You might find this additional information useful...

This article cites 43 articles, 20 of which you can access free at:
http://ajpregu.physiology.org/cgi/content/full/289/3/R729#BIBL

This article has been cited by 3 other HighWire hosted articles:

**Targeted enhancement of oleoylethanolamide production in proximal small intestine induces across-meal satiety in rats**
J. Fu, J. Kim, F. Oveisi, G. Astarita and D. Piomelli
[Abstract]  [Full Text]  [PDF]

**Postprandial increase of oleoylethanolamide mobilization in small intestine of the Burmese python (Python molurus)**
G. Astarita, B. C. Rourke, J. B. Andersen, J. Fu, J. H. Kim, A. F. Bennett, J. W. Hicks and D. Piomelli
[Abstract]  [Full Text]  [PDF]

**Highlights from the Literature**
[Full Text]  [PDF]

Updated information and services including high-resolution figures, can be found at:
http://ajpregu.physiology.org/cgi/content/full/289/3/R729

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:
http://www.the-aps.org/publications/ajpregu

This information is current as of October 16, 2008.
Mechanisms of oleoylethanolamide-induced changes in feeding behavior and motor activity

Karine Proulx,1,2 Daniela Cota,2 Tamara R. Castañeda,2 Matthias H. Tschöp,2 David A. D’Alessio,2 Patrick Tso,2 Stephen C. Woods,2 and Randy J. Seeley2

1Department of Neuroscience, University of Cincinnati, Cincinnati; and 2Department of Internal Medicine, and Department of Pathology and Laboratory Medicine, Genome Research Institute, Cincinnati, Ohio

Submitted 18 January 2005; accepted in final form 27 April 2005

Proulx, Karine, Daniela Cota, Tamara R. Castañeda, Matthias H. Tschöp, David A. D’Alessio, Patrick Tso, Stephen C. Woods, and Randy J. Seeley. Mechanisms of oleoylethanolamide-induced changes in feeding behavior and motor activity. Am J Physiol Regul Integr Comp Physiol 289: R729 –R737, 2005. First published May 5, 2005; doi:10.1152/ajpregu.00029.2005.—Oleoylethanolamide (OEA), a lipid synthesized in the intestine, reduces food intake and stimulates lipolysis through peroxisome proliferator-activated receptor-α. OEA also activates transient receptor potential vanilloid type 1 (TRPV1) in vitro. Because the anorexigenic effect of OEA is associated with delayed feeding onset and reduced locomotion, we examined whether intraperitoneal administration of OEA results in nonspecific behavioral effects that contribute to the anorexia in rats. Moreover, we determined whether circulating levels of other gut hormones are modulated by OEA and whether CCK is involved in OEA-induced anorexia. Our results indicate that OEA reduces food intake without causing a conditioned taste aversion or reducing sodium appetite. It also failed to induce a conditioned place aversion. However, OEA induced changes in posture and reduced spontaneous activity in the open field. This likely underlies the reduced heat expenditure and sodium consumption observed after OEA injection, which disappeared within 1 h. The effects of OEA on motor activity were similar to those of the TRPV1 agonist capsaicin and were also observed with the peroxisome proliferator-activated receptor-α agonist Wy-14643. Plasma levels of ghrelin, peptide YY, glucagon-like peptide 1, and apolipoprotein A-IV were not changed by OEA. Finally, antagonism of CCK-1 receptors did not affect OEA-induced anorexia. These results suggest that OEA suppresses feeding without causing visceral illness and that neither ghrelin, peptide YY, glucagon-like peptide 1, apolipoprotein A-IV, nor CCK plays a critical role in this effect. Despite that OEA-induced anorexia is unlikely to be due to impaired motor activity, our data raise a cautionary note in how specific behavioral and metabolic effects of OEA should be interpreted.

OEA is a structural analog of the endocannabinoid anandamide but does not activate cannabinoid receptors (32). It is rather an endogenous ligand for the peroxisome proliferator-activated receptor-α (PPAR-α), through which it has been reported to stimulate lipolysis (15) and inhibit feeding (13, 34). Indeed, OEA reduces food intake when administered peripherally (13, 27, 31, 34) but appears to be ineffective at doing so when administered centrally (34). In ad libitum-fed rats, the suppression of food intake is associated with increased latency to initiate a meal without change in meal size or postmeal interval, whereas in fasted rats OEA increases latency to initiate a meal and reduces size of the first meal (14). Furthermore, chronic administration of OEA can reduce the rate of body weight gain in both lean (34) and obese animals (15). All of these effects are absent in mice that lack PPAR-α, supporting a critical role for these receptors in the metabolic effects of OEA (13).

Rodriguez de Fonseca et al. (34) reported that although intraperitoneal administration of OEA reduces food intake without causing a conditioned taste aversion (CTA), it potently reduces locomotor activity in rats. Although the effects of OEA on feeding appear to be mediated via PPAR-α activation (13), the mechanisms by which it reduces locomotion are unknown. It is also unknown whether other PPAR-α agonists have independent effects on locomotor activity as well. On the other hand, OEA activates the transient receptor potential vanilloid type 1 (TRPV1) in vitro (1). This receptor is widely distributed in the nervous system, including on dopaminergic neurons of the substantia nigra (22), a region well known to be involved in the regulation of motor activity. Interestingly, capsaicin-induced activation of the TRPV1 causes a reduction in ambulation as well as other motor behaviors such as rearing and grooming in the open-field test (12).

Although the effects of OEA on feeding behavior are intriguing, consideration of OEA as a target for pharmacological treatment of obesity requires that several basic questions be addressed. Thus the fact that OEA reduces locomotor behavior (34) is noteworthy given that OEA reduces food intake by delaying meal initiation and reducing first meal size (14). Given these behavioral effects, we reassessed the possibility that intraperitoneal administration of OEA results in nonspecific behavioral effects, visceral illness, and/or aversion, which contribute to the reduced food intake. To examine this possibility, we used three very different paradigms: CTA, need-induced sodium appetite, and conditioned place aversion.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org 0363-6119/05 $8.00 Copyright © 2005 the American Physiological Society R729
(CPA). Furthermore, because of the potential involvement of reduced motor activity in the anorexigenic effect of OEA, we characterized the effects of OEA on motor behavior using computer-based video analysis and an open-field test. We explored whether TRPV1 receptor activation can alter motor behavior, and we predicted that a TRPV1 agonist would recapitulate the hypolocomotor effects of OEA, whereas a PPAR-α agonist would not. Assessing the metabolic consequences of OEA administration is also important in studying its role in energy homeostasis. Thus, because OEA upregulates uncoupling protein-2 (UCP-2) mRNA in both white adipose tissue (WAT) and skeletal muscle (15), we tested whether OEA increases energy expenditure using indirect calorimetry.

OEA shares some similarities with several gut hormones involved in the regulation of food intake. Like OEA, ghrelin, peptide YY (PYY), glucagon-like peptide 1 (GLP-1), apolipoprotein A-IV (apoA-IV), and cholecystokinin (CCK) are involved in the regulation of food intake. Like OEA, ghrelin, PYY, GLP-1, and apoA-IV at several time points after OEA administration. Given that CCK (25), like OEA (34), is synthesized in the intestine and mediates its anorexigenic signal via the vagus nerve, we tested a specific role for endogenous CCK in the effects of OEA with the use of a pharmacological antagonist of the CCK-1 receptor.

MATERIALS AND METHODS

Animals

Male Long-Evans rats, weighing 250–300 g at the beginning of the experiments, were individually housed and maintained on a 12:12-h light-dark cycle. All animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Drugs

OEA was purchased from Tocris Cookson (Ellisville, MO). Lorglumide, CCK-8, Wy-14643, and capsaicin were purchased from Sigma (St. Louis, MO). OEA was dissolved in 5% Tween 80, 5% propylene glycol, and 90% physiological saline. CCK-8, apolipoprotein A-IV (apoA-IV), and lorglumide were dissolved in 5% Tween 80, 5% propylene glycol, and 90% physiological saline. Lorglumide, CCK-8, and furosemide were dissolved in physiological saline. Lithium chloride (LiCl) was dissolved in sterile distilled water (except in experiment 1, where LiCl was dissolved in isotonic saline). The stock solution was then dried under nitrogen after addition of Tween 80 and reconstituted in physiological saline (Tween 80-saline; 1:16 vol/vol). Wy-14643 was dissolved in 100% ethanol. The stock solution was then dried under nitrogen after addition of Tween 80 and reconstituted in physiological saline. Capsaicin was first dissolved in 5% Tween 80, 5% propylene glycol, and 90% physiological saline. Capsaicin was first dissolved in 100% ethanol. The stock solution was then dried under nitrogen after addition of Tween 80 and reconstituted in physiological saline (Tween 80-saline; 1:16 vol/vol). Wy-14643 was dissolved in 70% DMSO and 30% physiological saline. Lithium chloride (LiCl) was dissolved in sterile distilled water (except in experiment 2), whereas lorglumide, CCK-8, and furosemide were dissolved in physiological saline. For each experiment, vehicle served as the control solution, except for LiCl where saline was used as a control. All drugs were injected intraperitoneally in a volume of 1 ml/kg, unless otherwise specified. OEA was administered at 5, 10, and 20 mg/kg (34) in experiment 1. Given that 20 mg/kg was the only dose that remained effective at reducing food intake 1 h after the injection, this dose was used in all subsequent experiments.

Experimental Design

Experiment 1: effect of OEA on food intake. Rats were assigned to one of four treatment groups (n = 8 or 9/group). Thirty minutes before the onset of the dark phase, 24-h fasted rats received an injection of either vehicle or OEA (5, 10, or 20 mg/kg) (34) and then received access to food. The food hoppers were weighed at 30 min and 1, 2, 4, and 24 h after the injection.

Experiment 2: effect of OEA on CTA. If rats become ill after consumption of a novel flavor, they will avoid consumption of this flavor at future opportunities (33). An example of this is reduced consumption of a flavor that has been previously paired with toxins such as LiCl (3). To test whether the anorexigenic effect of OEA is secondary to illness, rats were assigned to one of two groups (saline-LiCl or vehicle-OEA; n = 10/group), and preference ratios for novel flavors were determined after pairing with the drugs. The preference ratio for a drug is calculated as the intake of the drug-paired flavor over the total intake of both flavors and the critical comparison is whether the ratio for either flavor deviates from 0.5. All animals were trained for 10 days on a water deprivation schedule during which they had access to two water bottles for 1 h/day. On conditioning day 1, all rats had access to two bottles of saccharin-sweetened Kool-Aid (5.25 g of saccharin, 3,500 ml of water, and one packet of either cherry or grape Kool-Aid; both bottles contained the same flavor, in a counterbalanced manner across rats and groups) for 1 h. After access to flavor 1, half of the rats from each group received either saccharin-sweetened Kool-Aid (the other half of the rats received either LiCl or vehicle) for 1 h. The next day, rats had access to two water bottles for 1 h, as a rest day. On conditioning day 2, rats had access to two bottles of flavor 2 for 1 h. Rats that were injected with saline or vehicle on conditioning day 1 were then injected with LiCl or OEA and vice versa. Rats then had 2 rest days in which they had access to two bottles of water for 1 h. On the test day, rats received one bottle of each flavor, with the side of flavor presentation being switched at 30 min to avoid any side preference. Fluid intake was measured at 1 h, and water was returned at the end of the experiment.

Experiment 3: effect of OEA on need-induced sodium appetite. Another reliable index of visceral illness is failure to consume hypertonic saline solution in response to sodium depletion. For instance, when rats rendered sodium depleted are injected with emetic and/or toxic agents such as LiCl, they reduce their “need-induced” consumption of hypertonic saline (9). To assess whether OEA is aversive by this definition, rats were assigned to one of two treatment groups (vehicle or OEA, n = 7/group) and need-induced sodium appetite was assessed. For 7 days, rats had access to two bottles, one that contained water and another one that contained a 0.5 M NaCl solution. Twenty-four hours before the experiment, the saline bottle was removed, and the food hopper with regular diet was replaced with one that contained sodium-free rat diet (ICN Biochemicals, Cleveland, OH). Water remained available at all times. Rats were then weighed and received two subcutaneous injections of the diuretic furosemide (5 mg/kg), 1 h apart (4). Rats were weighed again 3 h after the first injection, and diuresis (and presumed sodium depletion) was confirmed by observing at least 18 g of body weight loss. Twenty-four hours after the first furosemide injection, rats received an intraperitoneal injection of isotonic LiCl (0.15 M in a volume equivalent to 2% of the rat’s body weight), an equal volume of physiological saline, OEA (20 mg/kg), or vehicle. Fifteen minutes later, rats received two bottles; one that contained distilled water and another one that contained a 0.5 M NaCl solution. Intake of both fluids was measured every 30 min for 2 h and 24 h after the injection.
to 2% of the rat’s body weight) or OEA (20 mg/kg) and were immediately placed on one side of the CPA box with the door closed. On the other set of alternate days, rats received either saline or vehicle and were immediately placed in the alternate side of the box from that paired with the drug on the previous day, and the door was closed. Conditions were counterbalanced across rats and groups. On the 7th day (test), rats were placed into the middle chamber and were allowed to explore the apparatus with both guillotine doors opened for 15 min. Rats were videotaped, and time spent in each chamber was scored by an investigator who was blind to the drug treatment. An animal was considered to be in a chamber when its head and forepaws were inside it. Results are expressed as the time spent in the drug-paired side.

Experiment 5: effect of OEA on heat expenditure. Rats were assigned to one of two treatment groups (vehicle or OEA, n = 8/group). Four hours before the testing procedure, rats were placed in indirect calorimeter chambers for habituation. They were then injected with either vehicle or OEA (20 mg/kg) 30 min before the onset of the dark and returned to the indirect calorimeter chamber immediately after the injection. Food was removed, but water remained available throughout the experiment. Heat production was recorded every 24 min for 24 h.

Experiment 6: comparison of the effects of OEA and capsaicin on behaviors in the home cage. This study examined the effects of OEA on behaviors displayed by rats in their home cage. Fifteen minutes before the onset of the dark phase, rats (n = 5–7/group) received an injection of OEA (20 mg/kg), capsaicin (1 mg/kg) (12), or their respective vehicles and were immediately returned to their home cage.

Experiment 7: comparison of the effects of OEA, capsaicin, and Wy-14643 in the open-field test. This experiment compared the effects of OEA, capsaicin, and Wy-14643 on motor behaviors. Rats (n = 5–7/group) were injected with OEA (20 mg/kg), capsaicin (1 mg/kg) (12), Wy-14643 (40 mg/kg) (13), or their respective vehicles 10 min before the testing. The dimensions of the open-field were 36 × 45 cm, and the height of the walls was 30 cm. The floor was divided into 20 squares (9 × 9 cm). Rats were placed in the middle of the open field, and they were videotaped for 10 min. Only the last 5 min of the tape were analyzed, with the first 5 min serving as a habituation period. Time spent in inactivity, ambulation (number of squares crossed), and spontaneous activity (number of rearing or grooming events) were measured (12). Rats were considered to have crossed a square when they placed their four paws into an adjacent square. Tapes were scored by an investigator who was blind to the drug treatment.

Experiment 8: effects of OEA on plasma levels of ghrelin, PYY, GLP-1, and apoA-IV. Rats were injected with either OEA (20 mg/kg) or vehicle 30 min before onset of the dark period and were killed at 15, 30, 90, and 120 min after the injection. An additional group of rats (n = 8) remained uninjected and served as baseline controls. Trunk blood samples were collected into tubes that contained an antiproteolytic cocktail (25 mM EDTA plus 500 kallikrein inhibitory units aprotinin and 80 U heparin/ml of blood). Blood was then centrifuged at 4°C for 10 min at 4,000 rpm followed by 2 min at 12,000 rpm. Separated plasma was stored at −20°C until assay. Plasma ghrelin (38) and PYY (36) concentrations were determined with the use of

---

Fig. 1. Mean ± SE food intake at different time points (A) and at 24 h (B) after an intraperitoneal injection of vehicle (Veh) or 3 doses of oleoylthanolamide (OEA) in 24-h fasted rats. *P < 0.05, **P < 0.01, and ***P < 0.001.

Fig. 2. Mean ± SE preference ratio of flavors previously paired with an intraperitoneal injection of saline (Sal), lithium chloride (LiCl), Veh, or OEA (20 mg/kg). ***P < 0.001.

Fig. 3. Mean ± SE 2-h sodium intake after an intraperitoneal injection of Sal, LiCl, Veh, or OEA (20 mg/kg) in sodium-depleted rats. ***P < 0.001.
commercial RIA kits according to the manufacturer’s directions (Phoenix Pharmaceuticals, Belmont, CA) as previously described. The ghrelin assay measures both acylated and desacetylated ghrelin, and the PPY assay measures both PYY-(1–36) and PYY-(3–36). Total plasma GLP-1 concentration was measured by RIA using antiserum 89390 (kindly provided by Dr. Jens Holst, Paanum Institute, Copenhagen, Denmark) from ethanol extracts of plasma as previously described (30). This antibody recognizes both the intact hormone GLP-1-(7–36)-NH₂ and the metabolite GLP-1-(9–36)-NH₂. The intra- and interassay coefficients of variation for these assays were, respectively, <5% and <13% for ghrelin, <7% and <11% for GLP-1, and <8.42% and <14.52% for PYY. Plasma apoA-IV concentration was measured by an ELISA as previously described (20).

Experiment 9: effect of the CCK-1 antagonist lorglumide on OEA-induced reduction of food intake. To test the involvement of CCK-1 receptors in the anorexigenic effect of OEA, rats were assigned to one of four treatment groups (saline + vehicle, saline + OEA, lorglumide + vehicle, or lorglumide + OEA; n = 6 or 7/group). Thirty minutes before the onset of the dark, 24-h fasted rats received an injection of either saline or lorglumide (1 mg/kg) (16). Fifteen minutes later, rats received a second injection of either vehicle or OEA (20 mg/kg) and then received access to food. The food hoppers were weighed at 30 min and 1, 2, 4, and 24 h after the last injection. As a positive control, we tested the ability of the same dose of lorglumide to block the anorexigenic effect of CCK-8. Rats were assigned to one of four treatment groups (saline + saline, saline + CCK-8, lorglumide + saline, or lorglumide + CCK-8; n = 8 or 9/group). Two hours before the onset of the dark, 22-h fasted rats received an injection of either saline or lorglumide (1 mg/kg) (16). Fifteen minutes later, rats received a second injection of either saline or CCK-8 (4 μg/kg) (24) and then received access to food. Food hoppers were weighed at 15, 30, and 45 min and 1 h after the last injection.

Statistical Analysis

Changes in food intake, sodium intake, and heat expenditure across time were analyzed by one-way or two-way repeated-measures ANOVA, unless otherwise specified in the results. Significant ANOVAs were followed by least significant difference post hoc tests. Preference ratios, time spent in the drug-paired side, hormones levels, and behaviors in the home cage and in the open field were analyzed

Fig. 4. Mean ± SE time that rats spent in the side previously paired with an intraperitoneal injection of Sal, LiCl, Veh, or OEA (20 mg/kg). *P < 0.05.

Fig. 5. Mean ± SE heat expenditure at different time points after an intraperitoneal injection of either Veh or OEA (20 mg/kg) (A) and mean ± SE heat expenditure per hour during the 12-h dark phase (B) in fasted rats. ***P < 0.001.

Fig. 6. Mean ± SE time that rats displayed an extended posture in the 2 h after an intraperitoneal injection of either Veh or OEA (20 mg/kg) (A) and either Veh or capsaicin (CAPS) (1 mg/kg) (B). **P < 0.01.
RESULTS

Experiment 1: Effect of OEA on Food Intake

OEA significantly reduced 30-min food intake compared with saline at 10 mg/kg (\(P < 0.05\); see Fig. 1A) and 20 mg/kg (\(P < 0.001\); Fig. 1A), with the 20 mg/kg suppressing 99.48% of food intake. One hour after the injection, only the 20 mg/kg dose caused a significant reduction in food intake (\(P < 0.01\); Fig. 1A), and this effect remained significant 24 h after the injection (\(P < 0.05\); Fig. 1B).

Experiment 2: Effect of OEA on CTA

The positive control LiCl caused a CTA, as indicated by the significantly lower preference ratio for the LiCl-paired flavor compared with the saline-paired flavor (\(P < 0.001\); Fig. 2). In contrast, there was no significant difference between the preference ratio of the flavor previously paired with OEA compared with the vehicle-paired flavor (\(P = 0.746\); Fig. 2), indicating that rats did not develop a CTA to OEA.

Experiment 3: Effect of OEA on Need-Induced Sodium Appetite

LiCl significantly reduced need-induced sodium appetite compared with saline at all time points (\(P < 0.001\); Fig. 3 for 2 h, data at other time points not shown) for at least 24 h (\(P < 0.05\)). Two-tailed \(t\)-test comparisons indicated that sodium intake was significantly suppressed by OEA relative to vehicle at 30 min (OEA: 4.37 ± 1.03 g vs. vehicle: 10.03 ± 0.91 g; \(P < 0.01\)), although to a lesser extent than by LiCl (LiCl: 1.67 ± 0.12 g; \(P < 0.05\)). In contrast to rats injected with LiCl, OEA-injected rats rapidly compensated for this acute reduction, as this effect had disappeared at 1 h (OEA: 8.43 ± 1.06 g vs. vehicle: 11.12 ± 2.66 g; \(P = 0.090\)). Indeed, one-way repeated-measures ANOVA revealed that OEA did not have a significant effect relative to vehicle over 24 h (\(P = 0.482\); Fig. 3 for 2 h, data at other time point not shown).

Experiment 4: Effect of OEA on CPA

Rats in the LiCl-saline group spent significantly less time in the LiCl-paired side compared with the saline-paired side (\(P < 0.05\); Fig. 4), indicating the formation of a CPA to LiCl. In

Fig. 7. Mean + SE time that rats spent in inactivity (A), number of times the rats crossed a square (B), and number of times the rats either reared or groomed (spontaneous activity) (C) after an intraperitoneal injection of OEA (20 mg/kg), CAPS, (1 mg/kg), Wy-14643 (40 mg/kg), or their respective Veh. *\(P < 0.05\), **\(P < 0.01\), and ***\(P < 0.001\).
contrast, OEA did not cause a CPA because rats in the OEA-vehicle group spent a similar amount of time in both the OEA-paired side and the vehicle-paired side ($P = 0.849$). This result together with the lack of a sustained reduction of sodium appetite and induction of a CTA suggests that acute administration of OEA does not have a toxic or illness-inducing effect in rats.

**Experiment 5: Effect of OEA on Heat Expenditure**

Two-tailed $t$-test comparisons revealed that OEA significantly reduced heat expenditure relative to vehicle when measured for the first 48 min after the injection ($P < 0.001$; Fig. 5A). However, this effect waned by 72 min ($P = 0.341$; Fig. 5A), and one-way repeated-measures ANOVA indicated that there was no significant effect of OEA treatment over the course of either the 12-h dark ($P = 0.367$; Fig. 5B) or the 12-h light phase ($P = 0.403$; data not shown).

**Experiment 6: Comparison of the Effects of OEA and Capsaicin on Behaviors in Home Cage**

OEA significantly increased the time that rats displayed an extended posture, that is, pushing their abdomen against the floor of the cage with splayed hindlimbs, compared with vehicle ($P < 0.01$; Fig. 6A), whereas capsaicin did not cause a significant effect on this behavior ($P = 0.334$; Fig. 6B). There was no significant change in the other behaviors analyzed after either OEA or capsaicin administration compared with their respective vehicles ($P > 0.05$).

**Experiment 7: Comparison of the Effects of OEA, Capsaicin, and Wy-14643 in the Open-Field Test**

Rats treated with OEA were significantly more inactive ($P < 0.001$; Fig. 7A) and had significant decreases in ambulation ($P < 0.01$; Fig. 7B) and spontaneous activity ($P < 0.001$; Fig. 7C) compared with vehicle-treated rats. The effect of OEA on inactivity was recapitulated by Wy-14643 ($P < 0.05$; Fig. 7A) but not by capsaicin ($P = 0.164$; Fig. 7A). However, neither Wy-14643 ($P = 0.420$; Fig. 7B) nor capsaicin ($P = 0.208$; Fig. 7B) had a significant effect on ambulation. Similar to OEA, both Wy-14643 ($P < 0.01$; Fig. 7C) and capsaicin ($P < 0.05$; Fig. 7C) significantly inhibited spontaneous activity compared with their respective vehicles.

**Experiment 8: Effects of OEA on Plasma Levels of Ghrelin, PYY, GLP-1, and apoA-IV**

OEA did not cause any significant change in plasma levels of ghrelin, PYY, GLP-1, and apo A-IV relative to vehicle, at any of the time points that were examined ($P > 0.05$; Fig. 8).
**DISCUSSION**

As previously reported (13, 34), intraperitoneal administration of OEA reduces food intake and, at 20 mg/kg, food intake is almost completely suppressed for the first 30 min. The anorexigenic effect of OEA is accompanied by a suppression of locomotor activity. In particular, the injection of OEA is followed by a unique behavior in which the rat takes on an extended posture (pushing its abdomen against the floor of the cage) with splayed hindlimbs and little ambulatory activity. This behavior resembles the “lying on belly” that is caused by toxins that produce visceral illness (3). However, on two sensitive assessments of visceral illness, OEA had a minimal effect, consistent with previous findings (34). Moreover, OEA did not induce the formation of a CPA. From these results, it is difficult to ascribe the anorectic effects of OEA to illness or aversion. Therefore, it seems likely that suppression of food intake by OEA involves specific actions on pathways regulating energy homeostasis.

Illness in rats manifests as a pattern of well-established stereotypic behaviors. CTA is one of the hallmark behaviors of visceral illness, and CTA tests rely on the ability to learn the association between a flavor and an aversive stimulus (33). In these studies, OEA did not support a CTA. However, other members of the fatty acid ethanolamide family, such as anandamide, have been reported to cause anterograde amnesia (2). Thus it is possible that OEA could induce visceral illness but not produce a CTA because of a separate effect to impair memory. To avoid being misled by this potential confounder, we used the additional measure of need-induced sodium appetite (9). Although OEA caused some reduction in sodium consumption compared with vehicle within the first hour after the injection, this effect dissipated more rapidly than the effect on food intake and was much shorter than the effect of LiCl. Together, these data are consistent with the hypothesis that the effect of OEA to suppress food intake is not secondary to visceral illness. Moreover, because the ingestion of the NaCl solution involves much of the same motor behavior as ingestion of food, it is unlikely that the anorexia is simply a result of the reduced motor activity, at least at time points beyond 1 h.

Given the negative results of the visceral illness and conditioned aversion tests, the reduced locomotor activity and unusual posturing that we observed after OEA administration require an alternative explanation. We therefore further characterized the behavior caused by OEA administration using computer-based video analysis. This assay confirmed that rats display an extended posture with splayed hindlimbs early after an intraperitoneal injection of OEA. This behavior is also followed by signs that resemble those of catalepsy. Although the pharmacological effects of OEA are not due to activation of any of the known cannabinoid receptors (32), it is noteworthy that we have observed comparable behavior in rats given intraperitoneal anandamide (unpublished data). Reduced locomotor activity, ataxia, and catalepsy are indeed commonly observed after administration of cannabinoid agonists (35). Interestingly, stearoylanethanolamide is another member of the fatty acid ethanolamide family that does not activate cannabinoid receptors but that causes cannabimimetic effects, including catalepsy (21).

In the open-field test, OEA not only reduced locomotion but also reduced grooming and rearing, while increasing time spent in inactivity. We compared these behavioral effects of OEA to agonists of either PPAR-α or TRPV1. Agonists of TRPV1 such as capsaicin have previously been reported to reduce motor activity (12), and our data indicate that this effect is similar to what occurs with OEA. Like OEA, capsaicin significantly reduced spontaneous activity. Capsaicin also showed a trend to reduce ambulation and to increase inactivity and extended posture. Because of this trend, we cannot rule out the possibility that this is a real effect of capsaicin that would be revealed with higher numbers of animals.

---

**Experiment 9: Effect of Lorglumide on OEA-Induced Reduction of Food Intake**

As reported in experiment 1, OEA significantly reduced food intake relative to vehicle (1-h intake: P < 0.01, 2-h intake: P < 0.001; Fig. 9A). At none of the time points examined was this effect significantly modified by pretreatment with lorglumide relative to saline (P > 0.05; Fig. 9A), whereas the same dose of lorglumide was effective at blocking the anorexigenic effect of CCK-8 relative to saline (15- and 30-min intakes: P < 0.05; Fig. 9B).

---

**Fig. 9.** Mean ± SE food intake at different time points after an intraperitoneal injection of either Sal or lorglumide (LORGL) (1 mg/kg), followed by an intraperitoneal injection of either Veh or OEA (20 mg/kg) (A) and either Sal or CCK-8 (4 μg/kg) (B) in fasted rats. *P < 0.05 and **P < 0.01.
Given that there have been no previous descriptions of the effects of PPAR-α to reduce motor behavior, we were surprised to see a potent effect of Wy-14643 to reduce motor activity. Although these observations are consistent with the distribution of TRPV1 and PPAR-α in areas associated with motor activity such as the substantia nigra compacta and the striatum (22, 26), both receptors are widely distributed; therefore, a firm conclusion about the mechanism for this effect is difficult. It remains unclear how OEA induces postural changes and suppresses motor activity.

Because activation of the PPAR-α is necessary for OEA’s effect on food intake (13), the present data raise the possibility that the locomotor effects are also the result of agonism of PPAR-α. Interestingly, it has been reported that capsaicin-induced activation of TRPV1 leads to OEA production in vitro (12). This raises the hypothesis that PPAR-α activation, by ligands of either endogenous (capsaicin-induced production of OEA) or exogenous (administration of either Wy-14643 or OEA) sources, could mediate the motor effects of OEA, capsaicin, and Wy-14643. Nonetheless, further experiments either with highly specific receptor antagonists or with targeted genetic disruption of these receptors will be necessary to conclude whether PPAR-α and/or TRPV1 are involved in the behavioral effects of OEA on motor activity.

Activation of PPAR-α has been associated with reduced body weight gain due to effects on lipid catabolism and energy expenditure. For instance, the PPAR-α ligands Wy-14643 and the fibrate drugs clofibrate, fenofibrate, and bezafibrate are all hypolipemic agents that reduce weight gain in rodent models (40). Moreover, fibrate treatment has been associated with elevation in UCP-1 mRNA in WAT and in UCP-3 in WAT and skeletal muscle in rodents (5). The effects of OEA are consistent with those of fibrates, since OEA upregulates UCP-2 mRNA in both WAT and skeletal muscle (15). However, contrary to what we have predicted, OEA did not increase energy expenditure in rats. We observed a significant reduction in heat during the first 24 min that followed the OEA administration compared with vehicle. This effect is likely the result of the reduced motor activity caused by OEA.

Although it is unlikely that the effects of OEA on food intake are merely by-products of its action on other motor behaviors, our results raise a cautionary note as to how specific behavioral and metabolic data should be interpreted in light of these potent actions. Recent data continue to point to a critical role in the control of food intake for a number of gut hormones whose secretion is either increased or decreased by nutrients being absorbed from the gastrointestinal tract (41). Thus the possibility that OEA could influence food intake by altering the secretion of one or more of these hormones was necessary to consider. We measured circulating plasma levels of ghrelin, PYY, GLP-1, and apoA-IV at several time points after the administration of OEA at a dose that reduces food intake. OEA did not have a significant impact on plasma levels of any of these hormones at any time point compared with vehicle-treated rats. These data are consistent with a recent report indicating that OEA failed to modulate either GLP-1 or ghrelin under free-feeding conditions (5). However, in this study, OEA did reduce plasma levels of acylated ghrelin in fasted animals. Conclusions from the present data are limited by the fact that rats did not have exposure to nutrients after the administration of OEA. Such a design was used to avoid the possibility that drug-induced changes in feeding could influence hormone levels. Consequently, we cannot completely rule out the possibility that OEA modulates the ability of nutrients to alter gut hormone secretion.

Another gut hormone that has been linked to the control of food intake is CCK (25). However, the critical form (CCK-8) is difficult to measure in plasma; in the rodent, several lines of evidence point to paracrine rather than endocrine action of CCK to reduce feeding (8). Thus, to examine whether there may be a role for increased CCK release in the anorexigenic effect of OEA, we took advantage of a well-validated CCK-1 receptor antagonist (16). Although pharmacological antagonism of CCK-1 receptors with lorglumide can block the anorexia induced by exogenous CCK-8, it cannot block the anorexia induced by OEA. Thus it seems unlikely that CCK mediates the effects of OEA on food intake.

**Perspectives**

Together, these data argue against the hypothesis that ghrelin, PYY, GLP-1, apoA-IV, or CCK plays a critical role in the effect of OEA to suppress food intake, at least under free-feeding conditions. Previous data have indicated that neither corticosterone, leptin, nor insulin plays a role in OEA-induced anorexia (34). Although the present experiments were not designed to test this specific hypothesis, it is tempting to speculate that OEA reduces food intake by regulating the expression of genes in the periphery that are involved in lipid metabolism. Intraperitoneal administration of the fatty acid ethanolamide stearoylethanolamide has been shown to cause anorexia via such mechanism (37). Furthermore, OEA increases the expression of PPAR-α (13), which represses the transcription of lipogenic genes (23), such as hepatic fatty acid synthase (17, 42). Growing evidence points to a role for lipid metabolism in the regulation of feeding (19, 29). These findings raise the possibility that ongoing modulation of lipid metabolism in the periphery is involved in the anorexigenic effect of OEA.

**ACKNOWLEDGMENTS**

The authors are grateful to Kay Ellis, Amin Yu, and Paul Pfleger for expert technical assistance.

**GRANTS**

This work was supported by several National Institute of Diabetes and Digestive and Kidney Diseases grants (DK-54080, DK-54890, DK-17844, and DK-59630) and a fellowship from Fond de la recherche en santé Québec (FRSQ4729 to K. Proulx).

**DISCLOSURES**

This work was partially funded by the Procter and Gamble Company.

**REFERENCES**


