Daily increase of fat ingestion mediated via mu-opioid receptor signaling pathway

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ABSTRACT
We investigated the involvement of opioid receptors such as the mu and delta receptors in the predominant elevation of corn oil appetite just after 5-day repeated treatment of corn oil ingestion. Rats were given 5% corn oil emulsified with 0.3% xanthan gum for 20 min at the same hour for 5 consecutive days. A strong appetite for fat was formed after the 5 days presentation, and it was inhibited by naloxonazine, a selective antagonist of the mu-1 receptor, at doses of 3 mg/kg, but not by antagonists of the opioid delta receptor. In days 6, after the formation of a strong appetite for corn oil, an additional injection of naloxonazine suppressed fat intake 0–30, 30–60, 60–90 and 90–150 min after the presentation of the corn oil, but antagonists of the opioid delta receptor did not. These data suggested that the opioid mu receptor is involved in the sharp elevation of corn oil appetite during repeated presentation of corn oil to rats.

Obesity resulting from excessive energy ingestion is a serious health issue. Despite warnings about the excessive ingestion of high-fat foods, there currently appears to be no decrease in the consumption of such foods (3, 11, 13, 16). In comparison to rats fed on regular chow diets, rats fed on a high fat diet showed a greater food intake and became overweight as a result (2). In addition, the reinforcing effects of corn oil on mice have been demonstrated in conditioned place preference (CPP) tests (6). Understanding why mammals, including humans, prefer high-fat foods will provide important information for regulating oil ingestion and thus maintaining good health.

We have discovered that beta-endorphin, an opioid peptide, is released just after dietary oil ingestion in rats (12). Moreover, the gene expression of POMC (a precursor of beta-endorphin) increased just before dietary oil ingestion (12). These results suggested that the desire for fat might be mediated via opioid receptors.

Naltrexone, a non-selective antagonist of opioid receptors, reduces the ingestion response to low concentrations of sucrose, sodium chloride and alcohol, and increases the aversive response in taste reactivity testing (4). In addition, naltrexone suppresses both the ingestion volume and velocity (lick rate) of sucrose solution (5). A similar antagonist, naloxone, blocks the acquisition of CPP for corn oil ingestion (7). These results indicate that the functions of the mu and delta opioid receptors may be related to the enhancement desire for food through the reinforcing effects.

In the present study we examined the effect of a subtype of the opioid receptor on the enhancement of appetite for oil.
MATERIALS AND METHODS

Animals. Nine week old male Wistar rats (Japan SLC, Hamamatsu, Japan) were kept in stainless wire mesh cages in a room controlled by a 12-hour light-dark cycle (dark phase: 15:00–3:00) and constant temperature (24 ± 1°C). They were housed separately for a week to allow acclimatization to the environment. They were fed distilled water and regular chow (MF; Oriental Yeast, Tokyo, Japan) ad libitum. This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and the Japan Neuroscience Society, and was in complete compliance with the National Institutes of Health: Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and to limit the experimentation to what was necessary to produce reliable scientific information.

Experimental Protocols. We first conducted 5-day training sessions with the rats during which daily water deprivation took place at 11:00 for 5 h and daily fasting took place at 14:00 for 2 h. One hour after the start of the dark phase (16:00), the rats were provided with 5% corn oil emulsion suspended in 0.3% xanthan gum aqueous solution, which masked the oil texture, for 20 min. The amount of intake during the 20-min period was measured for each rat.

On Day 6, after completion of the training sessions, a test session was administered to all rats. Water deprivation and fasting took place at the same hour as the training sessions. In the test sessions, the rats were provided with the 5% corn oil emulsion for 150 min. The amount of intake during the 150-min period was measured for each rat at intervals of 30 min.

Pharmacological tests. Drugs or the vehicle (saline or 10% dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were dissolved and intraperitoneally (i.p.) administered 1 h before the presentation of the corn oil emulsion in the above experimental protocols as follows. First, in order to examine the effects of drugs on the formation or enhancement of fat preference, drugs or the vehicle were administered in training sessions. Secondly, in order to examine the effects of drugs on fat ingestive behavior after the formation or enhancement of fat preference occurred at the training sessions, the vehicle was administered in training sessions, and then drugs or the vehicle were administered in the test sessions.

Drugs. A selective antagonist of the mu-1 receptor, naloxonazine dihydrochloride (Sigma-Aldrich Japan K.K., Tokyo, Japan), and a non-selective antagonist of the delta receptor, naltrindole hydrochloride (Sigma-Aldrich Japan K.K.) were dissolved in saline. Naloxonazine was i.p. administered at doses of 1 mg/kg body weight or 3 mg/kg body weight (Naloxonazine-1 or Naloxonazine-3 group). Naltrindole was i.p. administered at doses of 1 mg/kg body weight or 5 mg/kg body weight (Naltrindole-1 or Naltrindole-5 group). A selective antagonist of the delta-1 receptor, BNTX maleate (Tocris Cookson Ltd., United Kingdom) and a selective antagonist of the delta-2 receptor, naltriben mesylate (Tocris Cookson Ltd.) were dissolved in 10% DMSO. BNTX was i.p. administered at doses of 0.5 mg/kg body weight (BNTX group). Naltriben was i.p. administered at doses of 0.5 mg/kg body weight (Naltriben group).

Statistical Analysis. Data are expressed as mean ± standard errors (SE). For all measurement items, comparisons were separately analyzed by two-way repeated-measure analyses of variance (ANOVA) (group vs time). Comparisons between the 2 groups were made using an unpaired Student’s t-test. The comparisons between time with the other time in the same group were made using one-way ANOVA with Sheffé post hoc significance testing. All statistical tests were done with StatView (SAS Institute Inc., Cary, NC). Statistical significance was defined as p < 0.05.

RESULTS

Experiment 1
In the 5-day test session, in which 5% corn oil emulsion was given, the intake of 5% corn oil emulsion for 20 min was significantly lower in the Naloxonazine-3 group than in the Saline group (Sheffé’s test: p < 0.05, Fig. 1). No significant differences in the intake of 5% corn oil emulsion for 20 min were observed between the Naloxonazine-1 and Saline group in the 5-day test session (Fig. 1).

In the 5-day test session, no significant inhibition in the intake of 5% corn oil emulsion for 20 min was observed by Naltrindole-1 (low dose), Naltrindole-5 (high dose), BNTX nor Naltriben (Figs. 2A and 2B).
Fat ingestion mediated via mu receptor

Experiment 2

In an additional test session after the 5-day training period, which was conducted after the rats had learned that corn oil was palatable, the intake of 5% corn oil emulsion was significantly lower in the Naloxonazine-3 group than in the Saline group 0–30, 30–60, 90–150 min after the presentation of corn-oil emulsion (Sheffé’s test: $p < 0.05$, Table 1). No significant differences in the intake of 5% corn oil emulsion were observed between the Naloxonazine-1 and Saline groups in the test session.

No significant inhibition in the intake of 5% corn oil emulsion was observed by Naltrindole-1, Naltrindole-5, BNTX or Naltriben (Table 1).

DISCUSSION

In this study, we examined the involvement of a subtype of the opioid receptor on the formation or enhancement of preference of dietary fat by means of a pharmacological procedure. We previously reported that the i.p. injection of naloxone, a non-selective antagonist of the opioid receptor, inhibited ingestive behavior of 5% corn-oil emulsion in rats (12). To demonstrate the effect induced by subtypes of the opioid receptor, i.e., the mu- and delta-recept-
tors, we examined the intake of 5% corn-oil emulsion when rats were administered the selective antagonist of mu- and delta-receptors. As a result, we showed that dietary fat intake was inhibited when rats were administered naloxonazine, a selective antagonist of the mu receptor, but not a selective antagonist of the delta receptor. The effect of naloxonazine on fat intake in present study paralleled that of naloxone, a non-selective antagonist of the opioid receptor in our previous study (12).

The involvement of the opioid systems in the brain in the preference for dietary fat was demonstrated in previous studies. Imaizumi et al. used the CPP test and reported that corn oil is a reinforcer in rodents (6). The reinforcing (rewarding) effects of dietary fat disappear with the administration of naloxone, BNTX and naltriben (7). These results indicate that ligands binding to the mu and delta receptors are necessary for the reinforcing effects of dietary fat. However, our results showed that the administration of an antagonist of the delta receptor did not affect the intake of dietary fat. The difference was unclear in this study, but the mechanisms of the reinforcing effects in the CPP test and ingestive behavior may differ in the brain. For example, the CPP test in the previous study examined the reward effect with associative learning of the place and reinforcer, but not the ingestive test in this study.

We previously reported that beta-endorphin (beta-end), an opioid peptide binding to the mu- and delta-receptors with almost the same affinity, was released in rats’ cerebrospinal fluid and serum 15 min after the presentation of corn-oil emulsion (12). In addition, the intraventricular administration of beta-end to beta-end-deficient (beta-end−/−) mice increases their food ingestion, and this effect is abolished by the intraperitoneal injection of naloxone (1, 10). Although beta-end−/− mice were hyperphagic and obese compared with their wild-type counterparts, it is very interesting that they ate still more following the intraventricular injection of beta-end. These results suggest that the beta-end is involved in the induction of ingestion behavior of dietary fat. We suggested that binding of the beta-end to the mu receptor might play important role in the ingestive behavior of dietary fat.

Will et al. showed a significant increase in the ingestion of a high-fat diet by the administration of DAMGO, an agonist of the mu receptor, into the nucleus accumbens (NAc) when compared with saline injection (14). This increase was eliminated by the administration of Muscimol, an agonist of the GABA receptor, to the lateral hypothalamus (LHA), ventral tegmental area (VTA), the intermediate region of the solitary tract nucleus (NTS) and amygdala (14, 15). These results indicate that the mu receptors in the NAc are related to the increase in the ingestion of dietary fat and that the effects of opioids are transmitted via the projections from the NAc to the LHA, VTA, NTS and amygdala and are achieved by the depression of the activities of GABAergic neurons in each region. The activation of mu receptors in the GABAergic interneurons in the VTA depresses their activities and cancels the suppression of dopaminergic neurons (8, 9, 17). Therefore, the system is most likely involved with reward effects and ingestion behavior relating to dietary fat.

In conclusion, we demonstrated that the signaling pathway via the mu receptor but not the delta-receptor mediated a part of the ingestive behavior of dietary fat. The formation or enhancement of dietary

| Effect of an antagonist of mu and delta opioid receptor on the intake of 5% corn-oil emulsion for 150 min in the test session |
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|   | 0–30 | 30–60 | 60–90 | 90–150 |
| Saline | 5.62 ± 0.46 | 2.40 ± 0.25 | 2.29 ± 0.41 | 3.82 ± 0.41 |
| Naloxonazine-1 | 4.48 ± 0.56 | 3.19 ± 0.70 | 1.71 ± 0.66 | 5.59 ± 0.65 |
| Naloxonazine-3 | 2.47 ± 0.80* | 0.46 ± 0.20* | 1.19 ± 0.29 | 1.96 ± 0.62* |
| Naltrindole-1 | 4.59 ± 0.23 | 2.40 ± 0.43 | 2.19 ± 0.76 | 5.77 ± 0.44 |
| Naltrindole-5 | 5.67 ± 0.46 | 1.96 ± 0.52 | 1.84 ± 0.59 | 3.90 ± 0.66 |
| DMSO | 5.81 ± 0.78 | 2.75 ± 0.34 | 1.79 ± 0.29 | 4.23 ± 0.87 |
| BNTX | 5.61 ± 0.65 | 2.26 ± 0.61 | 2.25 ± 0.47 | 4.78 ± 0.49 |
| Naltriben | 5.98 ± 0.66 | 2.13 ± 0.48 | 2.22 ± 0.49 | 4.31 ± 0.76 |

Each value represents the mean ± standard errors (SE) for 8–12 rats. Asterisks (*) indicate a significant difference in comparison with the Saline or DMSO group (p < 0.05).
fat preference may occur via activation of the mu receptor.

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REFERENCES