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Dietary fat ingestion activates β-endorphin neurons in the hypothalamus

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ABSTRACT

The opioid system regulates food choice, consumption, and reinforcement processes, especially for palatable meals such as fatty food. β-Endorphin is known as an endogenous opioid peptide produced in neurons of the hypothalamus. In this study, we found that Intralipid (fat emulsion) ingestion increased c-fos expression in β-endorphin neurons. However, intragastric infusion of Intralipid only slightly increased c-fos expression 2 h after infusion. Further, dissection of glossopharyngeal nerve, innervating posterior tongue taste buds, partially but significantly decreased the Intralipid-induced c-fos expression. These results indicate that mainly the orosensory stimulation from fat may activate β-endorphin neurons, thereby promoting β-endorphin release.

1. Introduction

Dietary fat is an appealing food component because it makes foods more palatable. Such a hedonic response is supported by behavioral studies in rodents [1–3], and regulation of hedonic processes associated with food choice, consumption, and reinforcement have long been thought to involve the endogenous opioid circuit, especially the μ-opioid receptor [4–6]. Recently, our research group has reported that not only flavor, texture, and post-ingestive consequences of energy from fat, but also orosensory chemical sensing of fat may contribute to its palatability [7–10].

Several studies provide evidence that ingestion of a palatable meal is capable of altering β-endorphin neuronal activity [11–13]. However, these studies only measured β-endorphin concentration in the brain and cerebrospinal fluid, and thus, the actual response of β-endorphin neurons in the brain remains unclear. β-Endorphin is known as an endogenous opioid and μ-opioid receptor ligand generated by proteolysis of pro-opiomelanocortin (POMC) and is produced not only in the neurons of the arcuate nucleus, but also in the pituitary gland. For this reason, it is possible that β-endorphin present in the cerebrospinal fluid might be at least partially derived from the pituitary.

Here, the neuronal activity of β-endorphin-producing cells (POMC neurons) in the hypothalamic arcuate nucleus during fat ingestion was investigated. To achieve this goal, we assessed c-fos expression as a marker of neuronal activation. In addition, we determined whether taste stimulation could activate β-endorphin neurons.

2. Materials and methods

2.1. Animals

Eight-week-old male BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan) for each experiment. The mice were housed individually in a vivarium maintained at 23 ± 2 °C under a 12:12 h light/dark cycle (lights on 06:00–18:00 h). Commercial standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water were available ad libitum. All experiments were carried out at night (20:00–24:00 h). 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.

2.2. Reagents

Intralipid, an intravenous fat emulsion containing 20% soybean oil, 1.2% lecithin, and 2% glycerol, was purchased from Terumo (Tokyo, Japan). Mineral oil was purchased from Kaneda Company, Inc. (Tokyo, Japan). All other chemicals were purchased from

Abbreviations: POMC, pro-opiomelanocortin; GLX, glosspharyngeal nerve transection surgery

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Nacalai Tesque, Inc. (Kyoto, Japan). Mineral oil was used as non-nutritive oil with similar viscosity and hydrophobic properties of soybean oil. Likewise, saccharin was used as a non-nutritive sweet solution to compare with the nutritive sweet solutions sucrose and glucose.

2.3. Glossopharyngeal nerve transection (GLX) surgery

The bilateral glossopharyngeal nerves, which innervate taste buds on the posterior part of the tongue, were cut in the neck under the digastric muscle. Sham control mice received similar surgical operation, but both nerves were left intact. After surgery, all mice were allowed to recover for at least 10 days. At the end of the experiment, the circumvallate papillae of each mouse was removed and stained with hematoxylin and eosin for verification that the surgery was successful. Immunohistochemical staining of taste buds using rabbit anti-α-gustducin antibody was also performed (Supplementary Fig. 1).

2.4. Gastric cannula implantation

The surgical implantation of gastric cannula into the stomach was performed as previously described [14] with a few modifications. Briefly, the gastric cannula (Silascon tubing, #00, Kaneka, Osaka, Japan) was inserted into the stomach through a 1-mm incision and sutured to the stomach wall. A 4-mm square piece of Bard™ Mesh (DAVOL Inc., Warwick, RI) was placed around the base of cannula for reinforcement. The end of the cannula was then exteriorized from an abdominal cavity through a stab wound about 0.5 cm to the left of the midline. Next, it was carefully routed up the neck of the animal through a subcutaneous tunnel, exteriorized from the skin, and sutured to underlying tissue. The cannula was flushed with saline daily to prevent occlusion. After at least 10 days of recovery, animals were used in experiments.

2.5. Fluid intake and sample collection for immunohistochemistry

During training sessions, the mice were given access to the sample solution in their home cages for 10 min every other day (Days 1–5). Thirty minutes after removal of food and water, the mice were offered a test fluid for 10 min. Similar training was also conducted on animals who received GLX surgery and gastric cannula implantation surgery.

On the test day (Day 6), mice were offered one of several test fluids ($n = 5$ for each test fluid; both sham-operated and GLX groups, $n = 6$) for 60 min. Between 1 and 2 h after the start of test fluid ingestion, mice were deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused via the aorta with PBS followed by 4% PFA. The brains were then removed and fixed in 4% PFA. Samples were sliced into 25-μm-thick sections and collected in PBS. After preincubation with Block Ace (Yukijirushi, Sapporo, Japan), sections were incubated overnight with rabbit anti-c-fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and chicken anti-POMC antibody (Chemicon International, Temecula, CA) at room temperature. After several rinses in PBS, the sections were incubated with Alexa Fluor 546-conjugated donkey anti-rabbit IgG and Alexa Fluor 488-conjugated goat anti-chicken IgG (Molecular Probes, Eugene, OR). Immunostained sections were observed under a confocal laser scanning microscope.

Since an anti-β-endorphin antibody applicable for double immunostaining with rabbit anti-c-fos antibody was not available, a chicken anti-POMC antibody was used instead for the labeling of β-endorphin neurons. The specificity of the chicken anti-POMC antibody was confirmed by double immunohistochemistry, which revealed that β-endorphin-immunoreactive cells in the arcuate nucleus were also labeled with anti-POMC antibody confirming their co-localization within the same cell (Supplementary Fig. 2).

For gastric administration, 1.2 ml of water ($n = 5$) or Intralipid ($n = 5$) were administered through the gastric cannula using a syringe pump at a 0.12 ml/min infusion rate for 10 min. Between 1 and 2 h after the start of administration, brains were collected as described above. As a positive control group ($n = 5$), Intralipid was offered to animals who received gastric cannula implantation surgery for 10 min without any gastric administration. The amount of test fluid administered to the stomach was determined based on the amount of Intralipid consumed by the positive control group for 10 min on test day (1.18 ± 0.05, $n = 10$).

2.6. Quantitative assessment of co-localization of c-fos and POMC

Quantitative assessment of immunostaining was performed on mouse brain coronal sections containing mid-to-caudal-arcuate nucleus that corresponds to −1.70 to −1.94 mm posterior from the bregma (according to the Paxinos and Franklin Mouse Brain Atlas). All nuclei with intense or medium-intensity c-fos labeling (i.e., showing the most prominent c-fos activation) and POMC-expressing cells were counted bilaterally in 4–5 sections from each individual and averaged. Images were recorded digitally to prevent multiple counts using Photoshop software (Adobe, San Jose, CA).

3. Statistical analyses

All values are presented as the mean ± S.E.M. The effects of the fluid intake on the number of c-fos-immunoreactive nuclei, c-fos/POMC double-labeled cells, and% of c-fos/POMC double-labeled cells ((c-fos + POMC double-labeled cell)/total POMC cells) were examined using one-way ANOVA with Dunnett's multiple comparison test or an unpaired *t*-test (Prism 4.0; GraphPad Software, Inc.; San Diego, CA, USA).

4. Results

4.1. Effect of Intralipid ingestion on c-fos expression in POMC neurons

Compared to naïve mice (before ingestion, non-treatment group), water ingestion did not affect the number of c-fos-immunoreactive-(IR) nuclei (Fig. 1). In contrast, the number of c-fos-IR nuclei significantly increased in the Intralipid-ingested group 1–2 h after ingestion compared to both the non-treatment and the water-ingested groups (Fig. 1A and B). In addition, c-fos + -POMC double-labeled cells and% of (c-fos + POMC)/POMC cells were also increased by Intralipid ingestion. No significant changes in the number of POMC neurons per section were observed between any of the groups (data not shown).

Glossopharyngeal nerve transection surgery partially but significantly suppressed Intralipid-induced c-fos expression in POMC neurons compared to sham-operated animals (Fig. 2). Intake of Intralipid for 60 min was also decreased by the surgery.

4.2. Effect of intragastric Intralipid administration on c-fos expression in POMC neurons

Intragastric administration of Intralipid (1.2 mL) did not affect the number of c-fos + POMC double-labeled neurons 1 h after administration (Fig. 3). In contrast, oral ingestion of Intralipid for 10 min (positive control group) significantly increased the number of c-fos + POMC double-labeled cells. Two hours after administration, the number of c-fos + POMC double-labeled cells in the Intralipid-administered group was significantly higher than in the water-administered group. However, no significant differences

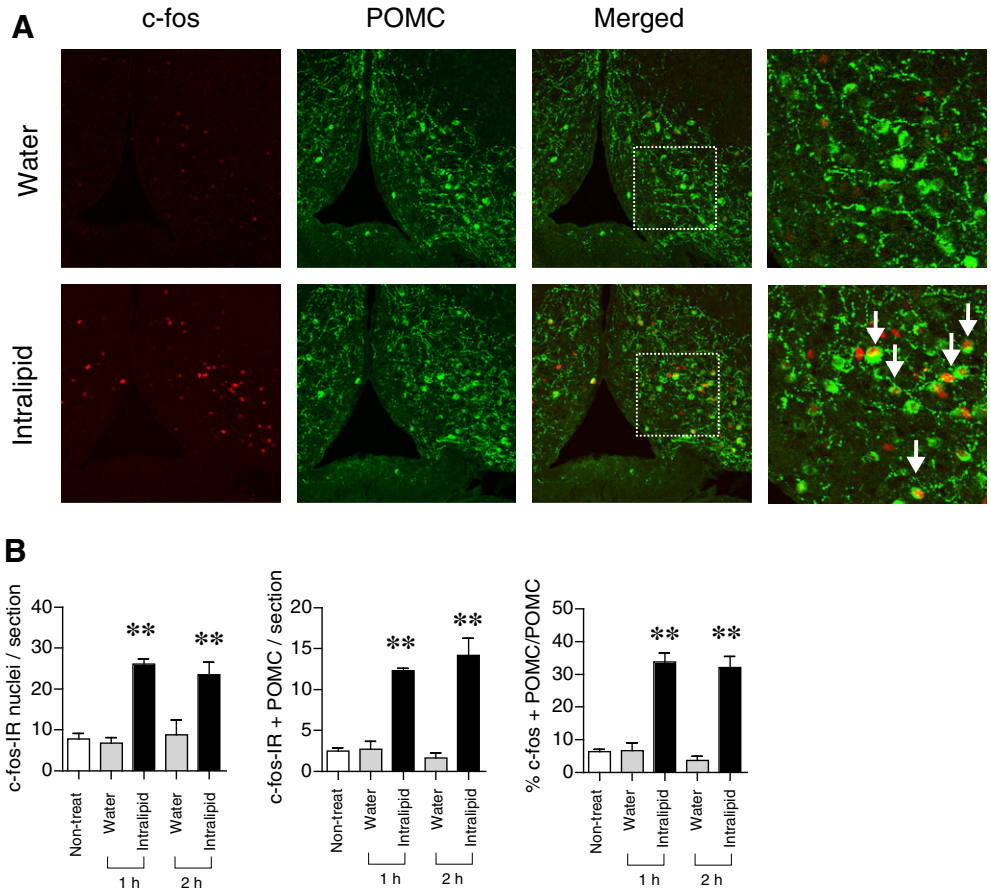

Fig. 1. Effect of Intralipid ingestion on c-fos expression in POMC neurons in the hypothalamic arcuate nucleus. (A) Immunohistochemical staining of c-fos (red) and POMC (green). White arrows indicate double immunoreactive cells. (B) Number of c-fos expressing POMC neurons in the hypothalamic arcuate nucleus. Values are presented as the mean ± S.E.M.; $n = 5$ ($^{*}P < 0.05$, $^{**}P < 0.01$ for non-treated vs. Intralipid (Dunnett's post hoc test)).

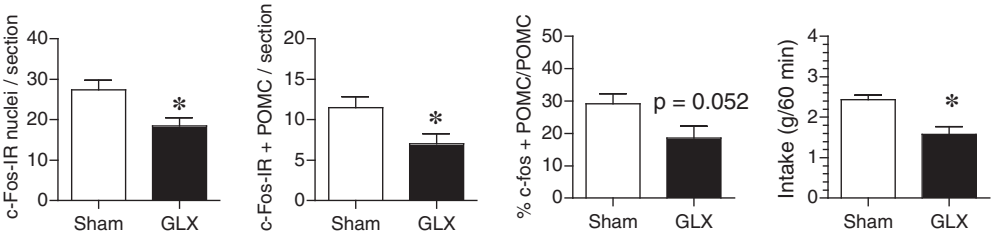

Fig. 2. Effect of glossopharyngeal nerve transection surgery (GLX) on c-fos expression in POMC neurons in the hypothalamic arcuate nucleus. Values are presented as the mean ± S.E.M.; $n = 6$ ($^{*}P < 0.05$, for sham vs. GLX (unpaired t-test)).

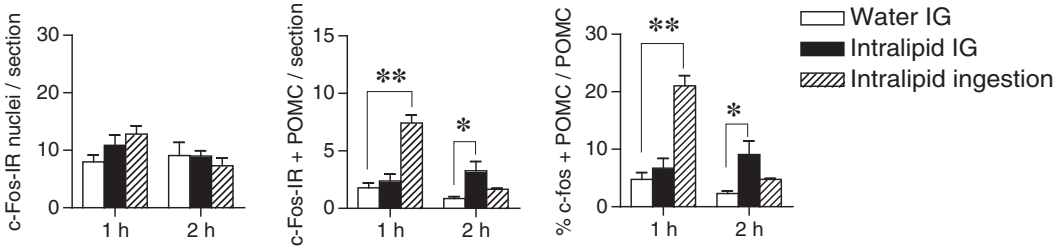

Fig. 3. Effect of intragastric administration of Intralipid on c-fos expression in POMC neurons in the hypothalamic arcuate nucleus. Values are presented as the mean ± S.E.M.; $n = 6$ ($^{*}P < 0.05$, $^{**}P < 0.01$, for Intralipid-administered group, Intralipid ingestion group vs. water-administered group (Dunnett's post hoc test)).

were observed between the Intralipid-administered group and the Intralipid oral ingestion group. Unlike Intralipid oral ingestion for 1 h (Fig. 1), Intralipid ingestion for 10 min did not affect the number of c-fos + POMC double-labeled neurons 2 h after ingestion. A similar result was observed in mice that did not receive surgery after Intralipid ingestion for 10 min (Supplementary Fig. 4).

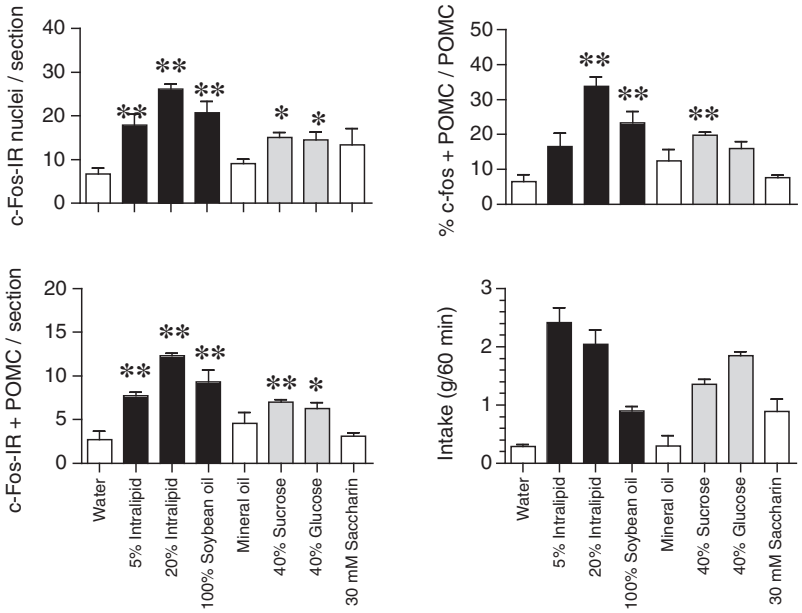

Fig. 4. Effect of ingestion of various test fluids on c-fos expression in POMC neurons in the hypothalamic arcuate nucleus. Values are presented as the mean ± S.E.M.; $n = 5$ ($^{*}P < 0.05$, $^{**}P < 0.01$ for water vs. test fluid (Dunnett's post hoc test)).

4.3. Effect of test fluid ingestion on c-fos expression in POMC neurons

Similar to 20% Intralipid, both 5% Intralipid and soybean oil ingestion increased the number of c-fos-IR nuclei and c-fos + POMC double-labeled cells (Fig. 4). Furthermore, 40% sucrose and glucose ingestion increased the number of c-fos-IR nuclei and c-fos + POMC double-labeled cells as well. In contrast, mineral oil and 30 mM saccharin solution did not affect c-fos expression compared to the water ingestion group. With regard to intake, 5% Intralipid caused a greater amount of intake than all other test fluids, while no significant differences between intake of mineral oil, saccharin, or water were observed.

5. Discussion

Intralipid (20%) ingestion induced the strongest c-fos expression in POMC neurons; however, Intralipid (5%) induced the highest intake for 60 min. On the other hand, caloric intake calculated from the start of ingestion was the highest with 100% soybean oil, while mineral oil, which has a similar texture and viscosity to soybean oil, did not induce c-fos expression. By a 2-bottle preference test, preference order was Intralipid 20% > soybean oil = Intralipid 5% (Supplementary Fig. 2). In this test, the number of c-fos-IR nuclei correlated with test fluid preference.

Since the fatty acid binding protein CD36 is only seen in the taste buds of circumvallate papillae located in posterior tongue [7], the effects of glossopharyngeal nerve transection surgery were determined. This nerve transection partially suppressed Intralipid-induced c-fos expression in POMC neurons. In contrast, chorda tympani nerve transection surgery could not suppress c-fos expression (data not shown) suggesting that any residual response may be attributed to another taste nerve such as the greater superficial petrosal nerve, laryngeal nerve, or postoral factors.

In contrast to oral intake of Intralipid, intragastric administration of Intralipid did not affect c-fos expression in POMC neurons at least 1 h after administration. However, the number of c-fos + -POMC double-labeled cells in the Intralipid-administered group was higher than that of the water-administered group 2 h after administration. An fMRI study from another group showed that intragastric administration of corn oil emulsion activates the area around the arcuate nucleus [15]. Thus, it is possible that Intralipid moderately activates POMC during post-ingestive processes. Previously, we have demonstrated that induction of place preference by corn oil requires both gustatory palatable stimulus and post-ingestive factors [9,10]. Concerning these results, both gustatory palatable stimulation by fat and post-ingestive factors seems to be required to activate POMC neurons potently.

Similar to oral intake of Intralipid, the sweet solutions glucose and sucrose both increased c-fos expression in the same fashion. Considering that the insulinotropic action of glucose is greater than that of sucrose, insulin, incretin, or blood glucose levels are unlikely to stimulate POMC neurons. In contrast, saccharin did not change c-fos expression. A high concentration of saccharin is known to present as a weak bitter taste, and thus, it should be considered that the bitter taste contained in the saccharin solution might inhibit POMC neuronal activity.

POMC neurons express insulin and leptin receptors, whose activity are hormonally regulated [16,17]. POMC is a precursor protein of not only β-endorphin, but also of ACTH and melanocortins, and POMC neurons integrate peripheral and central information related to energy status and metabolism. In addition to previous findings, our current results indicate that ingestive behavior or, more specifically, taste stimulation might also regulate energy metabolism.

6. Conclusions

Spontaneous ingestion of dietary fat (Intralipid) and sugar (glucose and sucrose) activated POMC neurons in the hypothalamic arcuate nucleus via gustatory systems. It could be speculated that activated POMC neurons release β-endorphin to promote consummatory behavior of fatty and sugary foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.03.028.

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