Oleoylthanolamide: a new player in energy metabolism control. Role in food intake

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Oleoylthanolamide (OEA) is a lipid amide produced by enterocytes upon the absorption of dietary fat and participates in the induction of satiety. Through indirect pathways, probably depending on the local activation of peroxisome-proliferator-activated receptor-alpha and involving afferent vagus nerve fibers, OEA signal is transmitted to the brain-stem and the hypothalamus, where it stimulates the release of oxytocin from magnocellular neurons.

OEA mechanism might, thus, provide a novel target for the design of therapies controlling appetite.

Introduction
The spread of ‘obesity epidemic’ and the poor efficacy of many anti-obesity therapies highlight the need to identify novel mechanisms controlling feeding and energy balance. One such mechanism involves oleoylthanolamide (OEA), the monounsaturated analogue of the endocannabinoid anandamide. OEA is one of the N-acylethanolamides (NAEs) present in all living organisms that are synthesized from the precursors N-acylphosphatidylethanolamines (NAPEs), through the actions of the enzymes N-acyltransferase and NAPE-phospholipase D (NAPE-PLD), and hydrolyzed intracellularly by fatty acid amidase hydrolase enzymes \cite{1}. OEA has no affinity for cannabinoid receptors nor it induces cannabinomimetic effects and its biological role remained elusive for long. During the last decade it has clearly emerged that OEA is a gut-derived satiety factor controlling appetite and energy balance. The present review will summarize our knowledge on the role of OEA in the regulation of feeding, by focusing our attention on the gastrointestinal and the central levels.

OEA is characterized by satiety-inducing properties
OEA shows all of the defining characteristics of a ‘satiety factor’: (1) it inhibits feeding by prolonging the interval to the next meal; (2) its synthesis is regulated by nutrient availability and (3) its levels undergo circadian fluctuations.

Pharmacological studies have shown that OEA causes a dose-dependent and time-dependent decrease in food intake in starved and free-feeding rats and mice \cite{2–5}. These effects are structurally and behaviorally selective. They are not followed by compensatory hyperphagia, are not caused by the induction of visceral malaise, anxiety or stress-response, nor they are paralleled by alterations of the body temperature, pain threshold, plasma levels of glucose, insulin and leptin \cite{2}. Behavioral analyses of the feeding pattern of free-feeding rats and mice treated with OEA at the onset of the dark phase revealed that the hypophagic effect is due to the selective
OEA was also found to produce antinociceptive effects in animal models of visceral and inflammatory pain [16]. Like all TRPV1 agonists, particularly the lipophilic ones, OEA can also immediately desensitize this receptor [17], thus possibly explaining these discrepant observations. The involvement of TRPV1 receptor in OEA hypophagic action is suggested by the lack of effects in TRPV1 null mice [15], although opposite results were also reported [12].

Based on in vitro evidence, OEA was shown to activate the orphan receptor GPR119, with higher affinity (EC50 3 μM) than 1-oleoyl-lysophosphatidylcholine, previously known as its most potent endogenous agonist [18]. Recent data from C3H mice showed a positive correlation between GPR119 upregulation in the intestine and decrease of body fat pads induced by chronic oral administration of OEA [19]. However, not all GPR119 synthetic agonists mimic OEA effects on feeding. Whether this receptor is essential for the anorexiant action of OEA remains to be demonstrated (for review see [1]).

A large body of evidence indicate that OEA is able to engage peroxisome-proliferator-activated receptor-alpha (PPAR-alpha), a lipid-activated nuclear receptor that regulates several aspects of lipid metabolism and that appear to mediate the satiety-inducing effects of OEA. (1) OEA binds with high affinity and activates with high potency (EC50 120 nM) PPAR-alpha-driven transactivation in a heterologous expression system [11]. (2) The concentrations reached by endogenous OEA in the rodent small-intestinal mucosa after feeding (200–400 nM) [9] are sufficient to fully activate PPAR-alpha receptor, which is highly expressed in the small intestine [11] but not to affect the other putative OEA receptors, such as GPR119 and TRPV1. (3) OEA treatment in vivo affects the expression of different PPAR-alpha target genes, such as those encoding for PPAR-alpha itself, fatty acid translocase (FAT/CD36), fatty acid transport protein 1 (FATP1), inducible nitric oxide synthase (iNOS) [11]. Similar observations can be made in the small intestine of mice over-expressing NAPE-PLD in their duodenum [7]. (4) Intestinal PPAR-alpha expression parallels the circadian fluctuations of OEA levels, whereas the expression of its trans-repression target iNOS shows opposite pattern [11], thus suggesting that food-induced OEA production in the proximal small intestine may regulate satiety through a local autocrine or paracrine mechanism. (5) Synthetic PPAR-alpha agonists, such as the compounds GW7647 and Wy-14643, closely mimic OEA effects on both the intestinal expression of PPAR-alpha-regulated genes and on the eating pattern of free-feeding mice [11]. (6) PPAR-alpha knock out (PPAR-alpha−/−) mice do not respond to OEA or synthetic PPAR-alpha agonists [11,20], are more vulnerable to diet-induced obesity, and display an altered feeding pattern with respect to wild type mice [21]. In particular, free-feeding PPAR-alpha−/− mice on a standard lab chow were found to start earlier their nocturnal consummatory activity and with a higher meal frequency, while eating comparable amount of food at each meal, with respect to wt mice [22].

OEA receptors
OEA has been hypothesized to activate different receptors. OEA showed low efficacy for the vanillloid receptor TRPV1 (in vitro EC50 2 μM) and was able to excite vagal sensory neurons and induce visceral pain via activation of this receptor [14,15]. However, discrepant findings are reported, because OEA was also found to produce antinociceptive effects in
Notably, these behavioral differences were evident mostly during the first three hours of the dark phase and resulted symmetrically opposite to those elicited by NAPE-PLD overexpression in the small intestine [7]. This observation suggests that food-stimulated OEA signaling at intestinal PPAR-alpha receptors triggers a physiological satiety mechanism that controls meal timing and that primarily operates during the first hours of nocturnal feeding, when rodents eat the first and largest of their daily meals [21].

**Role of OEA at the gastrointestinal level**
The satiety-inducing properties of OEA may rely on its actions at various levels of the gastrointestinal tract. Pharmacological studies in rodents have shown that gastric emptying and small-intestinal transit are potently inhibited by OEA, with a temporal pattern that overlaps with the effects of OEA on feeding behavior (for review see: [22,23]). The mechanism responsible for such effect remains unknown, because it is not mediated by the activation of PPAR-alpha receptor, cannabinoid receptors CB1 and CB2, nor it is blocked by the antagonism of glucagon-like peptide-1 (GLP-1) [24]. It is worth nothing that many satiety signals, such as cholecystokinin (CCK), inhibit gastric emptying and this effect probably contributes to limit food ingestion by enhancing gastric mechanoreceptor stimulation [25].

Experimental evidence suggests that OEA stimulates fatty acid absorption in the gut, by enhancing the expression of FAT/CD36 and FATP1 [7,11,26], which mediate lipid transit. It is well known that intraduodenal infusion of dietary fat in rodents lowers meal frequency [27] reducing food intake. Therefore, the enhanced lipid absorption induced by OEA might account, at least in part, for its pro-satiety effects. By contrast, OEA mobilization in the proximal small intestine resulted strongly stimulated by duodenal infusion of fat, but not of protein or carbohydrates infusions [21]. In particular, OEA production in the intestine appeared to utilize dietary oleic acid as a substrate and it was disrupted in mutant mice lacking CD36 [21]. These observations suggest that small-intestinal OEA mobilization might serve as a molecular sensor linking fat ingestion to satiety. In accordance with this hypothesis, targeted disruption of CD36 or PPAR-alpha abrogated the satiety response induced by fat [21].

Different results on the modulation of OEA intestinal levels by dietary fat have been reported in chronic studies [25,28,29]. In particular, when animals were fed chronically with a high-fat diet, according to the protocol of diet-induced-obesity, the intestinal levels of several NAES, including OEA, decreased regardless of the diet energy density (for review see [29]). Conversely, an increase of OEA concentration was observed in the stomach of diet-induced obese mice and was paralleled by a delayed gastric emptying, with respect to lean control mice [25]. Enhanced levels of OEA were also observed in the duodenum of *ad libitum* fed Zucker rats, which are considered as model of dyslipidemia and other metabolic disturbances occurring during obesity [30]. Although further studies are needed to explain these observations, we can speculate that OEA, like most endogenous factors involved in food intake and metabolic control, can be deregulated following excessive and/or prolonged consumption of high-fat diets. Therefore, the changes in OEA levels 24 hours after a high-fat diet or following several weeks of high-fat diet cannot be expected to be similar to those induced acutely by a high-fat meal.

The molecular steps following OEA activation of PPAR-alpha are still incompletely understood. PPAR-alpha might act by influencing the local expression of satiety-inducing proteins or might repress pro-orexiant mechanisms.

For example, OEA was shown to stimulate the release of GLP-1 from intestinal L-cells [31], and of apolipoprotein A-IV in human hepatoma cells (for references, see [21]). Moreover, OEA was found to reduce plasma levels of ghrelin both in started and fed rats, without affecting plasma GLP-1 [32]. However, in other studies OEA did not cause any significant change in rat plasma levels of ghrelin, PYY, GLP-1, and apolipoprotein A-IV, nor the antagonism of CCK-1 caused any effects on OEA-induced anorexia [5].

**Role of OEA in the central nervous system**
Both OEA and PPAR-alpha are present in the brain, where they appear to be involved in several functions including the modulation of cognitive processes [33], the inhibition of drug rewarding properties [34], the neuroprotection [35] and the regulation of sleep-waking cycle [36]. Although it was recently observed that OEA can inhibit feeding when locally infused in the rat lateral hypothalamus [36], previous observations demonstrated that the anorexiant effect of OEA is mainly peripheral. In fact, it is completely lost when peripheral sensory fibers are removed by capsaicin treatment or by surgical dissection [2] and is absent when the drug is injected into the brain ventricles [2]. These observations, together with the finding that systemic administration of OEA is not followed by increased levels of the drug in the brain [6], suggest that peripheral and central compartments of OEA distribution are kept separated. This is presumably attributable to the high expression of its degrading enzyme, fatty acid amide hydrolase, in the blood-brain barrier [37].

Initial data obtained mapping mRNA levels for the activity regulated gene c-fos, by *in situ* hybridization of rat brain slices, showed that, after systemic administration, OEA evoked a highly localized increase in c-fos mRNA levels in the nucleus of the solitary tract (NST), the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) [2,20]. Similar effects on c-fos expression were observed following the administration of OEA precursors [38]. The stimulation of c-fos expression in the NST is consistent with the hypothesis that the primary site of action for OEA might be locally within the intestine,
and signaling with its neural targets might occur indirectly via peripheral sensory fibers. In fact, the NST is the site where vagal afferents from the gastrointestinal tract predominantly terminate and NST neurons are activated after feeding, gut distension, intragastric infusion of nutrients or peripheral CCK administration. Both PVN and SON are crucially involved in the regulation of feeding behavior and energy balance and their functions, mainly mediated by neuroactive peptides, are stimulated by several satiety signals [20,38]. In both nuclei c-fos was induced by OEA prevalently, albeit not exclusively, in oxytocin magnocellular neurons and it was paralleled by increased oxytocin immunoreactivity in the neurohypophysis and elevated circulating oxytocin levels [20].

OEA administration did not produce any effects on other hypothalamic neuropeptides, including vasopressin, thyrotropin-releasing hormone and pro-opiomelanocortin and the increase in oxytocin expression elicited by OEA resulted absent in mutant PPAR-alpha null mice [20]. Underscoring the role played by the oxytocinergic system in the satiety-inducing properties of OEA, pharmacological blockade of oxytocin receptors in the brain by intracerebroventricular infusion of the selective antagonist, L-368,899, was shown to prevent the anorexic effects of OEA [20].

Although the expression of PPAR-alpha receptor has been detected also in neurons of the enteric nervous system [24], the signal arising from the activation of PPAR-alpha receptor and recruiting sensory vagal afferents is still undefined. One possibility is that it may involve the transcriptional repression of intestinal nitric oxide production, which acts as a peripheral appetite-stimulating signal [11]. By contrast, the rapid onset of the OEA response (<30 min) might suggest, also, the involvement of a transcription-independent signal, which remains unidentified, though the ability of PPAR-alpha to elicit rapid non-genomic responses has been documented (see [21], for references).

Interestingly, a further possibility is that the modulation of vagal activity following PPAR-alpha activation might even occur at central level, in the NST, where PPAR-alpha receptor was recently found to be expressed and where very high levels of OEA were detected in Zucker rats [30]. Similarly to other satiety signals, it might be possible that more than one mechanism is involved in the modulation of the ‘gut-brain’ axis induced by OEA.

**Conclusions**

This review has been intentionally focused on the satiety-inducing effects of OEA, although this lipid mediator affects also several other aspects of the homeostatic system controlling energy balance. For example, the effects of OEA on lipid metabolism include the stimulation of lipolysis in white adipose tissue and liver, as well as the reduction of blood cholesterol and triglyceride levels [39]. This effect is probably involved in the reduction of body weight gain observed after repeated administration of OEA in different animal models of obesity [12].

Moreover, recent findings showed that OEA is able to strengthen long-term memory of emotional events [33], possibly linking the high caloric and hedonic value of fat-rich food to the formation of a stable imprint in our brain. This trace, when excessive, might lead to food cravings and compulsive eating, as suggested by the clinical findings that OEA signal is deregulated in patients who suffered from eating disorders [40]. Further support to this hypothesis derive from experimental results showing that OEA is able to modulate the mesolimbic dopaminergic response to addictive drugs [34], thus suggesting a possible extension of this effect toward the high addictive properties of fat-rich food.

Throughout species, food intake and energy balance are controlled as a homeostatic system. As food is ingested sensory and emotional cues are integrated in the brain with signals encoding for the energy state. As a consequence, the drive to eat decreases ensuring that the caloric load is sufficient to balance the energy needs while not exceeding what the body can safely handle. Although this system remained unchanged in the past 50 years, dramatic changes in the same period occurred in the sensory side of the control process, mainly due to increased palatability, availability, caloric density and constant advertisement of food. These signals became potent drivers of eating able to overwhelm the regulatory system and to trigger food overconsumption. In parallel with the changes in food supply, social norms related to eating have changed. Grazing, snacking, eating on the go became common and supply a substantial proportion of calories, thus greatly contributing to the modern spread of obesity epidemic.

The best opportunity to combat obesity might rely on the potentiation of the internal elements of the system, that may help the subject to redirect the eating habits toward a normal and healthier pattern. However, the integrated nature of energy homeostasis predicts that the efficacy of interventions targeting one subset or signal transduction pathway is inherently limited by compensatory responses elsewhere. This poses formidable challenges while clearly pointing at the emerging need of influencing more than one element jointly to achieve major weight reduction. Therefore, effective prevention or treatment of obesity may require therapies that target different components of energy homeostasis, satiety or food reward systems.

OEA signaling shows several characteristics fulfilling these requirements, thus suggesting that it might provides a chemical scaffold for the design of novel anti-obesity therapies.

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Figure 1. This figure illustrates the action of the lipid derived satiety factor OEA (green spheres), which is released from the intestine upon the intake of dietary fats (cheese). Through indirect pathways, probably depending from the local activation of PPAR-alpha receptors (sphere shadows) and involving afferent vagus nerve fibers (stairs) that reach the NST (door), OEA signal is transmitted to the brain, where it stimulates the release of oxytocin (chemical structure) from magnocellular neurons of the PVN and SON. Oxytocin is one of the numerous neuropeptides that mediate the central control (traffic light) of feeding behavior (lips) and the finding that OEA can modulate oxytocin release supports the role of this mediator in the complex network of the “gut-brain” axis.

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