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Altered orosensory sensitivity to oils in CCK-1 receptor deficient rats

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ABSTRACT

CCK-1 receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats are hyperphagic, which leads to subsequent obesity and diabetes. Additionally, they have increased sham intake and enhanced preference for sucrose solutions relative to control, Long Evans Tokushima Otsuka (LETO) rats. To determine the effects of oil on ingestion, we first measured real feeding of various concentrations of oil emulsions (12.5, 25, 50, 75, and 100%) in rats that were fed ad libitum. Secondly, to isolate the orosensory compontent of oils from post-ingestive consequences, as well as determine the contribution of energy status, we measured sham feeding in OLETF and LETO rats using one-bottle acceptance tests while non-deprived and overnight food deprived. Finally, to assess the orosensory effects of nutritive and non-nutritive oils, we used two-bottle preference tests in sham fed OLETF and LETO rats. We found that real feeding resulted in increased intake of high oil concentrations for OLETF rats relative to LETO rats. Similarly, OLETF rats consumed significantly more of higher concentration corn oils than LETO while non-deprived sham feeding. Conversely, OLETF rats overconsumed low concentration corn oil compared to LETO during overnight deprived sham-feeding tests. In two-bottle sham-feeding preference tests, both non-deprived OLETF and LETO rats preferred corn to mineral oil. Collectively, these results show that increased oil intake in OLETF rats is driven by both peripheral deficits to satiation and altered orosensory sensitivity.

1. Introduction

Substantial amount of evidence has placed the high amounts of fatty foods within the western diet as a cause for the obesity epidemic [1]. High-fat diets, due to their high palatability and energy density, stimulate voluntary energy intake leading to obesity in both animals [2] and humans [3]. Although fat ingestion is guided by orosensory and post-ingestive factors, the orosensory properties are sufficient for discrimination and ingestion independently of post-ingestive factors [4]. The orosensory properties of fats stimulate ingestion and this occurs in deprived and sated sham-feeding animals for nutritive (corn oil) as well as non-nutritive (mineral oil) stimuli [5,6]. Thus, ingestion of fats occurs in the absence of feedback with regard to energy status. Also, orosensory stimuli, such as fatty flavors, are rewarding, and promote approach behavior and operant learning even when post-oral feedback is minimized [4,7]. In some obese rat models, hyperphagia for palatable foods is related to the positive feedback from orosensory properties [8]. For example, we recently showed that increased food intake in obese Otsuka Long Evans Tokushima Fatty (OLETF) rats can be attributed, at least in part, to altered orosensory functions. Specifically, we found increased preference for high concentrations of sweet solutions [9], altered neural coding for sweet taste [10], and heightened reward sensitivity [11].

The OLETF rat, a rodent model for obesity and non-insulin dependent diabetes mellitus (NIDDM) carries a natural single point deletion of the CCK-1R [12,13]. They are hyperphagic compared to LETO controls and gradually become obese [14] during their life span. The underlying cause(s) of hyperphagia in this rat model has not been fully revealed. Whereas most deficits can be explained by the absence of the CCK-1R and alterations in peripheral and central CCK signaling [15,16], additional non-CCK deficits have been shown to be responsible for OLETF rat's chronic hyperphagia, including impairments in hypothalamic peptide signaling [15,17–19], and dopamine functions [9,20–22]. As well as having deficits in peripheral satiation and enhanced orosensory stimulation, the OLETF has altered levels of dopamine, which positively correlate in reward with palatable sources [11,22–24].

Similar to other obese rodent models [8,25], OLETF rats have an increased preference and intake of highly rewarding or preferred foods, especially fat. For example, OLETF rats have decreased intestinal sensitivity to both intestinal infusion of oils [26] as well as solid high

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fat foods [27]. However, whether OLETF rats prefer fats more than LETO rats based on oral stimulatory effects is not known. Physiological need-states, such as food deprivation [28,29] or limited access to palatable food (e.g. sweet or fatty meals [30]) results in increased intake when the stimuli become available again (i.e., "reward sensitization") (see [31] for a review). For example, relative to lean controls, OLETF rats overeat on meals that are normally preferred when food deprived (fat [27]; sugars [9]).

Therefore, to examine the relationship of the orosensory component with feedback inhibition from the gastrointestinal tract, we employed real feeding of increasing oil emulsion concentrations (12.5, 25, 50, 75, and 100%). Further, to examine the orosensory acceptance of oils in various feeding states in the pre-diabetic OLETF compared to LETO controls, we sham fed OLETF and LETO rats corn oil emulsions under both non-deprived and food deprived conditions. Finally, to determine preference for a nutritive to non-nutritive oil between the two strains, we performed brief access (30 min) two-bottle preference tests with 100% emulsions of corn and mineral oil.

2. Methods

2.1. Animals

OLETF and LETO male rats were obtained from the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. Rats were housed individually in wire floored, hanging steel cages in a temperature controlled vivarium on a 12:12 light:dark cycle (lights on at 0600 h). Rats were provided ad libitum access to pelleted rat chow (Purina 5001) and water, except the night before food deprived sham-feeding tests described below. Rats were handled for a minimum of one week during the acclimation period before experimental protocol began. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Pennsylvania State University.

2.2. Surgical procedure: gastric cannulation

Following overnight fasting, rats were anaesthetized with a combination of ketamine (50 mg/kg), xylazine (5 mg/kg) and acepromazine (1 mg/kg). Gastric cannulation was performed as described previously [32]. Briefly, after anesthesia, rats were surgically fit with chronic gastric cannulae on the non-glandular portion of the stomach. A puncture wound was made in the lateral section of the rat's torso through which the cannula exited. Bard mesh was then used to secure the cannula to the abdominal wall. Rats were given two weeks recovery from the surgery before any feeding experiments begun.

2.3. Real feeding of oil emulsions

Rats ($n=13$, 6 OLETF, 7 LETO) with average weights of 567 ± 14 g and 475 ± 15 g, respectively were allowed ad libitum access to chow until presentation of oils. At 0900 h, chow was removed from the cages, rats were weighed, placed back into their cages, and presented with burettes filled with 12.5, 25, 50, 75 and 100% oil emulsions. To achieve the desired concentrations of oils, 0.75 ml of Tween-80 (Sigma) was added to every 100 ml mixture of oil and tap water. All oil concentrations were tested at least twice and intake were manually recorded by an individual every 5 min for 60 min. Tests were conducted every other day.

2.4. Sham feeding of oil emulsions in the non-deprived condition

A separate group of rats ($n=13$, 6 OLETF and 7 LETO) weighing 601 ± 30 g and 477 ± 13 g, respectively were equipped with chronic gastric cannulae and fed ad libitum chow throughout the experiment. Rats underwent an acclimation period of three trials that consisted of 60-min sham feeding 25% corn oil in the morning (0900) after overnight (1700–0900) water and food deprivation. During testing, at 0900 h, rats were weighed and their stomachs lavaged with warm water until the drained contents were clear and devoid of any food particles. Rats were then fitted with sham-feeding tubes and placed in mesh wire floored Plexiglas sham-feeding boxes. Each concentration of corn oil (12.5, 25, 50, 75, and 100%) was presented to all rats a minimum of two times in both ascending and descending orders of concentration. Intake of oil emulsions was individually recorded every 5 min for 60 min. Sham-feeding tests took place every other day. In all sham-feeding tests, gastric drainage was collected in plastic containers placed beneath each sham-feeding box. Great care was taken to ensure patency of drainage tubes and flow of oil freely through the tubes. In the event gastric drainage did not occur while rats were sham feeding, a connector tube attached to a syringe was used to verify eventual blockage and clear the tube promptly. This becomes important, particularly when high concentrations of oils are used, which in combination with gastric mucus, form viscous, foam like secretions.

2.5. Sham feeding of oil following overnight food deprivation

Rats ($n=12$, 5 OLETF, 7 LETO) weighing 440 ± 7 g and 361 ± 8 g, respectively were sham fed after overnight (16-h) food deprivation. Before tests begun, rats were trained to sham feed oil following a 2-h water deprivation. After training, rats were sham fed the oil emulsions as described above.

2.6. Oil preference tests

Seventeen naïve rats (8 OLETF and 9 LETO) with average weights of 574 ± 21 g and 458 ± 6 g, respectively, equipped with gastric cannulae received ad libitum access to rat chow, except during two-bottle preference testing. For training, rats were sham fed with either 100% corn or 100% mineral oil for 30 min in blocks of two days for a total of 8 days: 4 cycles $\times$ 2 days. After training, preference tests were conducted for two consecutive days. Rats received concomitant access to burettes containing 100% corn and 100% mineral oil and sham intake was measured for 30 min. To avoid side preference, burette positions were alternated for each test.

2.7. Oral glucose tolerance test (OGTT)

A subset of overnight food deprived rats from each experimental condition (sham feeding and real feeding; $n=12$, 6 per strain) were tested for blood glucose levels after an oral gavage of a glucose load (2 g/kg) using 8-French tubing as described previously [9]. Tail blood was collected at 0, 30, 60, 90, and 120 min post glucose administration. Rats with blood glucose over 300 mg/dl at any time post-gavage or over 200 mg/dl at 120 min post-gavage were considered diabetic [33]. Blood was analyzed using a glucometer (Lifescan, One-Touch Basic).

2.8. Statistical analyses

For 60-min intakes, two-way repeated measures ANOVA (rmANOVA) was performed with strain and oil concentration as main factors. Separate one-way ANOVA was run for each oil concentration to reveal differences between strains. Also, one-way rmANOVA was used to analyze all cumulative 5-min intakes in both OLETF and LETO groups. There was no significant effect of the presentation order of oil concentrations (ascending vs descending) between strains, therefore data was pooled for statistical analysis. To determine the effect of food deprivation, oil intake of OLETF was calculated as percent difference from LETO oil intake for each condition and subjected to two-