Obesity is increasing in almost all countries (1, 2), and there is an increasing demand for pharmaceutical treatment of this lifestyle-associated disease (2, 3). Decreasing nutrient absorption, inhibition of appetite, and increasing thermogenesis are being considered as possible pharmacological methods of treatment. All of them have their drawbacks. Decreasing nutrient absorption (e.g., inducing fat malabsorption) may cause gastrointestinal discomfort. Inhibition of appetite is usually expected to involve actions on brain structures, thus leading to problems of brain targeting and contamination of other tissues. Increasing thermogenesis may also have serious side effects. Thus, a naturally occurring, orally active compound that will decrease appetite via a direct and local pharmacological/physiological effect on the intestine is a desirable drug candidate.

Oleylethanolamide (OEA) may be an endogenous regulator of food intake, and intraperitoneal injection of this compound decreases food intake in 24 h-starved rats (Rodriguez de Fonseca, F., M. Navarro, R. Gómez, L. Escuredo, F. Nava, J. Fu, E. Murillo-Rodriguez, A. Giuffrida, J. LoVerme, S. Gaetani, S. Kathuria, C. Gall, and D. Piomelli. 2001. An anorexic lipid mediator regulated by feeding. Nature. 414: 209–212). It is generally believed that this kind of lipid amide is rapidly catabolized in the gastrointestinal tract, thereby preventing its use as an oral antiobesity compound. We now show that oral OEA inhibits food intake dose dependently at 90 min after food presentation to starved rats. Food intake was reduced by 15.5% (P < 0.01) by administration of 10 mg/kg OEA. [3H]OEA was used to assess the degree of catabolism in the gastrointestinal tract. The endogenous level of this acylethanolamide was increased 11 times in the intestinal tissue (to 3.91 ± 0.98 nmol/g tissue, mean ± SEM) at 90 min after food presentation, based on the finding of 0.48% of the dose as intact OEA. These findings reveal unexpected properties of orally administered OEA, which may have potential as a cheap and safe antiobesity drug.—Nielsen, M. J., G. Petersen, A. Astrup, and H. S. Hansen. Food intake is inhibited by oral oleylethanolamide. J. Lipid Res. 2004. 45: 1027–1029.

Supplementary key words appetite • catabolism • rat

Methods

Animal experiments were approved by The Animal Experimentation Inspectorate, The Danish Ministry of Justice. Male Wistar rats (Tacomin M and B, Ry, Denmark) ~10 weeks of age (251 ± 10 g, mean ± SD, n = 79) were housed individually in metabolic cages in a temperature- and light-controlled stable (21 ± 1°C, 12 h light/dark cycle) for 4 days before the start of the experiment. Water and standard rat chow pellets (Formula 1314; Brogaarden, Gentofte, Denmark) were pulverized and available ad libitum. On the fourth day of adaptation, food was withdrawn and rats were fasted for 24 h with free access to water. Compounds and vehicle were injected intraperitoneally or given orally for 15 and 30 min, respectively, before food was introduced. The effect of intraperitoneally administered OEA on food intake was recorded at 60 and 120 min after food presentation, and the effect of orally administered OEA was recorded at 30, 90, and 150 min after food presentation. In addition, the effects of orally administered oleate and ethanolamine on food intake were examined. Rats were given doses of oleate or ethanolamine.
mine on an equimolar basis comparable to 10 mg of OEA per kilogram of rat. Food was introduced at 30 min after oral administration followed by measurement of food intake at 30 and 90 min later.

OEA (Sigma Aldrich Corp., St. Louis, MO) was dissolved in dimethyl sulfoxide, 0.7 ml/kg rat for injections. OEA, oleate, and ethanolamine were dissolved in 1% carboxymethylcellulose (Unichem, Copenhagen, Denmark) in 0.9% saline, 3 ml/kg rat for gavage administration. Control rats received vehicles.

Four rats were used for studying the catabolism of [3H]OEA (10 mg/kg) in the gastrointestinal tract. Rats were killed at 120 min after administration of [3H]OEA (90 min after food presentation), and stomachs with contents, intestinal contents, and intestinal tissue of each rat were collected separately. Samples were then homogenized (Ultra-Turrax T25; IKA Labortechnik, Staufen, Germany) and extracted with 20 vol of chloroform-methanol (2:1) per gram of sample. [3H]OEA from the CaCl2-treated extracts, including [14C]-labeled internal standard, were isolated by TLC using chloroform-methanol-ammonium hydroxide (80:20:2) for development. Spots were isolated, and radioactivity was counted in a liquid scintillation counter (Tri-Carb 2000CA; United Technologies Packard, Pangbourne, UK).

Synthesis of [3H]OEA was performed essentially as described elsewhere (9, 10) from [9,10(n)-3H]oleate (9.54 G/mmol) (Amersham Biosciences, Amersham, UK) and liquid ethanolamine (Sigma Aldrich Corp.). Synthesized [3H]OEA, with a specific activity of 4.89 × 10⁶ dpm/μmol, was purified by TLC using the upper phase of ethyl acetate-isooctane-acetic acid-water (90:10:1:2) for development. Spots were isolated, and radioactivity was counted in a liquid scintillation counter (Tri-Carb 2000CA; United Technologies Packard, Pangbourne, UK).

RESULTS AND DISCUSSION

Reproduction of the results of Rodriguez de Fonseca et al. (8) using the same experimental setup with intraperitoneal injections of 5 mg/kg OEA resulted in a significant reduction of food intake. After the first 60 min of food availability, food intake was reduced to 72.0 ± 4.5% (mean ± SEM, n = 8) of control values (P = 0.001) compared with ~40 ± 8% in the previous study (8).

Next, we gave varying doses of OEA (1, 10, and 100 mg/kg) orally at 30 min before food presentation to 24 h-starved rats and measured food intake at 30, 90, and 150 min. There was a significant dose-dependent effect of OEA at 90 min (Fig. 1; ANOVA, P = 0.03). According to Rodriguez de Fonseca et al. (8), OEA may act locally on sensory fibers in the intestine. Thus, we speculated on how much of the oral dose would arrive intact at the presumed target (i.e., the intestinal tissue). To clarify this, 3H-labeled OEA (10 mg/kg, n = 4) was administered orally followed by isolation of stomach with contents, intestinal contents, and intestinal tissue. The amount of intact [3H]OEA and 3H-labeled material (i.e., catabolized OEA) in the three samples was analyzed. The ratio of intact OEA to catabolized OEA decreased from the stomach with contents to the intestinal lumen to the intestinal tissue, indicating progressively more catabolism of OEA (Fig. 2).

Fig. 1. Oleoylethanolamide (OEA) decreases food intake dose dependently upon oral administration. Twenty-four hour-starved rats were administered varying doses of OEA at 30 min before food presentation, and food intake was recorded at 90 min thereafter. Values (means ± SEM) are presented as percentages of control (100% = 6.4 ± 0.9 g, n = 12), and the number of rats administered 1, 10, and 100 mg/kg were eight, seven, and four, respectively. * P < 0.01 (Student’s t-test). # P < 0.05 (post hoc ANOVA and Tukey’s honestly significant difference test).

Only 0.48 ± 0.13% (mean ± SEM, n = 4) of the oral dose of OEA was found as intact OEA in the intestinal tissue at 90 min after food presentation. In spite of this excessive...
catabolism, $[^3]H$OEA amounted to 3.91 ± 0.98 nmol/g (mean ± SEM, n = 4) in the intestinal tissue, which is 11 times higher than the endogenous level of 0.354 nmol/g (8). Thus, it is conceivable that this high tissue level, originating from the oral intake of 10 mg/kg OEA, caused the observed decrease in food intake. In free-feeding rats, injections of OEA have been demonstrated to delay feeding onset (12), and this effect also may occur in response to oral administration. It is very likely that oral OEA, after its intestinal action, is catabolized by the enzyme FAAH (13–15) to olate and ethanolamine. These degradation products could cause a decrease in food intake. However, we also tested the effect of oral ethanolamine (1.88 mg/kg = 30.72 μmol/kg) and olate (8.68 mg/kg = 30.72 μmol/kg) separately and found no effect on food intake, with 131.6 ± 26.8% (mean ± SEM, n = 4, P = 0.30) and 94.7 ± 9.2% (mean ± SEM, n = 12, P = 0.62), respectively, of the amount measured in control rats (n = 4, n = 12). Also, rat and human studies investigating the appetite-regulating effect of olate suggest that the dose of olate needed to elicit a hypophagic effect may be several times higher than the dose we used (16, 17).

From a pharmacological point of view, the conversion of OEA to olate and ethanolamine, two nontoxic, naturally occurring dietary compounds, is highly desirable because the degradation products of oral OEA are expected to cause low side effects. Although dietary changes and physical activity are preferred for the prevention of obesity, safe and effective pharmacological treatment of obese people is still needed. Our findings demonstrate that OEA is functional in reducing food intake upon oral administration in rats. The observation that this simple and naturally occurring compound holds potential for oral use could be greatly advantageous in the further development of an antiobesity medicine.

The study was supported by research grants from the Danish Medical Research Council. Michael Bonnichsen and Bettina Jensen are thanked for technical support.

REFERENCES

1. Swinburn, B., and G. Egger. 2002. Preventive strategies against weight gain and obesity. Obes. Rev. 3: 289–301.
2. Crowley, V. E. F., G. S. H. Yeo, and S. O’Rahilly. 2002. Obesity therapy: altering the energy intake-and-expenditure balance sheet. Nat. Rev. 1: 276–286.
3. Chiest, M., C. Huppertz, and K. G. Hofbauer. 2001. Pharmacotherapy of obesity: targets and perspectives. Trends Pharmacol. Sci. 22: 247–254.
4. Schmid, H. H. O., P. C. Schmid, and V. Natarajan. 1990. N-Acylated glycerophospholipids and their derivatives. Prog. Lipid Res. 29: 1–43.
5. Schmid, P. C., L. E. Wold, R. J. Krebsbach, E. V. Berdyhev, and H. H. O. Schmid. 2002. Anandamide and other N-acylphospholipids in human tumors. Lipids. 37: 907–912.
6. Hansen, H. S., B. Moesgaard, H. H. Hansen, and G. Petersen. 2000. N-Acylphospholipids and precursor phospholipids—relation to cell injury. Chem. Phys. Lipids. 108: 135–150.
7. Fu, J., S. Gaetani, F. Oveisi, J. Lo Verme, A. Serrano, F. Rodriguez de Fonseca, A. Rosengarth, H. Luecke, B. Di Giacomo, G. Tarzia, and D. Piomelli. 2003. Oleoylphospholipids regulate feeding and body weight through activation of the nuclear receptor PPAR-y. Nature. 425: 90–95.
8. Rodriguez de Fonseca, F., M. Navarro, R. Gómez, L. Escredo, F. Nava, J. Fu, E. Murillo-Rodriguez, A. Giuffrida, J. LoVerme, S. Gaetani, S. Kathuria, C. Gall, and D. Piomelli. 2001. An anorexic lipid mediator regulated by feeding. Nature. 414: 209–212.
9. Felder, C. C., A. Nielsen, E. M. Briley, M. Palkovits, J. Priller, J. Axelrod, D. N. Nguyen, J. M. Richardson, R. M. Riggin, G. A. Koppel, S. M. Paul, and G. W. Becker. 1996. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. FEBS Lett. 393: 231–235.
10. Giuffrida, A., L. H. Parsons, T. M. Kerr, F. Rodriguez de Fonseca, M. Navarro, and D. Piomelli. 1999. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat. Neurosci. 2: 358–363.
11. Hansen, H. S., L. Lauritzen, A. M. Strand, A. M. Vinggaard, A. Frandsen, and A. Schousboe. 1997. Characterization of glutamate-induced formation of N-acylphosphatidyethanolamine and Nacylphosphatidylethanolamine in cultured neocortical neurons. J. Neurochem. 69: 753–761.
12. Gaetani, S., F. Oveisi, and D. Piomelli. 2003. Modulation of meal pattern in the rat by anorexenic lipid mediator oleoylphospholipid. Neuroperopharmacology. 28: 1311–1316.
13. Schmid, P. C., M. L. Zuarte-Augustin, and H. H. O. Schmid. 1985. Properties of rat liver N-acylethanolamine amidehydroase. J. Biol. Chem. 260: 14145–14149.
14. Cravatt, B. F., and A. H. Lichtman. 2002. The enzymatic inactivation of the fatty acid amide class of signaling lipids. Chem. Phys. Lipids. 121: 135–148.
15. Katayama, K., N. Ueda, Y. KuraHashi, H. Suzuki, S. Yamamoto, and I. Kato. 1997. Distribution of anandamide amidohydrolase in rat tissues with special reference to small intestine. Biochem. Biophys. Acta. 1347: 212–218.
16. Cox, J. E., W. J. Tyler, A. Randich, G. R. Kelm, S. S. Bharaj, R. J. Jan-deck, and S. T. Meller. 2000. Suppression of food intake, body weight, and body fat by jejunal fatty acid infusions. Am. J. Physiol. 278: R604–R610.
17. French, S. J., C. A. Conlon, S. T. Mutuma, M. Arnold, N. W. Read, G. Meier, and J. Francis. 2000. The effects of intestinal infusion of long-chain fatty acids on food intake in humans. Gastroenterology. 119: 943–948.