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High dietary fat intake influences the activation of specific hindbrain and hypothalamic nuclei by the satiety factor oleoylethanolamide

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HIGHLIGHTS

- OEA inhibits food intake in rats fed with chow and a high fat diet.
- OEA increases *c-fos* mRNA in area postrema (AP) and nucleus of solitary tract (NST).
- The highest NST activation is in SolM, followed by SolC, SolDM and SolVL.
- OEA induces *c-fos* transcription in paraventricular (PVN) and supraoptic (SON) nuclei.
- Chronic exposure to a high fat diet alters *c-fos* expression in all these nuclei.

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ABSTRACT

Chronic exposure to a diet rich in fats changes the gastrointestinal milieu and alters responses to several signals involved in the control of food intake. Oleoylethanolamide (OEA) is a gut-derived satiety signal released from enterocytes upon the ingestion of dietary fats. The anorexigenic effect of OEA, which requires intestinal PPAR-alpha receptors and is supposedly mediated by vagal afferents, is associated with the induction of *c-fos* in several brain areas involved in the control of food intake, such as the nucleus of the solitary tract (NST) and the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON). In the present study we investigated whether the exposure to a high fat diet (HFD) alters the hindbrain and hypothalamic responses to OEA. To this purpose we evaluated the effects of OEA at a dose that reliably inhibits eating (10 mg/kg i.p.) on the induction of *c-fos* in the NST, area postrema (AP), PVN and SON in rats maintained either on standard chow or a HFD. We performed a detailed analysis of the different NST subnuclei activated by i.p. OEA and found that peripheral OEA strongly activates *c-fos* expression in the AP, NST and in the hypothalamus of both chow and HFD fed rats. The extent of *c-fos* expression was, however, markedly different between the two groups of rats, with a weaker activation of selected NST subnuclei and stronger activation of the PVN in HFD-fed than in chow-fed rats. HFD-fed rats were also more sensitive to the immediate hypophagic action of OEA than chow-fed rats. These effects may be due to a decreased sensitivity of vagal afferent fibers that might mediate OEA's actions on the brain and/or an altered sensitivity of brain structures to OEA.

1. Introduction

The adaptation to particular dietary regimens can alter the mechanisms through which the central nervous system (CNS) senses changes in intestinal nutrient availability as well as to regulate energy balance and modulate eating behavior [1–6]. For example, the consumption of a fat-enriched diet (HFD) seems to lower the sensitivity of the brain to signals involved in the homeostatic control of energy intake, such as cholecystokinin (CCK) [7], thus setting a positive feedback mechanism that sustains over-eating and increases body weight gain. This effect can be observed even after a short exposure to a fat-enriched diet when obesity has not developed yet, suggesting that such mechanisms might be involved in the early stages of adaptation [8,9]. Several signals generated in the gastrointestinal (GI)

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tract combine to promote meal termination by activating vagal afferents and/or by acting directly on the brain [10–12], for instance via circumventricular organs such as the area postrema (AP) [13]. Vagal afferents from the GI tract and projections from the AP connect to neurons of the nucleus of the solitary tract (NST), which, in turn, project extensively to other key regions involved in controlling food intake, including the hypothalamus, the amygdala, and the nucleus accumbens [9,14–17].

Oleoylethanolamide (OEA) is an endogenous lipid compound that induces satiety after intraperitoneal administration [18,19]. Produced by enterocytes upon the absorption of dietary fat [20–22], OEA activates intestinal peroxisome-proliferator-activated receptor-alpha (PPAR-α) [23] and excites vagal afferent neurons [24], which is supposed to ultimately inhibit eating. The exact mechanisms of OEA's eating-inhibitory effect are, however, still poorly understood. We previously showed that, similar to other anorexigenic gut-derived signals, peripherally administered OEA (10 mg/kg i.p.) selectively activates *c-fos* transcription in the NST, and the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus [25]. In both hypothalamic nuclei, OEA increased *c-fos* mRNA in neurons expressing oxytocin (OXY) [25]. This activation was paralleled by increased OXY mRNA levels, increased peptide neurosecretion, and elevated circulating OXY levels [25,26]. Also, we recently demonstrated that noradrenergic NST-PVN projections are involved in the activation of the hypothalamic areas induced by i.p. OEA, and that the ablation of these projections dampens OEA's satiety action [27].

In this study, we aimed at investigating whether the chronic exposure to a HFD can alter the effects induced by the acute administration of OEA on the activation of hypothalamic and brainstem neurons. To this aim, we assessed the magnitude of OEA-induced *c-fos* mRNA expression in the NST, AP, PVN and SON of rats maintained on either a standard diet (chow) or a HFD. In this study, the analysis of the NST activation was more detailed than previously reported [25,28] because the exact NST subnuclei activated by peripherally administered OEA remained unknown. Identifying them would help to understand how the OEA signal reaches the CNS. In fact, a large body of evidence indicates that there are distinct subpopulations of neurons in different subnuclei of the NST that have differential response properties to afferent fibers and to visceral signals [13]. For example, the gustatory fibers from the tongue and the posterior oropharynx terminate in the rostral tip of the NST [29]. At the level where the NST shifts dorsomedially, fibers from the esophagus terminate in a tight cluster, the central NST subnucleus [29]. Axons from parts of the vagus nerve that innervate the GI tract end more caudally in the medial part of the NST [30]. Finally, neurons in the gelatinous subnucleus and dorsomedial subnucleus are sensitive to gastric, but not duodenal, distension [31], whereas neurons in the subpostremal region show exactly the opposite reaction pattern, and neurons of the medial subnucleus are excited by both gastric and duodenal distension [31].

2. Materials and methods

2.1. Animals and housing

Male Sprague Dawley rats (Charles Rivers, Sulzfeld, Germany), weighing 180–200 g upon arrival, were individually housed in acrylic infusion cages in a climate-controlled room (22 ± 2 °C and 60% relative humidity) that was kept on a 12:12 h dark/light cycle. The rats were fed ad libitum standard chow (N 3433 diet, caloric density: 3.11 kcal/g, Provimi Kliba SA, Switzerland, http://www.kliba-nafag.ch/neutral/download/3433.pdf) or a high-fat diet (HFD, 60, 21, and 19% of energy from fat, carbohydrates, and protein, respectively; caloric density 5.23 kcal/g, Diet No. E15742, SSNIFF GmbH, Germany, http://www.ssniff.com/documents/gereinigte_diaeten_experimentaldiaeten.pdf). The animals were adapted to housing and diet conditions for at least 10 days before the catheter implantation (see below). All procedures were approved by the Veterinary Office of the Canton of Zurich.

2.2. I.p. catheter assembly

The i.p. catheters were made as described before [32,33]. They consisted of 20 cm silicone tubing (Dow Corning, Midland, MI; inner diameter (ID) × outer diameter (OD), 0.51 × 0.91 mm) connected to a polished L-shaped 20-gauge needle (Sterican, B. Braun, Germany). The connections between tubing and needles were shielded with 3 (ID × OD, 0.76 × 1.65 mm) and 2.2 cm (ID × OD, 1.02 × 2.18 mm) long pieces of silicone tubing as inner and outer layers, respectively.

2.3. I.p. catheter implantation

The catheter implantation was performed under aseptic conditions in rats adapted to the housing conditions for at least 10 days. Instruments were autoclaved and the catheters sterilized using ethylene oxide prior to use. A few hours prior to surgery, rats received subcutaneous (s.c.) injection of antibiotics (4 mg/kg body weight (BW) of trimethoprim and 20 mg/kg BW of sulfadoxine, Borgal 24%; Intervet/Schering-Plough Animal Health, Kenilworth, NJ) for infection prophylaxis. Fifteen minutes before surgery rats received an i.p. injection of atropine (0.05 mg/kg; Sintetica, Mendrisio, Switzerland) followed by the initiation of the 4–5% isoflurane and oxygen 1000 ml/min inhalation anaesthesia. The i.p. catheters were implanted as described previously [34]. Briefly, the proximal end of the catheter was led s.c. from the neck to a 4 cm midline incision in the abdomen and then inserted through a puncture hole in the abdominal cavity. The catheters were anchored on the left side of the abdominal wall with silk sutures. The abdominal muscle wall and skin were closed with absorbable sutures (3–0 and 5–0 Vicryl, respectively; Ethicon, Norderstedt, Germany). After surgery, rats received for 2 days s.c. injections of analgesic and antibiotic (5 mg/kg BW of carprofen (Rimadyl; E. Gräub, Bern, Switzerland) and 4 mg/kg Borgal 24%). To keep the catheters patent they were flushed every 2–3 days with 0.5 ml 0.9% sterile saline. All rats were allowed to recover from surgery for at least 2 weeks before starting the experiments.

2.4. Experiment 1: OEA effect on energy intake in rats fed with chow or HFD

About 2 weeks after i.p. catheter implantation, 26 rats fed either HFD (n = 13) or chow (n = 13) were adapted to the experimental procedure by receiving i.p. saline injections for 3 days and measuring baseline food intake. Food intake was recorded with an automated monitoring system as previously described [35]. Briefly food cups were placed on balances (XS4001S, Mettler-Toledo, Switzerland) connected to a computer with a custom-designed software allowing for continuous monitoring of food intake. OEA was dissolved in sterile saline/polyethylene glycol/tween 80 (90/5/5, v/v). On the experimental day food cups were closed 30 min prior to dark onset, and freshly prepared vehicle (2 ml), sterile saline/polyethylene glycol/tween 80 (90/5/5, v/v) or OEA (10 mg/2 ml/kg BW) solutions were administered in a single bolus injection via the i.p. catheter. The food cups were opened shortly before dark onset, and the program for cumulative food intake recordings was started. We selected this time point because our baseline food intake recordings indicated that animals hardly ate during the last light phase hour and because we tried to minimize disturbance of the animals during their light phase resting time. Specifically, the average onset of the last meal was 3.8 ± 0.9 h before dark onset and its average duration was 8.5 ± 1.2 min.

Vehicle and OEA were administered in a within-subject cross-over design with two intervening days between trials. Continuous recordings of food intake after the first trial showed that two intervening days were sufficient to avoid carry-over effects.