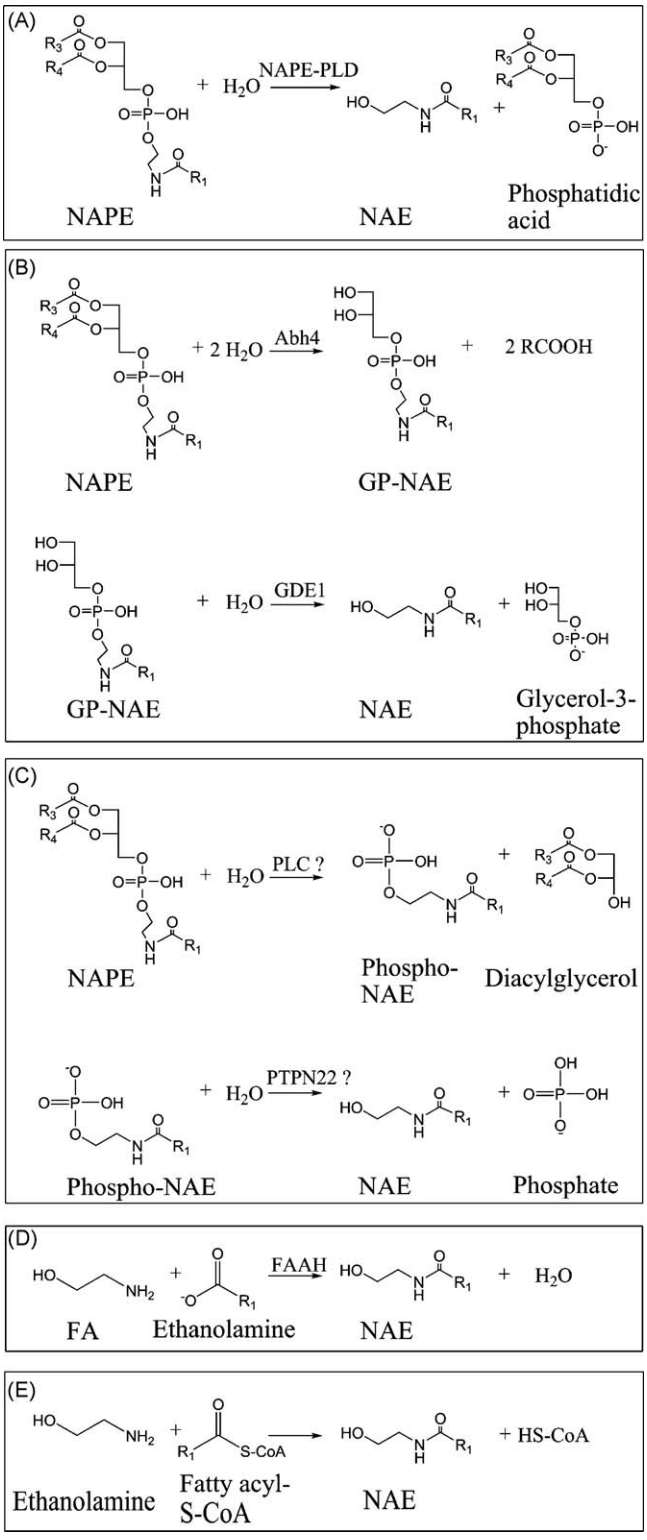

Fig. 2. Different pathways in the formation of NAEs.

the hydrolysis of lyso-NAPE by a lysophospholipase D does not exclude a major pool of the NAPE molecules as precursors for NAE biosynthesis.

Pathway C has been found in lipopolysaccharide-stimulated macrophages, where phospho-anandamide was detected, while NAPE-PLD at the same time was down regulated [45]. The anandamide level was increased when a non-receptor tyrosine phosphatase, PTPN22, was over expressed. Isolated PTPN22 converted phospho-anandamide to anandamide [45]. Phospho-anandamide has been detected in rodent brain [46] and these studies thereby suggest that a phospholipase C/phosphatase pathway exists for on-demand production of anandamide in the nervous system [45,46]. The NAPE-hydrolysing phospholipase C has not been identified and it is not known whether PTPN22 performs hydrolysis of phospho-anandamide *in vivo*. Anandamide can be phosphorylated by diacylglycerol kinase *in vitro* [47] but it is not known whether this contributes to endogenous formation of phospho-anandamide. It is furthermore unclear whether this pathway is specific for anandamide formation, or whether other NAEs can be formed in the same way. The physiological and pathophysiological significance of this pathway is at present unclear.

Pathway D has been found to proceed *in vitro* during conditions of high concentrations of ethanolamine and a fatty acid, *e.g.* arachidonic acid [19]. During tissue sampling, local ischemia and tissue injury are acutely produced and this may generate free fatty acid by the activation of phospholipase A_2. However, the level of free ethanolamine is usually not high enough to facilitate NAE formation. Thus, this pathway seems to be of no significant importance for *in vivo* levels of NAEs.

On the other hand, *pathway E* may possibly contribute to post-mortem levels of NAEs, especially anandamide and of other acyl amides in the brain. During brain ischemia, phospholipase A_2 is specifically activated, which thus liberates fatty acid (arachidonic acid) from the phospholipids. These fatty acids can be converted to fatty acyl-S-CoAs [21] that are highly reactive molecules, which may react with amino groups (aminolysis) [20]. It is also known that NAPE [48–50] and NAE accumulates with neuronal death [48,51,52] and some of the NAE can be hydrolysed by FAAH, thereby resulting in increased levels of ethanolamine [53], which can react with the increased levels of arachidonoyl-S-CoA. Thus, a selective increase in anandamide may in some cases occur during sampling of brain tissues that is dependent on the time and method of sampling.

NAEs can be hydrolysed by three enzymes: FAAH [54], FAAH-2 [55] and NAE-hydrolysing acid amidase (NAAA) [56]. FAAH is located in the endoplasmic reticulum in many cell types and it can hydrolyse all NAEs with great efficiency [18]. FAAH knockout mice have been generated with the phenotype of increased tissue levels of all NAEs [53,57]. Several inhibitors are available for this enzyme [18,58]. The FAAH-2 enzyme has only 20% sequence identity with FAAH and it hydrolyses anandamide and OEA with less efficiency than FAAH [55]. FAAH-2 is also inhibited by well-known FAAH inhibitors such as URB597 and OL-135. The *FAAH-2* gene is found in multiple primate genomes including human but it is not found in rodent genomes [55]. NAAA appears to have a lysosomal localization [59] predominantly in peripheral tissues, *e.g.* in macrophages [56,60]. NAAA seems to prefer saturated NAEs as substrates [56] and some inhibitors have been developed for this enzyme [61]. The significance of FAAH-2 and NAAA for catabolism of NAEs is not clear. In the rodent intestine, NAEs appear to be hydrolysed to a high degree by an enzyme other than FAAH [62], the identity of which needs to be clarified.

The unsaturated NAEs, *e.g.* anandamide, are *in vitro* substrates for lipoxygenases, cyclooxygenases and CYP 450 enzymes giving rise to a large number of oxygenated products of which several have biological activity [63,64] and some of these compounds have been shown to be generated *in vivo* in anandamide-injected animals [65]. The biological significance of these pathways of NAE metabolism is at present not clear.

3. Tissue levels of NAEs

The total levels of NAE in tissues are usually in the order of a few nmol/g of tissue with PEA stearoylethanolamide (SEA) and OEA as

the major species, *e.g.* in the brain [38,52,66]. In tissues other than the brain, linoleoylethanolamide (LEA) is also a major NAE species and in the intestine, it is actually the NAE having the highest concentration [17,33]. This near lack of LEA in the brain is caused by the very low percentage of linoleic acid in brain lipids as opposed to other tissues, where it is much more abundant [17].

Anandamide usually accounts for less than 5% of the total NAE level, being in the order of pmol/g of tissue [67,68], and most reports on tissue levels have measured only anandamide. This low level of anandamide is in agreement with a corresponding low level of *N*-arachidonoyl ethanolamine phospholipids (*N*-arachidonoyl-PE) that again is caused by the low prevalence of arachidonic acid in the *sn*-1 position of phospholipids [69,70]. Anandamide can accumulate post-mortem [22] and the time and method of sampling may thus influence the levels measured. Usually the formation and turnover of NAE is relatively slow, *i.e.* minutes, as compared to many other lipid-derived messenger molecules, *e.g.* signalling diacylglycerol and 2-arachidonoylglycerol that may be generated within seconds. There are reports of changes in one species of NAE while others were unchanged or changed in opposite direction [11,71–73]. Some of these differential changes may be explained by changes in the *N*-acyl composition of NAPE that is mirrored in the NAEs formed [72] while others may be caused by the involvement of the different biosynthetic and/or catabolic pathways. This indicates that some NAEs may be formed more or less specifically and that formation of one NAE is not always accompanied by an equivalent formation of another NAE.

4. Regulation of food intake by *N*-acylethanolamines

Several centers in the brain play a pivotal role in processes that regulate food intake and body weight. These centers receive information from the periphery either via humoral factors, sensory nerves and/or neuroendocrine signals and are capable of initiating an adequate behavioural and/or neurocrine response to counter regulate challenges to energy homeostasis [74]. NAEs are locally acting signalling molecules that can activate a number of different receptors, including cannabinoid receptor-1 (anandamide), vanilloid receptor/TRPV1 (anandamide and other NAEs), GPR119 (OEA and PEA), PPARα (anandamide and other NAEs) that may be involved in modulating food intake either by acting in the brain or in signalling from the gastrointestinal system. As opposed to many other types of anorexiants OEA acts by prolonging the latency between meals [15,75]. Anandamide is suggested to reduce gastric and intestinal mobility through activation of cannabinoid receptors in the tissues, an effect that may promote decreased food intake [76].

4.1. Via cannabinoid receptor-1 (CB1)

It is well known that activation of CB1 in the brain by exogenous anandamide [9] can stimulate food intake in rodents and a CB1 antagonist can decrease food intake in rodents [71,77,78] and in humans [79]. However, increasing the endogenous level of anandamide in the brain by a FAAH inhibitor or by knockout of FAAH in mice has not been reported to result in increased food intake, whereas it results in other CB1- and/or CB2-dependent behavioural effects, including reduction in pain sensation, inflammation, anxiety and depression [18]. Activators of CB1 are known to promote multiple behavioural effects in rodents, including analgesia, hypothermia and catalepsy (collectively called the tetraed test [80]). FAAH inhibitors are largely inactive in the tetraed test as they only cause analgesia [81], whereas inhibitors of 2-arachidonoyl glycerol degradation produce behavioural effects, including analgesia, hypothermia and hypomotility, that are classically associated with the pharmacology of cannabinoids [82]. Furthermore, increasing the anandamide level by several fold in the brain of mice by dietary arachidonic acid supplements did not increase food intake [83]. Thus, it is at present less likely that endogenous anandamide has a regulatory function on food intake mediated by central CB1.

Activation of CB1 on sensory vagal neurons as opposed to CB1 in the central nervous system has also been suggested to mediate some reduction in food intake [73,84]. LH-21 is a CB1 antagonist that is claimed to poorly pass the blood–brain barrier [85]. LH-21 is able to reduce food intake in obese Zucker rats suggesting a peripheral mechanism of action. However, CB1 null mice treated with LH-21 do not eat as much as wild type (WT), which indicates that the target of LH-21 is not only the CB1 receptor [86]. Furthermore, a recent study involving subdiaphragmatic vagotomy, subdiaphragmatic vagal deafferentation alone or in combination with complete celiac-superior mesenteric ganglionectomy of rats has shown that these peripheral neurons are not involved in regulating the CB1 antagonist reduction in food intake [87]. Thus, it is not likely that that endogenous anandamide may regulate food intake by CB1 on peripheral neurons.

4.2. Via vanilloid receptor (TRPV1)

TRPV1 is a ligand-gated non-selective ion channel that can be activated by heat, low pH and by capsaicin, the active ingredient in hot chilli pepper. All NAEs also appear to be able to activate TRPV1 *in vitro* [88,89], and this activation has been suggested to be involved in the anorexic effect of OEA [90]. TRPV1-null mice are protected against diet-induced obesity. No effect was observed in the cumulative energy intake of WT compared to TRPV1-null mice and the intestinal absorption of fat was also identical in the two groups of animals [91]. Therefore, the potential weight-reducing effect of vanilloid compounds is not due to reduction in the food intake or malabsorption. However, TRPV1-null mice exhibited a significantly greater thermogenic capacity apparently without upregulating uncoupling proteins. TRPV1-null mice injected *i.p.* with OEA (10 mg/kg) had reduced food intake up to 12 h after OEA administration as had WT mice, indicating that TRPV is not mediating the anorectic effect of OEA [92].

4.3. Via activation of GPR119

GPR119 has been characterized as an orphan receptor belonging to the G-protein-coupled receptors [93], which is coupled to adenylate cyclase via $G_{\alpha s}$ [14,94,95]. There is some discrepancy between the reported tissue distributions of GPR119. In humans, the primary site for GPR119 expression is in the gastrointestinal system with the greatest expression in the pancreas [94,96,97]. Furthermore, GPR119 positive cells have been found in the small intestine, colon [97] and in the stomach [96,97].

In rodents, some studies [94,98] have reported that the only site for GPR119 expression was in the pancreatic islet, more precisely in the polypeptide-secreting PP cells [98] and in the insulin producing β-cells [95]. However, other studies have shown that GPR119 also is expressed in the lung, duodenum [96], colon [96,97], ileum [97] and CNS [96]. Bonini reports that the highest level of GPR119 is observed in the CNS, especially in the hippocampal formation [96].

The function of GPR119 is debated but due to its presence in the digestive system, GPR119 could have a role in energy homeostasis/metabolism. The reported localization of GPR119 to the polypeptide Y-(PPY)-secreting cells of the pancreas [98] suggests that GPR119 plays a role in the release of the pancreatic hormone PPY. Double-immunofluorescence studies show that GPR119 is localized to the peripheral regions of the cells, whereas PPY is detected in the cytoplasmic parts of the cells. PPY is secreted in response to

food intake and has a wide range of actions, all associated with energy homeostasis. It inhibits pancreatic exocrine and endocrine secretion, regulates gall bladder motility and increases satiety and energy expenditure, all of which leads to body weight loss [99]. GPR119 and PPY mRNA expression in leptin-deficient *db*/*db* mice islets are markedly increased, indicating that they might be involved in the same pathways. It could therefore be speculated that the hypophagic effect of GPR119 agonists could partly be mediated by PPY formation.

Activation of GPR119 promotes glucose-dependent insulin secretion [94], a mechanism which could resemble the incretin hormone's action in the pancreas or act via increased release of incretin hormones [95,97]. Colocalization between GLP-1 positive cells and GPR119 [97], and observations that the GPR119 agonist AR231453 [95,100] administered orally but not intravenously increase glucose-dependent GLP-1 levels support the hypothesis that activation of GPR119 stimulate incretin release. Furthermore, GLP-1 antagonist exendin (9-39) [101] significantly reduced the efficacy of AR231453 [97], which further substantiate the role of GPR119 in incretin release. The first ligand to be identified for the GPR119 was lysophosphatidylcholine, with 1-oleoyl-lysophosphatidylcholine being the most potent species [94]. In 2006, Overton et al. [14] found that OEA could activate GPR119 in cells with higher affinity than 1-oleoyl-lysophosphatidylcholine. Dosing rats with 30 mg/kg OEA *i.p.* had an acute effect on reducing food intake (reduced food intake at 1 h and 2 h). The effect was similar to the one induced by an agonist of GPR119, PSN632408, which reduces: food intake, body weight gain and mesenteric fat deposition [14]. No changes in the locomotion/activity level in rats treated with GPR119 agonist were observed, although OEA has been reported both to reduce food intake and to reduce locomotor activity [11,102]. The TRPV1 agonist capsaicin has been reported to reduce locomotor activity. This could indicate that the OEA-mediated anorectic effect could be due to a dual effect: via TRPV1 [102] and via GPR119 [14]. The weight-reducing effect of GPR119 activation was not due to visceral malaise, or the production of aversive signals, which was tested by a conditioned taste aversion and a kaolin study [14]. Another synthetic activator of GPR119, AR231453, does not reduce food intake, thereby raising the question of whether GPR119 is involved in the anorectic effect of OEA [97]. Further studies must clarify this discrepancy.

4.4. Via activation of the transcription factor PPARα

OEA is a high affinity agonist of PPARα [15]. OEA injected into rats produces satiety and reduces body weight gain. However, this effect is not observed in PPARα-null mice [103]. OEA activates PPARα with an EC_{50} of 120 nM [15]. However, EC_{50} values ranging from nM to μM have been reported [15,17,104]. This discrepancy could be attributed to the use of PPARα clones obtained from different animal species [105]. Furthermore, to verify that only PPARα is involved in this weight-reducing effect, a PPAR β/δ agonist (GW501516) and a PPARγ agonist (ciglitazone) were found to be ineffective in reducing food intake in rats [15,103].

WT and PPARα-null mice were fed a high fat (HF) diet (60 energy% from fat) and treated for 4 weeks with OEA *i.p.* daily. This treatment reduced total food intake and suppressed body weight gain in WT mice, with no effect on null mice [15,103]. PPARα-null mice on HF diet are hyperphagic and have significantly increased body weight compared to WT on HF diet [103].

A meal pattern analysis comparing D-fenfluramine (serotonergic anorexiant) and cholecystokinin with different PPARα agonists showed different patterns. Whereas D-fenfluramine delayed feeding onset [75] and cholecystokinin reduced meal size [75], the PPARα agonists prolonged eating latency rather than changing meal size or post-meal interval [15]. OEA also prolonged eating latency [75].

Furthermore, OEA in the jejunum, duodenum and liver, but not in the ileum, increases the expression of three PPARα regulated genes: PPARα, FAT/CD36 and FATP1 (fatty acid transport protein 1) [15]. This regulation was not evident in the null mice [15]. The molecular mechanism for the PPARα–OEA regulated satiety could possibly involve intestinal nitric oxide production [15]. Nitric oxide can stimulate appetite [106] and OEA has been shown to inhibit the production of nitric oxide, by inhibiting iNOS function in epithelial cells. To sum up, there is clear evidence that OEA activates PPARα in the jejunum thereby modulating the feeding behaviour.

There is an acute reduction in food intake when OEA is administered, accounting for the primary weight-reducing effect of OEA. However, rats subjected to subchronic *i.p.* administration of OEA did not eat less on a daily basis but they gained significantly less weight compared to control animals [103,107]. It could therefore be speculated that exogenous OEA could activate several targets in the body; *e.g.* in the acute phase, OEA could activate GPR119 and GLP-1 secretion together with increasing glucose-dependent insulin secretion from the pancreas [94,98], whereas in the chronic phase, OEA could activate PPARα, favoring increased lipolysis in adipose tissue [103,108].

The exact mechanism by which OEA reduced food intake and decreased weight is still not clear. What is known is that PPARα-null mice are not responsive to OEA injection [103] and chemical destruction of the vagus nerve also abolishes the effect of OEA [11]. This indicates the involvement of a signal produced in the gut, which activates PPARα and downstream activates the peripheral nerves signalling to the brain. The effects of OEA are already evident 1 h after *i.p.* injection [11]. Therefore, this signal transduction must be rapid, which may be in conflict with PPARα being a transcription factor that functions more slowly. However, recent data indicate that PPARα can mediate rapid non-genomic effects [109] after OEA stimulation. These effects were observed in the brain but it could be speculated that the same mechanism of non-genomic effects of PPARα was present in the gut, leading to activation of peripheral nerve endings projecting to the brain, thereby inducing satiety.

4.5. Via inhibition of ceramidase

OEA is a well-known and widely used inhibitor of ceramidase and several studies have shown that it can increase ceramide levels in cell cultures [110–112]. Whether the anorectic effect of OEA also involves increased levels of ceramides is at present not known but this possibility must be taken into consideration.

5. Regulation of tissue levels of *N*-acylethanolamines by food intake

Fasting and feeding affect the levels of anandamide and OEA in the intestine, *i.e.* OEA levels decreased by fasting and increased by eating, which is in agreement with OEA having a putative role in controlling meal frequency. Levels of anandamide change in the opposite directions but the biological significance of this observation is not clear.

The endocannabinoids, anandamide and 2-arachidonoylglycerol, as well as the eicosanoids are derivatives of arachidonic acid, which is an essential fatty acid that belongs to the n-6 fatty acid family [113]. This means that extreme deficiency or extremely high dietary intake of arachidonic acid in the final analysis will affect the tissue levels of arachidonic acid esterified in phospholipids that serve as precursors for anandamide and other polyunsaturated NAEs.

Only a few studies have investigated the influence of dietary fat (type and amount) on endogenous levels of NAEs. A high fat diet (providing 60 energy%) for 14 weeks increased the level of

anandamide in mouse liver [114] and the authors suggest that this increased level could contribute to diet-induced obesity via activation of hepatic CB1. Sixty energy% of fat is far above the average intake of fat in the Western world (30–45 energy%). Another study has reported that feeding suckling piglets with a milk formula deficient in arachidonic acid resulted in a decreased level of anandamide and other polyunsaturated NAEs in the brain compared to piglets fed a milk formula containing small amounts of arachidonic acid and docosahexaenoic acid and to piglets fed sows milk that naturally contain these fatty acids [83]. In the same study, mice were supplemented for 58 days with 1 energy% arachidonic acid, corresponding to approximately 10-fold more than an average human intake [113]. These mice had had a 5.8-fold increased level of anandamide in the brain and an unchanged food intake. We have found that feeding adult rats with a pharmacological dose of arachidonic acid (4.9 energy%) for one week did increase levels of anandamide in liver and jejunum but not in the brain [17]. Likewise large doses of fish oil containing long-chain n-3 fatty acids can, when fed over longer time, decrease levels of arachidonic acid and anandamide [17,68]. However, it was unexpected that levels of OEA, LEA and anandamide in the brain were affected by dietary fats without corresponding changes in overall fatty acid composition of the brain. These changes were caused by the type of fat (rich in saturated, polyunsaturated or monounsaturated fatty acids) given to the animals. This raises the question whether different types of dietary fat in the human diet may have neurological effects mediated by changes in brain levels of NAEs. Recently, it was found that a ketogenic/caloric restricted diet that is known to attenuate seizure activity in epilepsy [115] also decreased the levels of OEA in the brains of mice but it is unclear whether this is associated with the decreased seizure activity [116]. Considering other dietary components, it is known that alcohol consumption can influence tissue levels of NAEs in different directions dependent on tissue, dose and time [117,118]. It is beyond the scope of this review to further discuss this dietary component.

In the rat intestine, all fat-enriched diets (45 energy%), as compared to the fat-poor rat chow (11 energy%), decreased levels of PEA, OEA and LEA in jejunum irrespective of the fatty acid composition when diets were fed for 7 days [17]. These three NAEs may all more or less function as anorectic lipids [119], which suggests that a high dietary fat may promote overeating by decreasing levels of OEA, PEA and LEA in the intestine. It is well known that high fat diets are used in research to develop diet-induced obesity. These decreased NAE levels were seen after one week feeding but more recent results indicate that this may occur already one day after introduction of this fat-enriched diet [120].

Schwartz et al. [121] have recently reported that short-term infusion of fatty acids into the rat intestine can increase intestinal levels of OEA and thereby mediate the well-known satiety effect (lower meal frequency) of intestinal fatty acids [122]. According to this model prolonged exposure that is common in human dietary habit to HF diets may induce a feedback mechanism that eventually decreases levels of OEA as we have observed in our one-week feeding study. Further studies are required to clarify this point.

6. Conclusion

At present, there is no clear role for endogenous anandamide in control of food intake via CB1, $^1/_2$1 neither centrally nor in the gastrointestinal tract. On the other hand, much evidence points to a role of OEA, PEA and perhaps also LEA in regulation of food intake. These NAEs seem to be formed locally in the intestine where they can activate PPARα located in close proximity to their site of formation. The rapid onset of the OEA response (decreased food intake) and its reliance on an intact vagus nerve suggest that activation of PPARα does not result in formation of a transcription-dependent signal but must rely on an unidentified non-genomic signal that translates to activation of vagal afferents. Whether GPR119, TRPV1 and/or intestinal ceramide levels also contribute to the anorectic and weight-reducing effect of exogenous OEA is less clear but the evidence is not strong.

High dietary fat may promote overeating by decreasing endogenous levels of OEA, PEA and LEA.

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