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The opioid system contributes to the acquisition of reinforcement for dietary fat but is not required for its maintenance

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HIGHLIGHTS

- Naltrexone reduced fat intake in mice well habituated to fat ingestion.
- Naltrexone suppressed daily increases of fat intake in mice naïve to fat ingestion.
- Naltrexone did not affect reinforcement for fat in well-habituated mice.
- Naltrexone suppressed the acquisition of reinforcement for fat in naïve mice.

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ABSTRACT

The opioid system plays an important role in ingestive behavior, especially with regard to palatable high-fat or sweetened foods. In the present study, we investigated the role of the opioid system in the regulation of ingestive behavior in mice with regard to dietary fat intake, reinforcement, and particularly the processes involved in development of these behavior types. Subcutaneous administration of the non-selective opioid receptor antagonist naltrexone (0.5 or 2.0 mg/kg body weight [BW]) reduced the spontaneous intake of fat emulsion (Intralipid). We investigated the effect of naltrexone on reinforcement by using an operant behavioral paradigm under a progressive ratio schedule in which the number of lever presses required to obtain a test sample increased progressively. Mice showed stronger reinforcement by Intralipid as a function of concentration. However, naltrexone (0.5 or 2.0 mg/kg BW) did not affect reinforcement at any concentration of Intralipid in mice that had repeatedly ingested Intralipid before testing was carried out. Intralipid ingestion also induced conditioned place preference (CPP), which is another evaluation index of reinforcement. High-dose naltrexone (2.0 mg/kg BW) administration during CPP conditioning suppressed the reinforcement induced by Intralipid ingestion, although the drug administration (0.5 or 2.0 mg/kg BW) during CPP testing did not affect reinforced behavior. These results suggest that the amount of fat ingestion and reinforcement for fat ingestion are separately regulated by the opioid system. Furthermore, our results indicate that the opioid system plays an important role in acquiring reinforcement for fat but is not required for maintenance of learned reinforcement.

1. Introduction

Dietary fats are attractive food components because they make foods palatable. Recent studies indicate that mice and rats prefer consuming not only fatty foods but also dietary fat itself [1–3]. We and several other researchers have revealed that rodents and humans have specific receptors for fat molecules in taste bud cells, which indicates that fat makes foods palatable not only through the olfactory system but also through the gustatory system [4–7]. In addition, we reported that the spontaneous ingestion of corn oil induces conditioned place preference (CPP) in a test that evaluated the reinforcing properties of a test sample [3,8]. In an operant responding paradigm, we demonstrated that the rewarding effect of corn oil is enhanced in proportion to its concentration [9] and that it is significantly stronger than that of xanthan gum solution (imitation of oil texture) or non-caloric oil. Thus, dietary fat is not only palatable but also reinforcing.

The endogenous opioid circuit has long been implicated in the regulation of appetite and, in particular, hedonic processes associated with food choice, consumption, and reinforcement [10]. There are mainly three subtypes of opioid receptors (μ-, δ-, and κ-opioid receptor); the μ-opioid receptor is especially involved in these behaviors. For example, the administration of non-selective and μ-selective opioid receptor

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agonists into the nucleus accumbens increases intake of highly palatable foods, such as sucrose solutions, fatty foods, and dietary fat [11–13]. It has also been reported that non-selective and μ-selective opioid receptor antagonists suppress ingestive behavior related to fatty foods [11, 14–16]. The opioid system does not affect ingestive behavior for water or standard laboratory chow, which exhibit hedonically neutral palatability [17–19], suggesting that the opioid receptor is involved only in ingestive behavior related to palatable foods. Recently, we reported that the ingestion of a sweet solution (glucose or sucrose) and dietary fat, but not water or laboratory chow activates β-endorphin neurons in the hypothalamus, thus promoting β-endorphin release [20,21]. β-Endorphin is a known endogenous μ-opioid receptor ligand; hence, β-endorphins released by palatable food ingestion might activate the opioid system and promote further ingestion of these foods.

Animals, including humans, show strong reinforcement (craving) toward eating fatty foods and sometimes over-consume them [22]. Repetitive ingestion and positive post-ingestion effects are thought to reinforce these types of behaviors. Previously, we reported that daily repetitive ingestion of dietary fat increases the intake and licking response for fat in mice [23,24]. In addition, as mentioned above, we observed that corn oil ingestion induces a reinforcing effect in a CPP test where mice could learn an association between a distinctive environmental cue and a novel and motivationally significant event or rewarding stimulus [8,25].

These combined results suggest that experience and learning could enhance fat preference and reinforcement for consumption behaviors. Some reviews have suggested that experience and learning are important components in the process of developing the palatability and reinforcement for ingestion [26,27]. It is possible that the opioid system is involved not only in the palatability of fat but also in developing these behavioral processes. Indeed, β-endorphin-deficient mice showed lower operant responses for fat as a reinforcer when compared to wild-type mice [28,29]. However, it is not clear whether the opioid system contributes to the learning and development of palatability and reinforcement or simply contributes to stimulation itself, because the experimental design in the previous study always inhibited the opioid system during the experimental period.

The present study was designed to investigate the physiological role of the opioid system that underlies the palatability of fat and fat's reinforcement properties in the learning process; we examined the effects of an opioid receptor antagonist (naltrexone) on these behaviors in mice that were either habituated or naïve to dietary fat ingestion.

2. Materials and methods

2.1. Animals

Eight-week-old male BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan) for each experiment. Newly purchased mice were used for all experiments and 286 mice were used in total. The mice were housed together in groups of 6 in one cage in an animal housing facility maintained at 23 ± 2 °C under a 12:12-h (reverse) light/dark cycle (lights off 06:00–18:00; lights on 18:00–06:00). Commercial standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water were available ad libitum. The mice were allowed to acclimate to their surroundings for at least one week after arrival before testing began. All experiments were carried out during the dark phase. This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and was in complete compliance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." All procedures used were approved by the Kyoto University Animal Care and Use Committee. Efforts were made to minimize the number of animals that were used and to limit experimentation only to that which was necessary to produce reliable scientific information.

2.2. Effect of naltrexone and naltrindole on Intralipid intake

Mice were used to investigate the effect of naltrexone (n = 12) and naltrindole (n = 10). All these mice were housed individually, and all training and tests were performed individually. During the training sessions, mice were given access to a bottle containing 20% Intralipid in their home cages for 10 min every alternate day. Mice that ingested 20% Intralipid at least 3 times were considered habituated and as having achieved a stable consumption level. To eliminate the effect of stress, before each training session, the mice were handled to simulate the conditions for injection. And then the mice were access to 20% Intralipid was allowed 30 min after food and water were removed from the cages. During the test session, mice received a subcutaneous (s.c.) injection of the non-selective opioid receptor antagonist naltrexone (0.5 or 2.0 mg/kg BW), the δ-opioid receptor antagonist naltrindole (2 or 4 mg/kg BW), or saline concurrent with the removal of food and water. The doses of each drug were determined according to previous reports [8,30]. After 30 min, the mice were offered 20% Intralipid, which was weighed at 0-, 10-, 30-, and 60-min time points, and the cumulative intake was calculated. Each drug administration was separated by at least 3 days, and mice were offered 20% Intralipid in the absence of either drug between each treatment to confirm that their intake had returned to the baseline amount. The order of testing naltrexone and naltrindole treatments was randomized across mice such that on each test day an approximately equal number of mice received each treatment.

After several exposures to fat ingestion, the animals ingested higher volumes of dietary fat and developed a preference and reinforcement to consume dietary fat [9,24,25,31]. To test whether the opioid system might also be involved in this behavior change, we administered naltrexone to mice that had never ingested 20% Intralipid and measured their daily 20% Intralipid intake. Newly purchased mice were used for each experimental group (n = 8). Training for fluid ingestion was carried out by using the same protocol described above but using a 20% sucrose solution instead of 20% Intralipid. During the test session, the mice received naltrexone (0.5 or 2.0 mg/kg BW) or saline via a single administration or 3 times by subchronic administration. In the single-administration group, on day 1, 30 min after administration, the mice were offered 20% Intralipid for 10 min. From day 2 to day 5, in order to exclude the possibility of non-specific effects of naltrexone (e.g., neophobia, aversion learning), intake of 20% Intralipid was recorded for 10 min using the same procedure as used on day 1 but without any drug/saline administration. For the subchronic-administration group, from day 1 to day 3 the mice were offered 20% Intralipid for 10 min every alternate day at 30 min after naltrexone administration. From day 4 to day 5, their intake of 20% Intralipid was recorded for 10 min by using the same procedure that was used on day 1 to day 3 but without any drug/saline administration.

2.3. Measurement of food intake

Newly purchased mice were used to investigate the effect of naltrexone (n = 10) and naltrindole (n = 10). Food intake was measured 8 h after the lights went off (10:00). The mice were housed individually and were allowed food and water ad libitum during the experiment. Naltrexone (0.5 or 2.0 mg/kg BW), naltrindole (2 or 4 mg/kg BW), and saline were administered subcutaneously. Preweighed food pellets in each cage were measured for 1-, 2-, 4-, and 8-h periods, and cumulative food intake was calculated. Spilled food was weighed, and food intake was corrected as necessary. Each drug administration was separated by at least 3 days. The order of testing with naltrexone and naltrindole treatments was randomized across mice such that on each test day an approximately equal number of mice received each treatment.