Oleoylethanolamide: Effects on hypothalamic transmitters and gut peptides regulating food intake

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ABSTRACT

Recently, it has been described the role of fatty acid ethanolamides in the control of feeding behavior. Oleoylethanolamide (OEA) is a member of this family of lipid mediators regulating feeding. OEA acts suppressing feeding behavior through, at least partially, a peripheral mechanism. However, the interaction between this acylethanolamide and other orexigenic or anorexigenic mediators is mostly not well characterized.

The aim of this study was to evaluate whether anorectic actions of OEA were mediated through the modulation of central and peripheral signals involved in the regulation of feeding. Experiments were performed in male Wistar rats under free-feeding and fasting conditions. We measured hypothalamic neuropeptides and monoamines by in situ hybridization and HPLC respectively as well as plasmatic levels of relevant endocrine signals. OEA administration induced changes in hypothalamic monoaminergic activity and in the anorexigenic neuropeptide CART expressed in the paraventricular nucleus (PVN) but lacked effect on neuropeptides expression in nucleus arcuatus. In addition, OEA induced peripheral changes in gut peptides, with marked effects on PYY and Ghrelin. These results further suggest that anorexigenic properties of OEA are mediated by peripheral signals and by central alterations in neuropeptides expressed by feeding-involved hypothalamic structures receiving input from peripheral sensory systems, such as the PVN.

1. Introduction

The worldwide increase in obesity and related diseases has focused attention on understanding the complex network of signals that control feeding behavior. The control of food intake and energy expenditure comprises a complex system of central and peripheral regulatory factors. The central regulation of feeding involves several signaling systems such as neuropeptidergic, monoaminergic or endocannabinoid system. The peripheral signals controlling feeding comprises several hormones, peptides and metabolic intermediates originated in the gastrointestinal tract, the pancreas, the liver and the white adipose tissue.

These peripheral signals are being intensively studied as a target of new medicines for obesity. Among them, the adipocyte-derived hormones leptin (the major adipocyte-derived hormone) and adiponectin, which are involved in the regulation of energy homeostasis, glucose and lipid metabolism (Hu et al., 1996; Scherer et al., 1995; Zhang et al., 1994). Similar to these adiposity signals, the hormones synthesized and secreted from gastrointestinal tract are currently evaluated in obesity. They include the peptide YY (PYY) (Battherham et al., 2002) and the hormone ghrelin (Kojima et al., 1999).

Some of these peripheral mediators reach the central nervous system by targeting the nucleus tractus solitarius (NTS) in the brainstem, via the activation of the vagus nerve. From the NTS, this sensory information reaches the hypothalamus, the major site for integration of peripheral and central signals involved in energy homeostasis. Several hypothalamic nuclei, including the arcuate (ARC) and paraventricular (PVN) nuclei, are implicated in the regulation of food intake and body weight. The ARC contains two different subsets of neurons controlling food intake. One group coexpresses two types of orexigenic peptides: neuropeptide Y...
ties Council Directive 86/609/EEC regulating animal research. In contrast to the NPY/AgRP neurons, the second subset of neurons coexpresses alpha melanocyte stimulating hormone and cocaine-and amphetamine-regulated transcript (CART), a potent inhibitor of food intake (Kristensen et al., 1998; Zigman and Elmquist, 2003). ARC neurons project to PVN, which plays a role in the integration of signals from several brain regions (Sawchenko and Swanson, 1983). In the hypothalamus, neuropeptides and peripheral hormones interact with monoamines to control the feeding behavior (Kalra et al., 1999). In fact, the release of monoaminergic neurotransmitters dopamine (DA), noradrenaline (NA) and serotonin (5-HT) is related to feeding (Brunetti et al., 1999, 2000; Ramos et al., 2005).

Together with these central and peripheral signals, fatty acid ethanolamides have emerged as another class of signaling lipids implicated in the control of feeding behavior. Oleoylethanolamide (OEA) is a structural analogue of the endocannabinoid arachidonylethanolamide (anandamide or AEA) but does not bind to or activates the cannabinoid CB1 receptor (Rodríguez de Fonseca et al., 2001). OEA is synthesized in astrocytes (Walter et al., 2002) and neurons (Cadas et al., 1997; Di Marzo et al., 1994), as well as in cells of the small intestine and adipose tissue where level of this lipid mediator is reduced by fasting and increased upon refeeding in an opposite pattern to that exhibited by AEA (Fu et al., 2007; Gómez et al., 2002; Rodríguez de Fonseca et al., 2001). OEA exerts a number of pharmacological effects, including induction of satiety, reduction of body weight gain and stimulation of lipolysis through activation of the peroxisome proliferators-activated receptor alpha (PPAR-α) (Fu et al., 2003; Guzmán et al., 2004; Rodríguez de Fonseca et al., 2001). OEA binds to this nuclear receptor with high affinity and its effects are absent in mice lacking PPAR-α (Fu et al., 2003; Guzmán et al., 2004). Recently, it has been suggested that the effects of OEA on food intake and body weight gain may be mediated in part by an orphan G-protein coupled receptor (GPR119) (Overton et al., 2006).

Previous studies have reported that central administration of OEA has no effect on feeding (Gómez et al., 2002; Rodríguez de Fonseca et al., 2001), but peripheral administration is accompanied by a discrete activation of brain structures, such as the PVN and NTS (Rodríguez de Fonseca et al., 2001). With respect to the OEA effect on gut peptides, recent studies have shown that OEA does not induce significant changes on plasma levels of PYY, ghrelin or glucagon-like peptide 1 under free-feeding conditions. However, OEA reduces plasma level of ghrelin in fasted animals (Cani et al., 2004; Proulx et al., 2005).

Thus, to get a better understanding of OEA mechanisms controlling food consumption, one of the objectives of this study was to determine whether the appetite-suppressing effects of OEA administration are associated with changes in hypothalamic neuropeptides mRNA levels and/or monoaminergic activity. The second objective was to determine whether anorexigenic effects of OEA are related to changes in the level of peripheral hormones involved in the regulation of appetite and body weight.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats weighing 200–300 g. Animals were initially housed in a humidity and temperature-controlled vivarium on a 12-h light/dark cycle (lights off 8:00 PM). Water and standard chow pellets (Prolab RMH 2500) were available ad libitum throughout the course of the studies, unless otherwise indicated.

All procedures were conducted in strict adherence to the European Communities Council Directive 86/609/EEC regulating animal research.

2.2. Drugs

N-oleoylethanolamide (OEA) was synthesized in the laboratory as previously described (Rodríguez de Fonseca et al., 2001). OEA was dissolved in a vehicle of 5% Tween-20 and 95% saline and administered intraperitoneally (i.p.) at doses of 5 and 20 mg/kg. Drugs were injected in a volume of 1 ml/kg.

2.3. Determination of OEA effect on food intake

The acute effect of OEA on feeding behavior was analyzed in food-deprived rats for 24 h with free access to water and individually housed. The bedding material was removed from the cage 30 min prior to the test. At beginning of the test, rats received an i.p. injection of OEA (5 mg/kg) or vehicle control (5% Tween-20 in saline) and a small amount of weighed standard food pellets was placed inside the cage. Chow pellets were weighed at 30, 60, 120 and 240 min after injections and the amount of food ingestion was controlled by a scale.

2.4. Free-feeding and fasting conditions for experimental procedures with OEA

For fasting condition, Wistar rats were deprived of food with free access to water for 24 h. Then, rats were administered peripherally with OEA (5 mg/kg, i.p.) or vehicle (5% Tween-20 in saline). A second group of rats was ad libitum-fed and received also an i.p. administration of OEA (5 mg/kg) or vehicle (5% Tween-20 in saline). After both experimental procedures, animals were sacrificed by decapitation to collect blood and remove the brains 2 and 6 h after drug injection. These tissues were used for hormonal determinations and in situ hybridization studies respectively.

There is a circadian expression of the main neuropeptides involved in food intake and body weight regulation, with high levels in the morning which get lower in the afternoon and start to increase again during the night. Due to the presence of these circadian changes, all the treatments were performed at the beginning of the light cycle because at this time the neuropeptide levels are still high and relatively stable (Stutz et al., 2007).

2.5. In situ hybridization in brain under feeding and fasting conditions

Brains were rapidly removed, frozen, and stored at –80 °C until cryosectioned and processed for in situ hybridization. Coronal brain sections containing the hypothalamus (16 μm thick) were cut on a cryostat, mounted on gelatin-coated slides, and immediately stored at –80 °C until hybridization. For NPY, CART and AgRP mRNA detection we used specific antisense oligodeoxynucleotide probes (for NPY, 5’-AGATG AGATGGGCGGAAAATCAGGAGAGAGAATTTCTAAT-3’, gene access number: M20373; for CART, 5’-CCGAGGACTGTACCCCTATCAAC-3’, gene accession number: NM017110; and for AgRP, 5’-GAGGGGAGGAGCACCTGCCC GGTTGTTGCTGACCTACCCCTCTCTCC-3’, gene bank accession number: AF098007). The probes were 3’-end labelled with [32P]-deoxy-ATP using terminal deoxynucleotidyl transferase. The specificity of the probes was confirmed by performing cohybridization studies, incubating the sections with an excess of unlabelled probes (data not shown).

In situ hybridization was performed as described previously (Señarís et al., 1996; López et al., 2002). Tissue sections were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature for 30 min, dehydrated through 70, 80, 90, 95%, and absolute ethanol (5 min each). Hybridization was carried out overnight at 37 °C in a moist chamber. Hybridization solution was applied onto each slide and contained 4 × SSC, 50% deionized formamide, 1 × Denhardt's solution, 10 μg/ml sheared single-stranded salmon DNA, 10% dextran sulfate, 0.1 M NaCl, 1 × 106 (NPY, CART) or 5 × 105 cpm (AgRP) per slide of the labelled probes. After hybridization, sections were sequentially washed in 1 × SSC at room temperature, four times in 1 × SSC at 42 °C (NPY and CART) or 55 °C (AgRP) (30 min/wash), and one in 1 × SSC at room temperature for 1 h, then rinsed in water and ethanol. Finally, sections were air-dried and exposed to Hyperfilm E-Max (Amer sham International, Little Chalfont, UK) at room temperature for 3–5 days for NPY and AgRP and for 7 days for CART. To compare anatomically similar regions, the slides were matched according to the rat brain atlas of Paxinos and Watson (1998). The slides from animals of all groups were always exposed to the same autoradiographic film. Autoradiographic signals were scanned and then analyzed using ImageJ-1.33b software (NIH, USA). The optical density of the hybridization signal was determined and subsequently normalized to the optical density of its adjacent background value. A rectangle, with the same dimensions in each case, was drawn enclosing the hybridization signal over each nucleus and over adjacent brain areas of each section (background). Data of the optical densities were expressed as mean ± standard error of the mean (SEM), and represented as percentage change in relation to values in fed animals treated with vehicle which were considered as control values in every autoradiographic film. Data were expressed in percentage in relation to the control group due to the different basal levels that can be found sometimes between different films and different repetitions of the experiment.

2.6. Measurements of hormonal parameters in blood under free-feeding and fasting conditions

Blood samples were obtained from the trunk of decapitated animals and collected into tubes containing EDTA-2Na (1 mg/ml blood) and aprotinin (500 units/ml blood).
Samples were immediately centrifuged, and the plasma was aliquoted and stored at –80 °C until determination of hormonal parameters.

2.6.1. Ghrelin
Plasma levels of ghrelin were assayed by a double-antibody radioimmunoassay using reagent kits and methods provided by Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA). All samples were assayed in duplicate within one assay, and results were expressed in terms of the ghrelin standard. The limit of the assay sensitivity was 2 pg/mL, the intra- and inter-assay levels were 5% and 12% respectively.

2.6.2. PYY
Plasma levels of PYY were assayed by a double-antibody radioimmunoassay using reagent kits and methods provided by Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA). The antibody cross-reacts 100% with PYY 1–36 and PYY 3–36 but not with pancreatic polypeptide, NPY or other known gastrointestinal hormones. All samples were assayed in duplicate within one assay, and results were expressed in terms of the PYY standard. The limit of the assay sensitivity was 2 pg/mL, the intra- and inter-assay levels were 5% and 13% respectively.

2.6.3. Adiponectin
Plasma levels of adiponectin were assayed by a double-antibody radioimmunoassay using a reagent kit and methods provided by Linco Research, Inc. (St. Charles, MO, USA). All samples were assayed in duplicate within one assay, and results were expressed in terms of the adiponectin standard. The limit of the assay sensitivity was 1 ng/mL, the intra- and inter-assay levels were 4.11% and 6.56% respectively.

2.7. Determination of monoamine neurotransmitters in hypothalamus with OEA
Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA), serotonin (5-HT) and 5-hydroxy-indolacetic acid (5-HIAA) were evaluated using male Wistar rats that received an acute dose of either vehicle or OEA (5 and 20 mg/kg, i.p.). Animals were sacrificed 60 min later and the brain of each animal was also quickly removed and frozen at –80 °C. On the day of analysis, the hypothalamus was dissected and assayed for monoaminergic parameters. DA, DOPAC, NA, 5-HT and 5-HIAA contents were analyzed using HPLC system with electrochemical detection as previously described (Rodríguez de Fonseca et al., 1995).

2.8. Statistical analysis
All data for graphs and tables are expressed as the mean ± SEM. The different experiments included 6–12 animals per group according to the assay. Statistical analysis of results was performed using the computer program GraphPad Prism version 4.0 (GraphPad Software Inc., San Diego, CA, USA). The significance of differences between groups was evaluated by two-way analysis of variance (ANOVA) followed by a post-hoc analysis for multiple comparisons (Bonferroni and Tukey test) or Student’s t test. A p-value below 0.05 was considered statistically significant.

3. Results
Several hypothalamic neuropeptides could be involved in the anorectic actions of OEA. Consequently in situ hybridization experiments were performed on hypothalamic tissue samples derived from rats treated with OEA under free-feeding or fasting conditions. Also we tested in these animals whether OEA administration resulted in changes on peripheral hormones involved in the regulation of energy metabolism which are released by gastrointestinal tract and adipose tissue. Additionally, we measured monoaminergic activity on hypothalamus after the administration of this fatty acid-derived.

3.1. OEA causes a time-dependent suppression of feeding
Previous studies have shown that the OEA administration causes a dose- and time-dependent suppression of food consumption (Rodríguez de Fonseca et al., 2001; Serrano et al., 2006a,b). The i.p. administration of OEA (5 mg/kg) to food-deprived animals resulted in a time-dependent reduction in food intake (Fig. 1A). The time course of procedures is given in Fig. 1B. According to the effects induced by OEA on food intake, we established the different points to collect the samples. For monoamine turnover we selected a short time (60 min after OEA injection), in accordance with previous analysis of the effect of other peripheral signals on hypothalamic monoamines (Rodríguez de Fonseca et al., 2000). However, we also checked whether the changes in neuropeptides and hormones were keeping after the disappearance of the acute effects of OEA on food consumption.

3.2. OEA does not affect orexigenic neuropeptides in the arcuate nucleus of the hypothalamus
Fasting for 24 h induced an increase of NPY and AgRP mRNA levels in ARC compared to fed animals when we administered i.p. vehicle (Fig. 2). In general, the peripheral administration of OEA (5 mg/kg) produced changes neither in NPY nor AgRP mRNA with regard to vehicle-treated animals, 2 and 6 h after the treatment. We also expected, fasting increased significantly the NPY mRNA expression (Figs. 2A, B and 4A); 2 h after injecting vehicle or OEA to food-deprived animals, an increase of 40–50% (*p < 0.05) was observed; and 6 h later an elevation of 50–70% (**p < 0.001) with respect to fed-vehicle animals. However, in comparison with fed OEA-treated animals, NPY mRNA increase was only significant at 6 h in fasted OEA-treated animals (#p < 0.05). Fasting also increased significantly AgRP mRNA expression compared to a free-feeding condition (Fig. 2C and D); 2 h after i.p. administration of OEA or vehicle to food-deprived animals, AgRP expression increased a 70–80% (**p < 0.001); and 6 h later about 100–110% (***p < 0.001) in comparison with vehicle-treated animal. Additionally, these differences between both conditions respecting fed-vehicle animals also resulted significant at 2 and 6 h compared to fed animals treated with OEA (###p < 0.001).

3.3. OEA affects mRNA levels of the anorectic neuropeptide CART in the paraventricular but not in arcuate nucleus
In ARC the results showed that food deprivation for 24 h induced a significant reduction (15–20%, p < 0.05) on CART mRNA expression in comparison with free-feeding condition, 2 h after an acute administration of vehicle or 5 mg/kg OEA (Fig. 3A). This decrease on CART expression between both conditions disappeared 6 h after vehicle or OEA administration (Fig. 3B). With respect to PVN, the
Fig. 2. NPY and AgRP mRNA expression in ARC with both fed and 24 h food-deprived Wistar rats. NPY mRNA expression 2 h (A) and 6 h (B) after a peripheral administration of OEA (5 mg/kg, i.p.) and vehicle. AgRP mRNA expression 2 h (C) and 6 h (D) after a peripheral administration of OEA (5 mg/kg) and vehicle. Data are expressed as % change in relation to fed-vehicle group. Bars represent means ± SEM (n = 6 animals per group). (*) p < 0.05 and (***) p < 0.001 versus fed-vehicle group; (#) p < 0.05 and (###) p < 0.001 versus fed-OEA group.

Fig. 3. CART mRNA expression in hypothalamic nuclei with both fed and 24 h food-deprived Wistar rats. 2 h (A) and 6 h (B) after a peripheral administration of OEA (5 mg/kg) and vehicle in ARC 2 h (C) and 6 h (D) after a peripheral administration of OEA (5 mg/kg, i.p.) and vehicle in PVN. Data are expressed as % change in relation to fed-vehicle group. Bars represent means ± SEM (n = 6 animals per group). (*) p < 0.05 versus fed-vehicle group; (#) p < 0.05 versus fed-OEA group.
reduction on CART expression 2 h after treatments was also present (25% decrease versus fed-vehicle group, *p < 0.05) but it was prevented in OEA-treated animals (Figs. 3C and 4B). 6 h after treating peripherally with vehicle or OEA to Wistar rats, there were observed no differences on CART mRNA levels in PVN neither when free-feeding state or treatment were considered (Fig. 3D).

3.4. OEA induces changes in hypothalamic monoaminergic activity

Table 1 shows hypothalamic monoamines (DA, NA and 5-HT) and monoamines metabolites (DOPAC and 5-HIAA) concentrations, as well as DA and 5-HT turnovers, 60 min after administration of vehicle or different OEA doses (5 and 20 mg/kg). DA and 5-HT turnovers are

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**Fig. 4.** Pseudocolour images of autoradiograms of *in situ* hybridization showing NPY and CART mRNA expression in hypothalamic nuclei with both fed and 24 h food-deprived Wistar rats, 2 h after a peripheral administration of OEA (5 mg/kg, i.p.) and vehicle. NPY mRNA expression in ARC (A): fed-vehicle group a), fed-OEA group b), fasted-vehicle group c) and fasted-OEA group d). CART mRNA expression in PVN (B): fed-vehicle group a), fed-OEA group b), fasted-vehicle group c) and fasted-OEA group d). Labeling densities are indicated by color following this scale: (low) blue-green-yellow-red (high). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
expressed by the ratios DOPAC/DA and 5-HIAA/5-HT, respectively. OEA increased in a dose-dependent manner the absolute DA levels by about 30% compared to the vehicle group (**p < 0.01 versus vehicle group). DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; NA, noradrenaline; 5-HT, serotonin; 5-HIAA, 5-hydroxy-indolacetic acid.

### 3.5. Effects of OEA on peripheral hormones

#### 3.5.1. Ghrelin

Fasting condition induced a weak increase on circulating ghrelin level at 2 h after i.p. administration of vehicle or OEA (5 mg/kg), although these differences were not significant (Fig. 5A). Contrarily, there were differences on ghrelin at 6 h after OEA treatment (Fig. 5B). An acute administration of OEA to fasted animals caused a strong and significant decrease about 40% and 50% on serum ghrelin concentration in comparison with fed animals (***p < 0.01 versus fed-vehicle group and ####p < 0.001 versus fed-OEA group, respectively). Additionally, a peripheral injection of OEA to fed animals induced a no significant increase on ghrelin compared to vehicle-treated animals. Hence, we observed that OEA acutely reduced serum ghrelin concentration on food-deprived rats.

#### 3.5.2. Peptide YY

Fig. 5 shows the PYY level in animals treated with vehicle or OEA (5 mg/kg) under free-feeding and fasting conditions, 2 and 6 h elapsed after receiving these drugs. When we compared vehicle animals during fed and fasted states, only there were differences at 6 h with a significant decrease to 31% (**p < 0.001) on PYY in fasted rats compared to free-feeding ones (Fig. 5D). By contrast, the OEA administration induced strong changes on this circulating peptide, specifically reductions. Thus 2 h after this treatment (Fig. 5C) there was a PYY decrease in OEA-treated animals in comparison with vehicle groups, resulting significant in fasted animals (40% and 45% compared with fed and fasted vehicle respectively). At 6 h (Fig. 5D) PYY level in fed OEA-treated rats was reduced to about 55% in comparison with fed-vehicle group (**p < 0.01). With respect to fasted animals, there were no differences between treatments but both groups showed a significant decrease compared to fed-vehicle group.

#### 3.5.3. Adiponectin

We observed that 24 h food deprivation promotes an important reduction on plasma adiponectin concentration with reference to fed state one (38% and 54% decrease in 2 and 6 h vehicle-treated animals respectively; ***p < 0.001 both decreases in comparison with fed-vehicle group) (Fig. 6A and B). With regard to the OEA treatment, its injection to fed animals showed a tendency to decrease adiponectin levels in both 2 and 6 h groups. By contrast, we observed a remarkable effect on adiponectin level in fasted rats group at 2 h since OEA prevented the adiponectin decrease. This
preventive effect had disappeared 6 h after administration because a significant reduction in fasted animals was observed in comparison with fed-animal groups (**p < 0.001 versus fed-vehicle group; ###p < 0.001 versus fed-OEA group).

**4. Discussion**

Acylethanolamides are being identified as major transmitters involved in feeding regulation. Such as it has been previously reported (Rodríguez de Fonseca et al., 2001), OEA is involved in the peripheral regulation of feeding. However, the physiological mechanisms through which this lipid mediator acts on feeding behavior remain to be fully elucidated. The present study shows how OEA interacts with several peripheral signals and peptides involved in the complex regulation of feeding behavior, suggesting that these interactions may contribute to the anorectic effects induced by OEA. The results presented here showed that OEA mainly affects peripheral signals controlling feeding and metabolism, while the impact on neuropeptides involved in feeding regulation in the hypothalamus is minor, especially on orexigenic signals from ARC.

It is known that gastrointestinal signals together with the adipose tissue and pancreatic hormones play an important role in the control of energy homeostasis. OEA induces glucose intolerance in rats in vivo (González-Yanes et al., 2005) and stimulates lipolysis and fatty acid oxidation (Guzmán et al., 2004; Serrano et al., 2006a,b), which suggests that OEA is involved in the peripheral control of energy expenditure.

OEA can be produced in the adipose tissue and previous data suggest that this acylethanolamide is involved in the regulation of adipocyte metabolism. Recently, the OEA action has been described on glucose transporter phosphorylation, decreasing glucose uptake by adipocytes (González-Yanes et al., 2005). On the other hand, OEA stimulated the lipolysis both in vivo and in vitro (Guzmán et al., 2004). This availability control of energy resources from adipose tissue may play a critical role in both anorexic actions and weight gain blockade.

Concerning other hormones produced by adipocytes, it is noteworthy both leptin and adiponectin. Leptin is secreted from adipocytes and regulates appetite and body weight by decreasing food intake and increasing peripheral energy expenditure (Elmqquist et al., 1999; Friedman, 1998). However, it has not been observed any OEA action on leptin levels after an exogenous administration (Rodríguez de Fonseca et al., 2001). Regarding adiponectin, fatty acid oxidation in liver and skeletal muscle is increased by this hormone which induces an increase in insulin sensitivity (Berg et al., 2001; Fuebris et al., 2001; Yamauchi et al., 2002). Our results showed that OEA did not have any effect on adiponectin under free-feeding condition. However, 24 h food-deprived rats showed a decrease on adiponectin levels compared to free-feeding state, and the OEA administration was able to block this decrease at 2 h. Previous data have shown that a short-term fasting (48 h) does not affect circulating levels of adiponectin (Zhang et al., 2002) and also it has been suggested that the diurnal feeding/fasting cycle has no effect on serum level of adiponectin but other factors such as leptin could be involved (Oliver et al., 2006). The present results suggest that OEA could be one of these factors. Nevertheless, we need studies of long-term OEA administration in order to analyze the impact of this acylethanolamide on adiponectin regulation.

Similar to signals from the adipose tissue, peripheral regulation of feeding includes appetite or satiety signals originated from the gastrointestinal tract such as ghrelin and PYY. Ghrelin is a potent stimulant of short-term feeding and its plasma concentration increases during fasting and falls after food consumption, which is predominantly released from cells lining the fundus of the stomach (Cummings et al., 2001). Whereas, PYY is a gut hormone mainly released in small intestine and acts as a satiety signal that inhibits appetite (Batterham et al., 2002). Therefore, plasma levels of ghrelin and PYY were measured after a peripheral administration of OEA. During ad libitum-feeding condition, OEA did not have any effect on circulating level of ghrelin but induced a significant decrease on PYY at 6 h after its administration. Previous data suggest that ghrelin, PYY or other gut hormones do not play a significant role in the anorexigenic actions of OEA in a feeding state (Cani et al., 2004; Proulx et al., 2005). Under mentioned condition, these authors do not observe any effect on plasma levels of ghrelin or PYY at 2 h after a peripheral administration of OEA (20 mg/kg, i.p.). According to these previous studies, in our study a low dose of OEA (5 mg/kg) did not induce any change on both hormones at 2 h after an injection, but at 6 h serum PYY was decreased suggesting a regulation on this gut factor mediated by OEA. In fact, it is known that OEA release from the small intestine is reduced during fasting (Rodríguez de Fonseca et al., 2001). With respect to fasting condition, we did not observe any significant change on the ghrelin level compared to fed rats, although plasma concentration of PYY decreased during fasting. In such condition, OEA reduced plasma levels of both hormones. A similar effect of OEA on ghrelin level has been reported previously (Cani et al., 2004).

All these data suggest a physiological interaction between OEA and peripheral signals involved in feeding regulation. This lipid mediator may play a critical role in the modulation of the secretion of peripheral orexigenic and anorexigenic signals, contributing in that way to its actions as a satiety endogenous mediator.

The regulation of body weight and food intake involves interactions between peripheral organs and the central nervous system through vagal and sympathetic nerves. Peripheral signals reach...
NTS in the brainstem, which projects to higher neural centre involved in the regulation of feeding behavior and energy balance, such as PVN or ARC in the hypothalamus (Berthoud et al., 1990). Previous data show that systemic administration of OEA increases c-fos mRNA expression in NTS and PVN (but not in ARC) and it has not effect in capsaicin-treated animals, suggesting that this fatty acid-derived needs peripheral sensory fibers to exert anorectic effects (Rodríguez de Fonseca et al., 2001). Because the OEA effect is associated with the discrete activation of brain regions involved in the control of satiety, in the present study we examined whether peripheral administration of OEA induced changes in the expression of hypothalamic neuropeptides. We did not observe any effect on neuropeptides expression in ARC. Nevertheless, in PVN the reduction on the CART mRNA expression induced by fasting was restored after a systemic administration of OEA. These changes on CART levels in PVN may be related to the activation of sensory fibers targeting PVN through ascending NTS connections.

It is known that CART is localized in oxytocin-containing neurons in PVN (Elias et al., 2001). A recent study has suggested a role of the central regulator oxytocin in the satiety induced by OEA (Gaetani et al., 2010). Such study has demonstrated, using in situ and immunohistochemical experiments, that the OEA administration induces an elevation in the oxytocin expression in neurons of PVN. These findings are consistent with the present results and suggest that neuropeptides expressed in PVN might be implicated in mediating the anorexigenic effects of OEA.

Together with neuropeptides and peripheral hormones, monoaminergic neurotransmitters are involved in the control of food intake. Regulatory activity of appetite by some peripheral hormones could be explained, in part, because they are involved in the modulation of monoamines in the hypothalamus (Brunetti et al., 2005). OEA administration had a dose-dependent effect on DA and NA levels, but did not change 5-HT or its metabolite. The use of the metabolite to neurotransmitter ratios also showed that OEA decreased the DA turnover in the hypothalamus. These results suggest that OEA may be involved in the regulation of the activity of DA and NA neurons projecting into this area and which are crucial in the regulation of pituitary hormones involved in appetite and glucose homeostasis, including prolactin, GH and ACTH. These changes may be also a reflection of direct inputs into the hypothalamus of ascending projections from the brainstem, so they may reflect the integration of peripheral sensory systems of information with the neuroendocrine regulation of energy and metabolism.

4.1. Conclusions

In summary, this study shows new evidence for a role of OEA in feeding regulation and metabolism. These results suggest that OEA is involved in the complex system of central and peripheral signals, which regulates energy homeostasis. In the periphery, OEA interacts with circulating factors and metabolic mediators from gastrointestinal tract and adipose tissue related to appetite and energy balance. Such peripheral signaling pathway, through sensory fibers, reaches the brain modulating at the hypothalamic level, at least, CART neuropeptide expression and monoamine activity. The main peripheral component of OEA activity is very attractive in terms of therapeutic approaches to eating disorders and obesity, since the lack of central effects of this new type of signals may give a secure therapy devoid of unwanted central nervous system side effects.

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