Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration

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Abstract

Oleoylethanolamide (OEA) is an endogenous lipid that contributes in important ways to the peripheral regulation of food intake. When administered intraperitoneally, OEA is a potent satiety-inducing anorexiant in rats and mice [Nature 414 (2001) 209; Neuropsychopharmacology 28 (2003) 1311; Nature 425 (2003) 90]. In the present study, we show that oral administration of OEA in pH-sensitive enteric-coated capsules produces a profound and long-lasting inhibition of food intake in free-feeding rats. This effect is accompanied by a marked elevation in OEA levels in the small intestine, but not in brain or muscle.

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1. Introduction

Oleoylethanolamide (OEA) is an endogenous lipid mediator that participates in the control of feeding behavior. When administered intraperitoneally, OEA is potent at decreasing food intake in rats and mice [1–3]. This effect is due to a selective change in the onset and frequency of feeding—a strong indication of increased satiety—and is not accompanied by aversive or stress-related responses [1,2]. In addition, the anorexiant effect of OEA is mediated by visceral sensory fibers of the vagus nerve, and is accompanied by activation of brain structures—nucleus of the solitary tract, hypothalamic paraventricular nucleus—that are intimately involved in the control of energy balance [1]. The role of OEA as a satiety factor is further highlighted by the physiological regulation of its biosynthesis in the small intestine, which is influenced both by the natural circadian feeding rhythms and by forced starvation [1,3].

The molecular mechanism of action of OEA has been recently elucidated [3]. OEA binds to and activates with high potency the peroxisome proliferator-activated receptor-α (PPAR-α), a nuclear receptor that controls multiple aspects of energy balance [3]. As a result of PPAR-α activation, OEA not only produces satiety, but also reduces body-weight gain and serum lipid levels in genetically or diet-induced obese rats and mice [3]. These findings suggest that OEA may be useful in the pharmacotherapy of obesity, particularly if it were proven to be orally active. Because one of the sites of action of OEA is likely localized within the small intestine [1,3], oral activity might be achieved using pH-sensitive capsules, which are designed to release their content at the higher pH values of the small intestine. Therefore, the aim of the present study was to evaluate the effects of OEA administration in free-feeding rats using capsules coated with a pH-sensitive resin.

2. Methods

2.1. Animals

Adult male Wistar rats (250–300 g) were housed in groups of three in standard plexiglas cages at room temperature (22 °C). A 12-h light/12-h dark cycle was set with light on at 5.30 a.m. Water and standard chow pellets (Prolab RMH 2500) were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

2.2. Drugs

OEA was synthesized in the laboratory by the reaction of oleoyl chloride (Nu-chek Prep, Inc., Elysian, MN, USA)
with ethanolamine (Sigma-Aldrich) [4]. Briefly, oleoyl chloride was dissolved in dichloromethane (10 mg/ml) and allowed to react with 10-fold molar excess of ethanolamine for 15 min at 0–4°C. The reaction was stopped by adding purified water. After vigorous stirring and phase separation, the upper aqueous phase was discarded and the organic phase was washed twice with water to remove unreacted ethanolamine. This reaction results in the quantitative formation of OEA, which was concentrated to dryness under a stream of N₂ and stored at −20°C until used. Identity and chemical purity (∼98%) of the synthesized OEA was determined by thin-layer chromatography (TLC), using chloroform/methanol/ammonium hydroxide 9/10.1 (v/v) as mobile phase and high-performance liquid chromatography/mass spectrometry (HPLC/MS) as described [4].

For gavage administration, OEA was dissolved in saline/polyethylene glycol/Tween 80 (90/5/5, v/v; 1 ml kg⁻¹) and administered (0, 50, 100, and 200 mg kg⁻¹) to free-feeding rats. Enteric-coated capsules containing OEA powder (0, 25, and 50 mg kg⁻¹) were administered to free-feeding rats as described [5].

2.3. Preparation of enteric-coated capsules

Empty gelatin mini-capsules (Torpac, Fairfield, NJ, USA) were manually filled with OEA powder and coated with cellulose acetate hydrogen phthalate (CAP) as described [6]. Briefly, the capsules were immersed in a solution of 20% CAP in acetone/95% ethanol (9/1) for 5 min, followed by drying at room temperature for 12 h. The procedure was repeated twice.

2.4. Dissolution studies

To test the pH-sensitivity of the coating, capsules were filled with a colored powder, coated, and incubated in simulated gastric juice (20 ml, 0.08N HCl containing 0.2% NaCl, pH 1.2) for 3 h at 37°C with shaking at 200 rpm [6]. Undissolved capsules were dried and incubated in 10 mM phosphate buffer (20 ml, pH 4) at 37°C with shaking at 200 rpm. After 3 h of incubation, the pH was gradually increased every 30 min up to pH 8, and capsule dissolution was monitored every 5 min.

2.5. Analysis of feeding behavior

Food intake was recorded with an automated system (Scipro Inc., New York, NY, USA) as described [2]. Rats were habituated to the test cages for 3 days prior to trials. OEA was administered to free-feeding animals either by gavage or in enteric-coated capsules, 2 h prior to dark onset. In both experiments food intake was recorded for 24 h. The following parameters were measured:

- Cumulative food intake (g kg⁻¹), defined as food ingested per kilogram body weight per hour;
- Number of meals consumed during the 24 h test period, adopting a minimum inter-response interval separating two meals of 10 min [2];
- Meal size (g kg⁻¹), expressed as the average amount of food consumed during a meal;
- Post-meal interval (min), measured as the average time interval separating two consecutive meals.

2.6. Fatty acid ethanolamide analysis

In a separate experiment, rats that had received OEA capsules (50 mg kg⁻¹) were anesthetized with halothane and sacrificed. Blood samples were collected by cardiac puncture for plasma isolation and tissues were excised and immediately frozen in liquid nitrogen. All samples were stored at −80°C until analyzed. Fatty acid ethanolamides (FAEs) were isolated and quantified by HPLC/MS as described [4]. A HP 1100 Series HPLC/MS system equipped with an octadecylsilica Hypersil column (100 mm × 3 mm × 4.6 mm, i.d., 5 μm) was used. Reversed-phase separations were carried out by using linear increases of methanol (B) in water (A) (25% A, 75% B for 2 min; 15% A, 85% B for 3 min; 5% A, 95% B for 20 min; 100% B for 5 min) at a flow rate of 0.5 ml/min. Column temperature was kept at 20°C. Under these conditions, FAE standards eluted from the column with the following retention times: anandamide (AEA), 14.9 min; OEA, 17.8 min; palmitoylethanolamide (PEA), 16.7 min (Fig. 1). MS analyses were performed with an electrospray ion source set in the positive ionization mode. Capillary voltage (V.cap) was set at 3.5 kV, and fragmentor voltage was varied from 80 to 100 V. Nitrogen was used as drying gas at a flow rate of 121 min⁻¹. The drying gas temperature was set at 350°C and the nebulizer pressure at 50 psi. For quantitative analyses, sodium adducts of the molecular ions [M + Na]⁺ were detected in the selected ion monitoring (SIM) mode.

2.7. Statistical analyses

Twenty-four hours total food intake was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett’s test as post hoc. Cumulative food intake, measured hourly across the test period, was analyzed by two-way ANOVA using dose and time as the two factors, followed by Dunnett’s test for individual comparisons. Two-tailed t-test was used to evaluate the effects of treatments on meal parameters. FAEs levels in tissues and plasma were analyzed by unpaired one-tailed or two-tailed t-test, where appropriate. All the analyses were conducted by using the Graph Prism program (Graph Pad, 2.0), and differences were considered significant if P < 0.05.
3. Results

3.1. Capsule dissolution

Initial in vitro assays, conducted at 37 °C, revealed that enteric-coated capsules released their content within 10–15 min of incubation at pH 6.0 (data not shown).

3.2. Feeding

Oral administration of OEA caused a significant inhibition of food intake in free-feeding rats at doses of 200 mg kg⁻¹ (gavage) and 50 mg kg⁻¹ (capsules) (Fig. 2). One-way ANOVA of 24 h total food intake gave the following results: F(dose) = 176.73, d.f. = 2/314, P < 0.0001; F(time) = 213.75, d.f. = 22/314, P < 0.0001; F(dose × time) = 1.57, d.f. = 44/314, P < 0.05.

Administration of OEA capsules (50 mg kg⁻¹) was associated with a series of characteristic alterations in average meal parameters (Fig. 4A–C). In particular, the treatment decreased the number of meals consumed (4–8 h) (Fig. 4A; t = 2.314, d.f. = 10, P < 0.05), increased the post meal interval (4–8 h) (Fig. 4B; t = 2.45, d.f. = 10, P < 0.05), and had no effect on meal size (Fig. 4C).

3.3. OEA distribution

After OEA capsule administration (50 mg kg⁻¹), OEA levels were significantly higher than baseline levels in sules. Two-way ANOVA for cumulative food intake measured hourly gave the following results: F(dose) = 176.73, d.f. = 2/314, P < 0.0001; F(time) = 213.75, d.f. = 22/314, P < 0.0001; F(dose × time) = 1.57, d.f. = 44/314, P < 0.05.

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the upper portion of the gastrointestinal tract (i.e. stomach, duodenum, and jejunum; Fig. 5A-C), but not in the ileum (Fig. 5D). Unpaired one-tailed t-test gave the following results: stomach = 2.33, d.f. = 8, P < 0.05; duodenum = 1.804, d.f. = 13, P < 0.05; jejunum = 2.068, d.f. = 13, P < 0.05. In plasma, OEA concentrations were increased 4h after dosing (Figs. 6A and 7; t = 2.17, d.f. = 16, P < 0.05). Similar elevations were observed in liver and epididymal fat (Fig. 6B and C; t_{liver} = 2.94, d.f. = 16, P < 0.01; t_{fat} = 2.78, d.f. = 15, P < 0.01). By contrast, no changes in OEA content were observed in the brain and soleus muscle (Fig. 7A and B). OEA administration did not significantly affect the levels of two additional FAEs, anandamide and PEA (Table 1).
4. Discussion

The main finding of this study is that oral administration of OEA to free-feeding rats causes a persistent inhibition of food intake, demonstrating that this compound is an orally active anorexiant. The results confirm our previous observation that OEA suppresses food intake after intraperitoneal administration, and that this effect is not followed by a compensatory hyperphagia [1]. When administered by gavage, the lowest effective dose of OEA (200 mg kg\(^{-1}\)) produced a 20% inhibition of food intake over a 24-h period. A similar inhibition was obtained with a four-fold lower dose when OEA was administered in capsules coated with a gastroprotected pH-sensitive resin. The effects of OEA capsules were significantly different from controls 5 h after administration: this delay is likely due to the transit of the capsules from the stomach to the small intestine [3]. In agreement with this conclusion, in vitro tests showed that the enteric-coated capsules opened and released their content at pH value 6, which correspond to those found in the initial tract of the small intestine [1,2]. Together, these findings support the hypothesis that OEA reduces feeding by acting at a site localized within the small intestine [1,2].

Previous studies have shown that intraperitoneal OEA inhibits food intake by inducing satiety (the tonic state of inhibition over eating) rather that satiation (the phasic termination of feeding resulting from the act of food ingestion). To test whether this is also the case after oral administration of OEA, we investigated the effects of OEA capsules on three meal parameters: average number of meals, post meal interval, and meal size [2]. We found that OEA produced a temporary decrease in the number of meals from 5 to 8 h after administration, and that this effect was accompanied by an increase in post-meal interval, but not in meal size. We interpret these results to indicate that oral OEA, like intraperitoneal OEA, induces a transient state of satiety.

To determine the tissue distribution of exogenous OEA at the time of its maximal activity, we measured OEA levels in various tissues by HPLC/MS 4 h after administration of OEA capsules (50 mg kg\(^{-1}\)). The results revealed that OEA levels in the initial segment of the gastrointestinal tract (stomach, duodenum, and jejunum) were up to 50-fold higher than those of vehicle-treated animals, whereas the levels of OEA in plasma and liver were only 2-fold higher than controls. These results support the hypothesis that OEA acts on PPAR-\(\alpha\) receptors present within the gastrointestinal tract, rather than in other visceral organs such as the liver [7]. Moreover, the lack of penetration of OEA into the brain further underscores the peripheral site of action of this compound [1].

Obesity has reached epidemic proportions in industrialized countries and there is an urgent need for safe and effective anti-obesity drugs. In this regard, a natural compound such as OEA, which is involved in the physiological control of feeding and energy balance, is of considerable interest. Our results, showing that OEA is an orally active anorexiant, further underscore its potential for drug development.

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Table 1

| Tissues     | PEA (pmol g\(^{-1}\)) | AEA (pmol g\(^{-1}\)) |
|-------------|----------------------|----------------------|
|             | Vehicle              | OEA                  | Vehicle              | OEA                  |
| Stomach     | 266.8 ± 142.3        | 857.8 ± 358.6        | 52.0 ± 24.8          | 557.7 ± 19.5         |
| Duodenum    | 453.2 ± 73.6         | 454.2 ± 39.5         | 52.7 ± 6.9           | 579.7 ± 7.5          |
| Jejunum     | 502.9 ± 59.6         | 523.3 ± 39.9         | 53.4 ± 11.0          | 521.5 ± 7.1          |
| Ileum       | 214.0 ± 46.0         | 275.5 ± 30.9         | 11.6 ± 1.5           | 111.9 ± 1.6          |
| Liver       | 15.3 ± 1.1           | 161.4 ± 2.2          | 3.0 ± 0.2            | 3.0 ± 0.2            |
| Fa          | 206.3 ± 27.3         | 194.8 ± 11.9         | 7.2 ± 1.1            | 97.6 ± 1.4           |
| Brain       | 757.4 ± 56.7         | 606.7 ± 61.0         | 87.4 ± 4.9           | 87.7 ± 16.7          |
| Muscle      | 91.9 ± 7.1           | 123.1 ± 21.2         | 182.6 ± 25.4         | 117.7 ± 17.0         |

Fig. 7. OEA concentrations in the brain (A), and soleus muscle (B) of rats sacrificed 4 h after the administration of OEA capsules (50 mg kg\(^{-1}\)). Data are expressed as mean ± S.E.M. (n = 4–10).
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