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Oleylethanolamide Stimulates Lipolysis by Activating the Nuclear Receptor Peroxisome Proliferator-activated Receptor α (PPAR- α)*

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Amides of fatty acids with ethanolamine (FAE) are biologically active lipids that participate in a variety of biological functions, including the regulation of feeding. The polyunsaturated FAE anandamide (arachidonylethanolamide) increases food intake by activating G protein-coupled cannabinoid receptors. On the other hand, the monounsaturated FAE oleylethanolamide (OEA) reduces feeding and body weight gain by activating the nuclear receptor PPAR- α (peroxisome proliferator-activated receptor α). In the present report, we examined whether OEA can also influence energy utilization. OEA (1–20 μ M) stimulated glycerol and fatty acid release from freshly dissociated rat adipocytes in a concentration-dependent and structurally selective manner. Under the same conditions, OEA had no effect on glucose uptake or oxidation. OEA enhanced fatty acid oxidation in skeletal muscle strips, dissociated hepatocytes, and primary cardiomyocyte cultures. Administration of OEA *in vivo* (5 mg kg⁻¹, intraperitoneally) produced lipolysis in both rats and wild-type mice, but not in mice in which PPAR- α had been deleted by homologous recombination (PPAR- α ^{-/-}). Likewise, OEA was unable to enhance lipolysis in adipocytes or stimulate fatty acid oxidation in skeletal muscle strips isolated from PPAR- α mice. The synthetic PPAR- α agonist Wy-14643 produced similar effects, which also were dependent on the presence of PPAR- α . Subchronic treatment with OEA reduced body weight gain and triacylglycerol content in liver and adipose tissue of diet-induced obese rats and wild-type mice, but not in obese PPAR- α ^{-/-} mice. The results suggest that OEA stimulates fat utilization through activation of PPAR- α and that this effect may contribute to its anti-obesity actions.

Amides of long-chain fatty acids with ethanolamine (FAE)¹ are a family of lipid mediators produced through the concerted action of two enzymes present in mammalian cells: *N*-acyltransferase, which transfers a fatty acid from the *sn*-1 position

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¹ The abbreviations used are: FAE, fatty acid with ethanolamine; OEA, oleylethanolamide; PPAR, peroxisome proliferator-activated receptor; FAT/CD36, fatty acid translocase; UCP-2, uncoupling protein-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

of a donor phospholipid to the free amine in phosphatidylethanolamine, producing *N*-acyl-phosphatidylethanolamine; and phospholipase D, which converts *N*-acyl-phosphatidylethanolamine to FAE (1, 2). The FAE are hydrolyzed intracellularly to fatty acids and ethanolamine by the action of fatty acid amide hydrolase enzymes (3–5).

Although the FAE were first described four decades ago (6), they did not attract much attention until the discovery that a polyunsaturated member of this family, anandamide (arachidonylethanolamide), is an endogenous ligand for cannabinoid receptors, G protein-coupled receptors targeted by the marijuana constituent Δ^9 -tetrahydrocannabinol (7). Anandamide is now established as a brain endocannabinoid messenger (8) and multiple roles for other FAE have also been proposed (9–11). One emerging function of these lipid mediators is the regulation of feeding behavior. Anandamide causes overeating in rats because of its ability to activate cannabinoid receptors (12). This action is of therapeutic relevance: cannabinoid agonists such as Δ^9 -tetrahydrocannabinol are currently used to alleviate anorexia and nausea in AIDS patients, whereas the CB₁ antagonist rimonabant (SR141716A) was recently found to be effective in late-stage clinical trials for the treatment of obesity (12).

In contrast to anandamide, the monounsaturated FAE oleylethanolamide (OEA) decreases food intake and body weight gain through a cannabinoid receptor-independent mechanism (11, 13). Pharmacological and molecular biological experiments have demonstrated that these effects result from the high affinity binding of OEA to, and consequent activation of, the nuclear receptor PPAR- α (peroxisome proliferator-activated receptor α) (14). Because PPAR- α serves an essential function in the regulation of lipid metabolism (15, 16) we investigated whether OEA may also influence nutrient utilization by altering fat storage and catabolism.

EXPERIMENTAL PROCEDURES

Chemicals—FAE synthesis was conducted as described (17). For *in vitro* experiments, drugs were dissolved in dimethyl sulfoxide and used at a final dimethyl sulfoxide concentration of 0.1–0.2 (v/v). For *in vivo* experiments, drugs were dissolved in a vehicle of 70% dimethyl sulfoxide/sterile saline (acute administrations) or 90% sterile saline, 5% polyethylene glycol, 5% Tween 80 (subchronic administrations).

Cell and Tissue Preparation—Hepatocytes (18) and epididymal adipocytes (19) were isolated by collagenase digestion of freshly isolated tissue from free-feeding male Wistar rats (150–200 g, Charles River, Wilmington, MA) or PPAR- α ^{-/-} mice (C57.129S4-*Ppara*^{tm1Gonz}; Taconic M&B, Ry, Denmark) and their corresponding wild-type C57BL/6N controls. Heart myocytes were prepared by collagenase digestion of embryonic rat hearts and maintained in primary culture as described (20). Rat plasma was prepared by centrifugation of EDTA-treated (0.02 M) blood obtained by cardiac puncture.

Lipolysis—Glycerol was determined spectrophotometrically in cell incubation media or plasma using a commercial kit (Sigma). Fatty acid release was measured in cells labeled by incubation with [³H]oleic acid (2 μ Ci per well, Amersham Biosciences) for 1 h at 37 °C. After labeling,

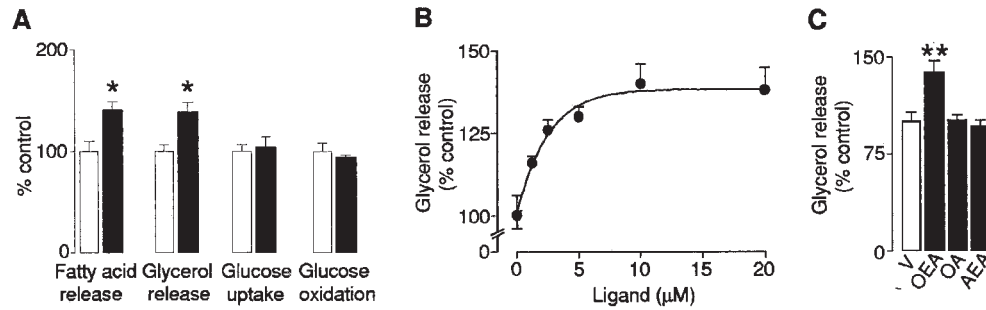


FIG. 1. **OEA stimulates lipolysis *in vitro*.** A, fatty acid release, glycerol release, glucose uptake, and glucose oxidation in isolated adipocytes incubated for 30 min with vehicle (open bars) or OEA (10 μ M, closed bars). Baseline levels were as follows: [3 H]fatty acid release, 21,968 \pm 3,207 cpm well $^{-1}$ h $^{-1}$; glycerol release, 262 \pm 23 nmol well $^{-1}$ h $^{-1}$; [3 H]deoxyglucose uptake, 128 \pm 14 nmol million cells $^{-1}$ h $^{-1}$; [14 C]glucose oxidation, 79 \pm 7 nmol million cells $^{-1}$ h $^{-1}$. B, concentration-dependent effects of OEA on glycerol release from adipocytes. C, effects of vehicle (V), OEA, oleic acid (OA), or anandamide (AEA), each at 10 μ M, on glycerol release from adipocytes. Significantly different from vehicle incubations: *, $p < 0.05$; **, $p < 0.01$; $n = 4-6$.

the cells were washed with ice-cold Krebs-Ringer buffer supplemented with 20 mM HEPES, 2 mM glucose, and 2% fatty acid-free bovine serum albumin (pH 7.4) and incubated for 30 min at 37 $^{\circ}$ C in 1 ml of buffer; the incubation medium was separated by centrifugation and fatty acids were isolated by solvent extraction followed by thin-layer chromatography (21). Plasma fatty acids were measured with a commercial kit (Wako Chemicals, Richmond, VA).

Fatty Acid Oxidation—Fatty acid oxidation was measured using [1- 14 C]oleic acid as a substrate (0.4 mM, 0.5 μ Ci per sample; American Radiolabeled Chemicals, St. Louis, MO). In soleus muscle strips and heart myocytes, fatty acid oxidation to CO $_2$ was determined by trapping released [14 C]CO $_2$ as bicarbonate in wells containing filter paper soaked with benzethonium hydroxide (1 M in methanol) (22). In hepatocytes, [14 C]ketone bodies, which constitute about 90% of total fatty acid oxidation products, were determined as non-volatile acid-soluble products (23).

Glucose Uptake and Oxidation—Glucose uptake was measured by using 2-D-[3 H]deoxyglucose as a substrate (1 μ Ci per sample, Amersham Biosciences). Cells in suspension (adipocytes or hepatocytes) were rapidly separated from the incubation medium by centrifugation, whereas in the case of attached heart myocytes and soleus muscle strips the medium was removed by aspiration. Samples were washed with ice-cold buffer, and radioactivity was counted in perchloric acid extracts. Glucose oxidation was measured by using D-[U- 14 C]glucose as a substrate (1 μ Ci per sample; Amersham Biosciences), trapping [14 C]CO $_2$ on filter paper, as described above.

Tissue Lipids and Protein—Tissue triacylglycerols were extracted with chloroform:methanol:NaCl (1 M) (1:1:0.5), suspended in a solvent of *tert*-butanol:methanol:Triton X-114 (3:1:1), and measured using a commercial kit (GPO-Trinder; Sigma). Tissue protein content was determined using a commercial kit (Bio-Rad).

Polymerase Chain Reaction—Reverse transcription of 2 μ g of total RNA was carried out with 0.2 μ g of oligo(dT) $_{12-18}$ primer for 50 min at 42 $^{\circ}$ C, and real time quantitative polymerase chain reaction was done with an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). Primer/probe sets were designed using Primer Express software (Applied Biosystems) and gene sequences available from GenBank $^{\text{TM}}$ data base. Primers and fluorogenic probes were synthesized at TIB Molbiol (Adelphia, NJ).

The primer/probe sequences for the rat genes were as follows. For PPAR- α : forward, 5'-TGGTGGACCTCCGGCA-3'; reverse, 5'-TCTTCT-TGATGACCTGCACGA-3'; and probe, 5'-CTGGTCACGGAGCATGCC-CAG-3'. For fat fatty acid-binding protein: forward, 5'-GGCTTCGCC-ACCAGGAA-3'; reverse, 5'-CCCTTCTACGCTGATGATCAAGT-3'; and probe, 5'-TGGCCGGTATGGCCAAGCCC-3'. For fat fatty acid translocase (FAT/CD36): forward, 5'-CATCGGCATGAGAAAGCA-3'; reverse, 5'-ACCAGGCCAGGAGCTTTA-3'; and probe, 5'-AAATGTTCA-GAAACCAAGTGACCGGAAA-3'. For uncoupling protein-2 (UCP-2): forward, 5'-CCAGAGCACTGTGCAAGCC-3'; reverse, 5'-TTCCAGAG-GCCCGGA-3'; and probe, 5'-ACAAGACCATTGCACGAGAGGAAGG-G-3'. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5'-AAGTATGATGACATCCAAGAAGGTGGT-3'; reverse, 5'-AG-CCCAGGATGCCCTTTAGT-3'; and probe, 5'-AAGCAGCGCGCCGAG-GGC-3'. RNA levels were normalized by GAPDH as an internal standard, and measurements were made as described (24).

Diet-induced Obesity Model—Animals (either 6-week-old male Wistar rats (Charles River) or 7-week-old male C57Bl/6J, PPAR- $\alpha^{-/-}$ (129S4/SvJae-Ppara $^{\text{(tm1Gonz)}}$) and wild-type 129S1/SvImj mice (Jackson

Labs, Bar Harbor, ME) were fed a high-fat diet (fat was 35% weight or 60% kcal; Research Diets Inc., New Brunswick, NJ). After 8 (rats) or 7 (mice) weeks, the animals received for 2 (rats) or 4 (mice) additional weeks daily intraperitoneal injections of vehicle or OEA (5 mg kg $^{-1}$) 30 min before dark. The pair-fed group of rats received only vehicle injections. Food intake and body weight were measured daily. At the end of the experiments, the animals were food-deprived for 16–18 h prior to blood and tissue collection.

Other Analytical Methods—Commercial kits were used to measure plasma glucose and 3-hydroxybutyrate (Sigma), as well as glucagon and insulin (Linco Research, St. Charles, MO).

Statistics—Results are expressed as mean \pm S.E. of n separate experiments. The significance of differences among groups was evaluated using Student's t test or analysis of variance followed by a Tukey's multiple comparisons test or a Dunnett's post-hoc test, as appropriate.

RESULTS

OEA Stimulates Lipolysis *in Vitro*—Incubation of freshly dissociated rat adipocytes in the presence of OEA (10 μ M, 30 min) significantly increased the release of non-esterified fatty acids and glycerol into the bathing medium (Fig. 1A). By contrast, OEA had no effect on adipocyte glucose uptake or oxidation, irrespective of whether the cells were isolated from free-feeding (Fig. 1A) or 18-h starved rats (data not shown). Moreover, OEA did not affect insulin-stimulated glucose uptake (insulin 100 nM, 69 \pm 17% stimulation; insulin plus OEA 10 μ M, 79 \pm 22% stimulation; $n = 3$). The lipolytic effects of OEA were concentration-dependent (half-maximal effective concentration, EC $_{50}$, \approx 2 μ M)(Fig. 1B) and structurally selective, as oleic acid and anandamide had no such effect (Fig. 1C).

Because lipolysis is primarily controlled through cAMP-dependent protein phosphorylation (25), we examined whether this second messenger pathway participates in the effects of OEA. At a concentration that produced maximal lipolysis (10 μ M), OEA did not change either basal or forskolin-stimulated cAMP levels (in %: vehicle, 100 \pm 11; OEA, 96 \pm 8; forskolin, 0.5 μ M, 127 \pm 11; OEA plus forskolin, 0.5 μ M, 124 \pm 9; forskolin, 25 μ M, 287 \pm 40; OEA plus forskolin, 25 μ M, 280 \pm 70). Moreover, the Ca $^{2+}$ ionophore ionomycin (5 μ M) had no effect on basal, OEA-stimulated or forskolin-stimulated lipolysis (data not shown). The results suggest that OEA promotes lipolysis in isolated rat adipocytes through a mechanism that is independent of cAMP or Ca $^{2+}$.

OEA Stimulates Lipolysis *in Vivo*—Systemic administration of OEA to rats (5 mg kg $^{-1}$, intraperitoneal) produced a rapid elevation in the circulating levels of non-esterified fatty acids and glycerol, which was followed by a rise in the concentration of 3-hydroxybutyrate, a marker of hepatic fatty acid oxidation (Fig. 2A). This response was dose-dependent (half-maximally effective dose, ED $_{50}$, \approx 2.6 mg kg $^{-1}$) (Fig. 2B) and accompanied by a decrease in the triacylglycerol content of epididymal fat

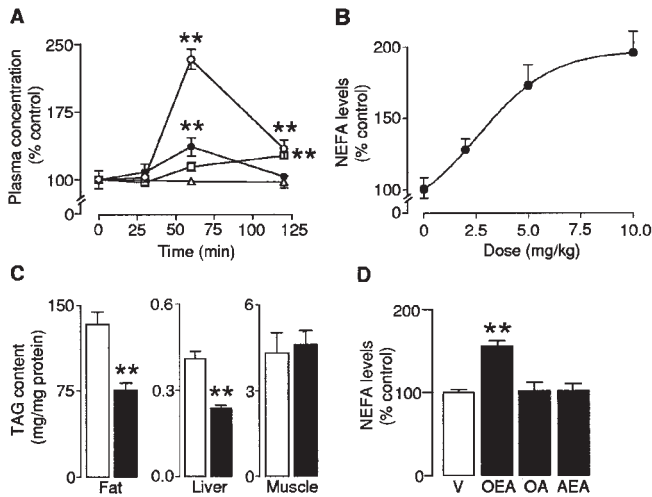


FIG. 2. OEA stimulates lipolysis *in vivo*. *A*, time course of the effects of OEA (5 mg kg^{-1} , intraperitoneal) on plasma fatty acids (*open circles*), glycerol (*closed circles*), 3-hydroxybutyrate (*open squares*), and glucose (*open triangles*). Baseline levels were as follows: fatty acids, $0.10 \pm 0.01 \mu\text{mol ml}^{-1}$; glycerol, $0.32 \pm 0.01 \text{ mg ml}^{-1}$; 3-hydroxybutyrate, $0.040 \pm 0.002 \text{ mg ml}^{-1}$; and glucose, $1.11 \pm 0.04 \text{ mg ml}^{-1}$. *B*, dose-dependent effects of OEA (1 h) on plasma non-esterified fatty acid (NEFA) levels. Baseline levels were 0.17 ± 0.01 . *C*, triacylglycerol (TAG) content in epididymal fat, liver, and soleus muscle of rats treated with vehicle (*open bars*) or OEA (5 mg kg^{-1} , 1 h; *closed bars*). *D*, effects of vehicle, OEA (5 mg kg^{-1}), oleic acid (OA, 20 mg kg^{-1}), or anandamide (AEA, 20 mg kg^{-1}) on plasma fatty acids 1 h after injection. Levels in vehicle-treated rats were $0.16 \pm 0.01 \mu\text{mol ml}^{-1}$. Significantly different from the respective controls: **, $p < 0.01$; $n = 3-6$.

and liver, but not skeletal muscle (Fig. 2*C*). By contrast, plasma glucose levels remained unchanged after OEA injection (Fig. 2*A*), as did those of insulin and glucagon (insulin, in ng ml^{-1} : vehicle, 1.7 ± 0.3 ; OEA, 30 min, 1.5 ± 0.1 ; OEA, 60 min, 1.6 ± 0.1 ; glucagon, in pg ml^{-1} : vehicle, 116.5 ± 8.3 ; OEA, 30 min, 135.7 ± 21.1 ; OEA, 60 min, 95.9 ± 13.6). Oleic acid and anandamide had no significant lipolytic effect *in vivo* at any of the doses tested (up to 20 mg kg^{-1} , intraperitoneal) (Fig. 2*D*).

PPAR- α Mediates OEA-induced Lipolysis—As previously seen in rats, administration of OEA (5 mg kg^{-1} , intraperitoneal) to C57Bl/6 mice significantly reduced liver triacylglycerol content 1 h after injection (Fig. 3*A*). To test the role of PPAR- α in this response we used three distinct approaches. First, we examined the effects of the synthetic PPAR- α agonist Wy-14643, which is structurally unrelated to OEA (26). At a dose that fully activates PPAR- α *in vivo* (20 mg kg^{-1} , intraperitoneal) (14), Wy-14643 reduced liver triacylglycerol levels to the same extent as did 5 mg kg^{-1} OEA (Fig. 3*A*). Second, we examined whether genetic deletion of PPAR- α affects the lipolytic response to OEA and Wy-14643 *in vivo*. In striking contrast with their effects in wild-type mice, both drugs increased liver triacylglycerol levels in mice in which PPAR- α had been deleted by homologous recombination (PPAR- $\alpha^{-/-}$) (27) (Fig. 3*A*). We did not investigate the bases for this paradoxical stimulation, although an intriguing possibility is that, in the absence of PPAR- α , OEA might activate other lipogenic nuclear receptors (28). Third, we investigated whether genetic deletion of PPAR- α affects the lipolytic response to OEA or Wy-14643 *in vitro*. Incubation of adipocytes isolated from wild-type mice with either compound stimulated lipolysis (Fig. 3*B*), whereas no such effect was observed in adipocytes isolated from PPAR- $\alpha^{-/-}$ mice (Fig. 3*B*).

To further explore the role of PPAR- α in the response to OEA, we fed wild-type and PPAR- $\alpha^{-/-}$ mice for 7 weeks with a high-fat chow and then treated them for 4 additional weeks with OEA (5 mg kg^{-1} , once daily, intraperitoneal), a drug

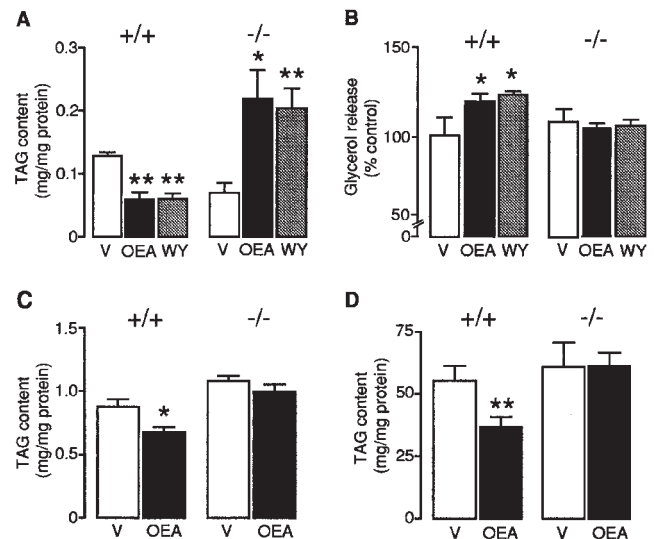


FIG. 3. PPAR- α mediates OEA-induced lipolysis. *A*, effects of vehicle (*open bars*), OEA (5 mg kg^{-1} , *closed bars*), or Wy-14643 (20 mg kg^{-1} , *shaded bars*) on triacylglycerol (TAG) levels in liver of wild-type (+/+) or PPAR- $\alpha^{-/-}$ mice 1 h after drug injection. *B*, glycerol release in adipocytes incubated for 40 min with either vehicle (*open bars*), OEA ($10 \mu\text{M}$, *closed bars*), or Wy-14643 ($50 \mu\text{M}$, *shaded bars*), isolated from wild-type (+/+) or PPAR- $\alpha^{-/-}$ mice. *C* and *D*, effects of vehicle (*open bars*) or OEA (5 mg kg^{-1} , intraperitoneal once daily for 4 weeks; *closed bars*) on triacylglycerol levels (*C*) in liver and epididymal fat pad (*D*) of diet-induced obese wild-type (+/+) or PPAR- $\alpha^{-/-}$ mice. Significantly different from the respective controls: *, $p < 0.05$; **, $p < 0.01$; $n = 3-9$.

regimen that was previously shown to reduce body weight gain (14). In wild-type mice, this treatment caused a significant decrease in liver and fat triacylglycerol content (Fig. 3, *C* and *D*), but had no such effect in PPAR- $\alpha^{-/-}$ mice (Fig. 3, *C* and *D*). To investigate the mechanism by which these changes might occur, we assessed the effects of OEA on the expression of various genes involved in lipid metabolism. In rats, 6 h following a single injection of OEA (10 mg kg^{-1} intraperitoneally), we found increased levels of PPAR- α mRNA in adipose tissue (Fig. 4*A*) and skeletal muscle (Fig. 4*F*). Concordantly, two downstream genes under the regulation of PPAR- α , fatty acid-binding protein, and fatty acid translocase (FAT/CD36), which encode for proteins that participate in the cellular transport of fatty acids (29), were elevated by OEA treatment in adipose (Fig. 4, *B* and *C*) and muscle tissue (Fig. 4, *G* and *H*). These changes were paralleled by an increase in the expression of UCP-2 (Fig. 4, *D* and *I*), which is thought to play a key role in mitochondrial energy utilization (30, 31).

OEA Stimulates Fatty Acid Oxidation *in Vitro*—PPAR- α plays a pivotal role in the control of fatty acid oxidation, suggesting that OEA may also affect this process. In support of this possibility, we found that stimulation of rat soleus muscle strips with OEA enhanced fatty acid oxidation in a concentration-dependent manner ($\text{EC}_{50} \approx 2 \mu\text{M}$) (Fig. 5, *A* and *B*), whereas having no effect on glucose uptake or oxidation (Fig. 5*A*). To test the role of PPAR- α in this response, we examined the effect of OEA on soleus muscle strips isolated from PPAR- $\alpha^{-/-}$ mice and wild-type controls. OEA stimulated fatty acid oxidation in tissue excised from wild-type (Fig. 5*C*), but not PPAR- $\alpha^{-/-}$ mice (Fig. 5*C*). As is the case with lipolysis, oleic acid and anandamide had no effect on fatty acid oxidation (Fig. 5*D*). The ability of OEA to enhance fatty acid oxidation was not limited to skeletal muscle, as similar results were obtained in primary cultures of rat heart myocytes and in dissociated rat hepatocytes (Fig. 5*E*). Although modest ($21 \pm 4\%$ stimulation after a 30-min incubation), $10 \mu\text{M}$ OEA-induced ketogenesis in hepa-

FIG. 4. OEA regulates gene expression in adipose tissue and soleus muscle. A–J, effects of vehicle (V) or OEA (O; 10 mg kg⁻¹, intraperitoneal) on mRNA levels (6 h after injection to rats) of PPAR- α (A and F), f-FABP (B and G), FAT/CD36 (C and H), UCP-2 (D and I), and GAPDH (E and J) used as an internal control in rat adipose tissue (A–E) and soleus muscle (F–J). mRNA levels are expressed in arbitrary units. Significantly different from the respective controls: *, $p < 0.05$; **, $p < 0.01$; $n = 3$.

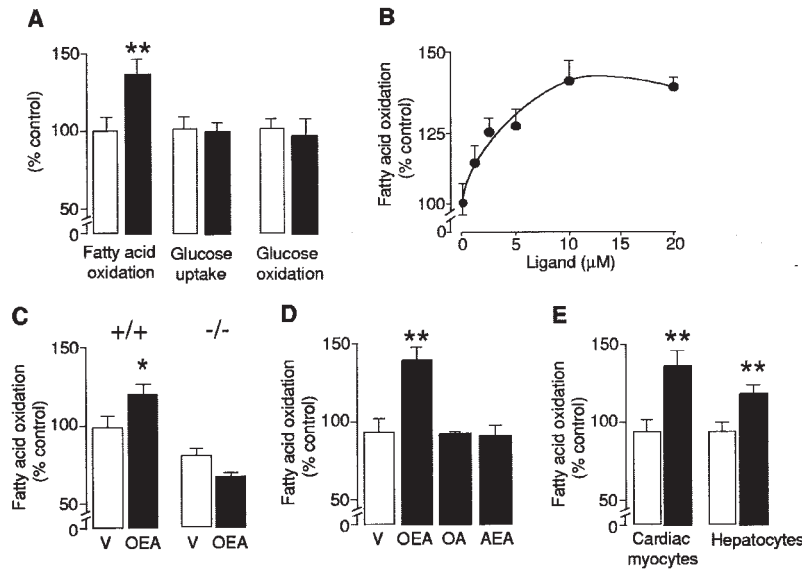
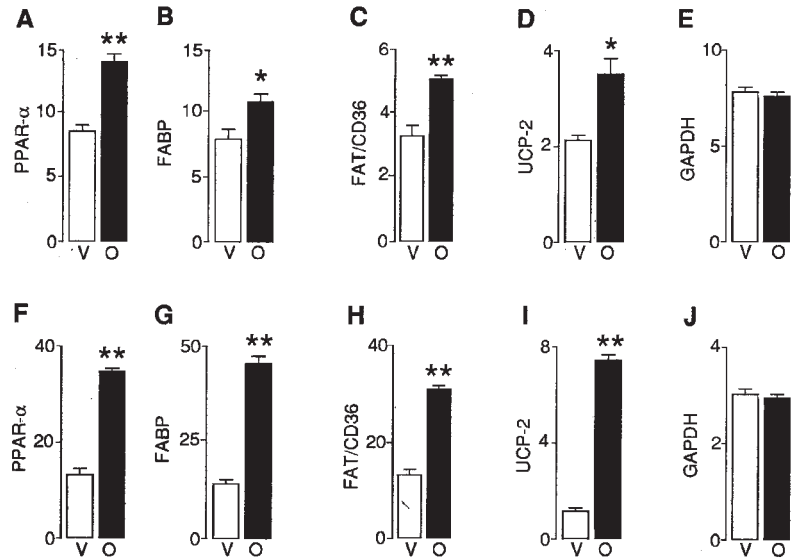


FIG. 5. OEA stimulates fatty acid oxidation in muscle, heart, and liver cells. A, fatty acid oxidation, glucose uptake, and glucose oxidation in soleus muscle strips incubated for 30 min with vehicle (open bars) or OEA (10 μ M, closed bars). B, concentration-dependent effects of OEA on soleus muscle fatty acid oxidation. C, fatty acid oxidation in soleus muscle strips incubated for 100 min with either vehicle (open bars) or OEA (10 μ M, closed bars), obtained from wild-type (+/+) or PPAR- α (-/-) mice. D, effects of vehicle (V), OEA, oleic acid (OA), or anandamide (AEA), each at 10 μ M, on soleus muscle fatty acid oxidation. E, fatty acid oxidation in primary cultures of cardiac myocytes incubated for 30 min with vehicle (open bars) or OEA (10 μ M, closed bars), and in isolated hepatocytes incubated for 10 min with vehicle (open bars) or OEA (10 μ M, closed bars). Significantly different from vehicle incubations: *, $p < 0.05$; **, $p < 0.01$; $n = 4-6$.

toocytes was comparable with that produced by 20 nM glucagon, a potent ketogenic stimulus ($33 \pm 5\%$ stimulation; $n = 4$).

Role of Energy Utilization in the Anti-obesity Effects of OEA—Subchronic administration of OEA to obese rats or mice inhibits food intake and body weight gain (11, 14), however, it remains unclear whether the weight-suppressing effects of this compound may be entirely attributed to its anorexiatic properties. To address this problem we first induced obesity in rats by feeding them with a high-fat diet for 8 weeks and then treating them once daily with OEA (5 mg kg⁻¹, intraperitoneal) or vehicle for 2 additional weeks. In keeping with previous results (11, 14), we found that OEA produced a substantial attenuation of body weight gain (Fig. 6A), which was accompanied by a small, but significant reduction in the amount of food consumed throughout the treatment period (Fig. 6B). The compound also reduced visceral fat mass (Fig. 6C) and triacylglycerol content in both white fat (Fig. 6D) and liver (Fig. 6E).

It seemed unlikely that the modest reduction in food intake caused by prolonged OEA treatment could account for the observed changes in body weight gain and visceral fat mass. To clarify this point, we incorporated in the experiment a third group of rats, which received the same amount of food consumed by OEA-treated animals along with vehicle injections. These pair-fed rats gained as much weight as did control,

free-feeding animals (Fig. 6A). Moreover, in these animals visceral fat mass (Fig. 6C) and triacylglycerol content in adipose tissue (Fig. 6D) and liver (Fig. 6E) were identical to those of free-feeding controls. Thus, whereas OEA reduces body weight gain in lean rats primarily by inhibiting food intake (11), the actions of this compound in obese animals might involve a distinct mechanism, possibly linked to its ability to stimulate fatty acid utilization.

DISCUSSION

The main finding of this study is that OEA stimulates lipolysis and fatty acid oxidation through activation of the nuclear receptor PPAR- α . The effects of OEA were observed both *in vitro* and *in vivo*, and the role of PPAR- α in such effects was supported by two complementary observations. First, OEA was potent at stimulating lipolysis and fatty acid oxidation in wild-type mice, but not in mutant mice deficient in PPAR- α ; second, the synthetic PPAR- α agonist Wy-14643 exerted similar effects, which were also contingent on PPAR- α expression. These results are consistent with the proposed role of OEA as a high affinity agonist for PPAR- α (14) and reveal a regulatory role of this lipid mediator in two distinct aspects of energy balance, namely feeding (11, 14) and fat utilization (present study).

We have previously (11, 14) reported that OEA, by activating

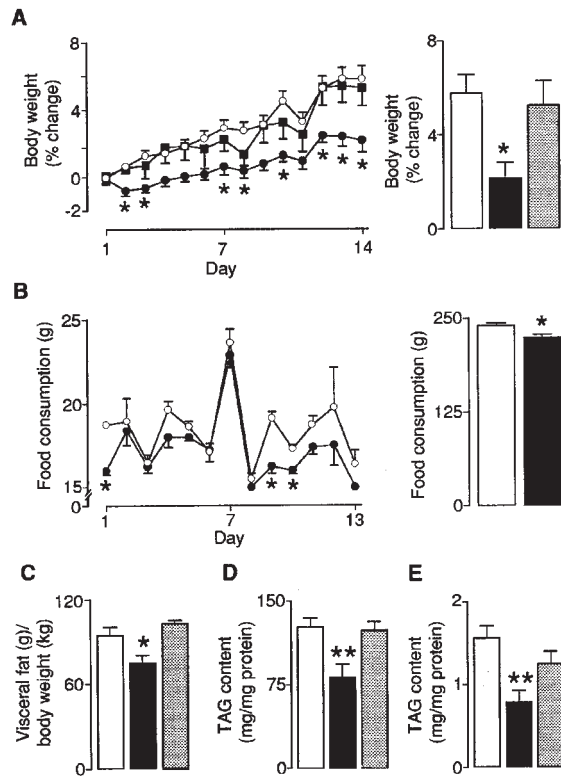


FIG. 6. Role of energy metabolism in the anti-obesity effects of OEA. A, time course of the effects of vehicle (open circles), OEA (5 mg kg⁻¹, intraperitoneally once daily; closed circles), or pair-feeding (closed squares) on body weight change in obese rats. Right panel, cumulative effects of vehicle (open bars), OEA (closed bars) or pair-feeding (shaded bars) on body weight change. B, time course of the effects of vehicle (open circles) or OEA (5 mg kg⁻¹, intraperitoneally once daily; closed circles) on food intake in obese rats. Right panel, cumulative effects of vehicle (open bars) or OEA (closed bars) on food intake. C–E, effects of vehicle (open bars), OEA (closed bars), or pair-feeding (shaded bars) on visceral fat mass (inclusive of epididymal, retroperitoneal, and mesenteric fat pads) (C), triacylglycerol (TAG) levels in epididymal fat (D), and triacylglycerol levels in liver (E). Significantly different from control animals: *, $p < 0.05$; **, $p < 0.01$; $n = 6–8$.

PPAR- α , reduces body weight gain in various rodent models. The present findings raise the question of whether PPAR- α -mediated fat utilization contributes to these effects (14). The answer to this question appears to depend on the particular model under consideration. Pair-feeding experiments indicate that induction of satiety may account for the weight-reducing actions of OEA in lean rats (11), but not in rats made obese by a high-fat diet (present study). Thus, in the latter model OEA-mediated changes in lipid metabolism might play a role. This hypothesis is confirmed by the marked ability demonstrated by OEA to lower tissue triacylglycerol levels. The stimulatory effects of this lipid mediator on fatty acid oxidation may also be significant in this regard, as OEA is able to induce the expression of PPAR- α -regulated genes that participate in fatty acid utilization (FAT/CD36, FABP) and energy balance (UCP-2).

Another pressing question raised by our results relates to the molecular mechanism underlying the rapid onset lipolytic actions of OEA. Although PPAR- α activation regulates expression of lipid transporters and lipid-metabolizing enzymes (15, 16), the time course of OEA-induced lipolysis suggests that it may be initiated by non-genomic signaling events. Such events, and particularly the activation of membrane phosphatidylinositol-3-OH kinase, have been shown to contribute in important ways to the action of estrogen receptors (32), but they have not yet been documented for PPAR- α or other members of the PPAR family. Elucidating these events may not only help to

understand the mechanism by which OEA induces lipolysis, but also provide important clues on the ability of the compound to induce satiety (11, 13).

The physiological significance of the metabolic actions of OEA is still undefined, although the relatively high levels of OEA found in white fat (rats, 106 \pm 9 pmol/g; mice, 316 \pm 46 pmol/g; $n = 5–6$) suggest that this compound may play a local modulatory role in this tissue, possibly complementary to those of adipocyte-derived signaling proteins such as leptin (33) and adiponectin (34). Noteworthy, the magnitude of OEA-induced stimulation of fatty acid oxidation in skeletal muscle, the tissue responsible for the bulk of whole body fatty acid oxidation, is comparable with that exerted by leptin (35) and adiponectin (36). However, unlike the latter, our findings suggest that OEA may not have a significant impact on glucose metabolism.

Synthetic PPAR- α agonists such as the fibrates (e.g. fenofibrate) are currently used in the clinic as lipid-lowering and anti-atherogenic drugs. However, the potencies of these compounds at PPAR- α are 500–900 times lower than that of OEA (14) and, consequently, they do not significantly affect food intake (14, 37). The pharmacological profile of OEA suggests that this natural lipid may provide a scaffold for the design of novel anti-obesity drugs with combined anorexiatic and lipolytic properties.

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