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Short communication

Intrajejunal infusion of 2-monoacylglycerol reduced food intake without inducing diarrhea in rats

Chihiro Okuma^{a,*}, Takeshi Ohta^a, Makoto Ito^a, Hironobu Tadaki^a, Tomohiro Oda^a, Shinichi Kume^b, Jun Nishiu^a, Makoto Kakutani^a^a Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan^b Laboratory of Animal Physiology and Functional Anatomy, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

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ABSTRACT

Some nutrients, such as carbohydrate, fat and protein, are known to stimulate satiety. However, the effect of sn-2-monoacylglycerol (2-MG), one of the digestive products of triglycerides, on food intake is still unclear. In the present study, the effects of 2-MG on food intake and diarrhea were evaluated and compared with long-chain fatty acid (LCFA) in rats by intrajejunal infusion.

Intrajejunal infusion of 2-MG reduced food intake. In addition, 2-MG did not induce diarrhea at the condition that it comparably reduced food intake as compared with LCFA. These results suggest that 2-MG stimulates satiety without inducing diarrhea, different from LCFA.

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The physiological regulation of food intake involves signals from the gastrointestinal tract as well as from the central nervous system. Indeed, dietary fat, carbohydrate and protein stimulate satiety signals (1–3). Understandably, since overconsumption of nutrients consequently causes an over intake of calories and obesity, this satiety signal may be against overeating. On the other hand, overconsumption of nutrients also causes malabsorption, which leads to gastrointestinal symptoms.

Long-chain fatty acid (LCFA) and sn-2-monoacylglycerol (2-MG) are both digestive products of dietary triglyceride (TG) hydrolysis. Typically, these digestive products are absorbed in the upper intestine, however, if these products reach to the distal intestine, they may stimulate a feedback signal for satiety (4–6). The function of LCFA, one of the digestive products of TG, in the intestine has been well investigated. LCFA reportedly suppresses appetite in humans (7) and rats (8,9). The mechanism of induction of satiety by intestinal LCFA is mainly understood via gastrointestinal peptide secretion such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK). On the other

hand, LCFA stimulates not only satiety, but gastrointestinal (GI) symptoms. The malabsorption of LCFA is known to induce diarrhea (10). In contrast, the function of 2-MG in the intestine is still unclear. In the present study, the effect of 2-MG on food intake and diarrhea was evaluated and compared to the effect of LCFA in rats. To correctly investigate the effect in the intestine, 2-MG and LCFA were directly infused into the jejunum.

Male Sprague Dawley rats were purchased from Charles River Laboratories (Yokohama, Japan). Rats were maintained with free access to water and a normal chow diet (CRF-1, Charles River Japan) and housed in a room controlled for temperature at 23 ± 3 °C and humidity of $55 \pm 15\%$ in 12-h light/dark cycles (lights on from 8:00 AM to 8:00 PM). All procedures were conducted according to guidelines from Japan Tobacco's Animal Care Committee.

Prior to surgery, the rats were fasted overnight and then anesthetized with 50 mg/kg of intraperitoneal pentobarbital sodium (Abbott Laboratories, Chicago, IL). After abdominal celiotomy, a polyethylene tube (SP28; I.D. 0.4 mm, O.D. 0.8; mm, Natsume, Tokyo, Japan) was inserted into the duodenum (5 cm from the pylorus). The end of the tube reached the proximal jejunum (10 cm from the pylorus). The tube was fixed to the entrance of the intestine with silk sutures. The opposite end of the tube was threaded through the opening in the abdominal wall and tunneled subcutaneously to the dorsal surface of the neck, where a Dacron mesh

* Corresponding author.

E-mail address: Chihiro.okuma@jt.com (C. Okuma).

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anchor button (DC95; Instech Solomon, Plymouth Meeting, PA) was implanted. The tube was also fixed to the ventral abdominal wall and the anchor button mesh using silk sutures. The rats were allowed to recover for 1 week and become sufficiently adapted to the continuous infusion apparatus (Instech Solomon) before experiments were performed.

In the feeding test, the chow was removed and immediately after that, 2-MG (2-monolein, synthesized by Japan Tobacco Central Pharmacological Research Institute), LCFA (linoleic acid, commercial grade (~60% linoleic acid, ~30% oleic acid), Sigma, St. Louis, MO), or saline were infused intrajejunally for 4 h in rats. Since rats mainly feed during the dark period, the feeding was restarted at beginning of dark period (1 h after starting the infusion). Cumulative food intake was measured for 3 h, 6 h and 23 h.

In the experiment 1, we evaluated the effect of L2-MG and LCFA on food intake in three separate crossover studies. 2-MG at a rate of 200 $\mu\text{L}/\text{h}$, 300 $\mu\text{L}/\text{h}$ and LCFA at a rate of 200 $\mu\text{L}/\text{h}$ were infused to separate animals. The jejunal infusion of respective lipids or saline was performed on separate days. Statistical analysis was performed using paired t-tests. In experiment 2, stool characteristics were observed after infusion of respective lipids to evaluate diarrhea induction. Similar to experiment 1, jejunal infusions of 2-MG, LCFA or saline were performed on separate days. Statistical analysis was performed using Fisher's exact test. In experiment 3, to evaluate water contents, whole cecal contents were collected from scarified rats at 4 h after infusion of respective lipids or saline. Total water

contents were calculated from the differences in cecal contents weight before and after drying. Statistical analysis was performed using Turkey's multiple comparison tests.

In experiment 1, the intrajejunal infusion of 2-MG resulted in reduced food intake at both infusion rate of 200 $\mu\text{L}/\text{h}$ and 300 $\mu\text{L}/\text{h}$ in rats (Fig. 1). This effect in the 300 $\mu\text{L}/\text{h}$ 2-MG treated group appeared to be clearer than in the 200 $\mu\text{L}/\text{h}$ 2-MG treated group. The effect in the 300 $\mu\text{L}/\text{h}$ of 2-MG treated group was continuously observed after the completion of infusion. Similar to another report (8), the intrajejunal infusion of LCFA reduced food intake in our experiment. The efficacy in the 300 $\mu\text{L}/\text{h}$ 2-MG and the 200 $\mu\text{L}/\text{h}$ LCFA treated groups seemed comparable.

As mentioned above, nutrients are known to stimulate satiety to prevent overeating. Considering this, 2-MG, one of the digestive products of triglycerides, may stimulate satiety signals as a feedback mechanism for overeating. In the preliminarily study, we evaluated portal plasma GLP-1 levels in 2-MG (300 $\mu\text{L}/\text{h}$) and LCFA (200 $\mu\text{L}/\text{h}$) treated animals. At 2 h after starting the infusion (1 h after starting feeding), the rats were anesthetized with diethyl ether and blood was collected from the portal vein into siliconized tubes on ice containing approximately 5% of EDTA, aprotinin, and a dipeptidyl peptidase IV (DPP-IV) inhibitor (final concentrations of EDTA, aprotinin and DPP-IV inhibitor (Millipore Corporation) were 6 mmol/l, 1×10^3 KIU/ml and 50 $\mu\text{mol}/\text{l}$, respectively). Plasma was isolated by centrifugation at $10,000 \times g$ for 30 min at 4 °C. Plasma active GLP-1 levels were measured via a sandwich enzyme

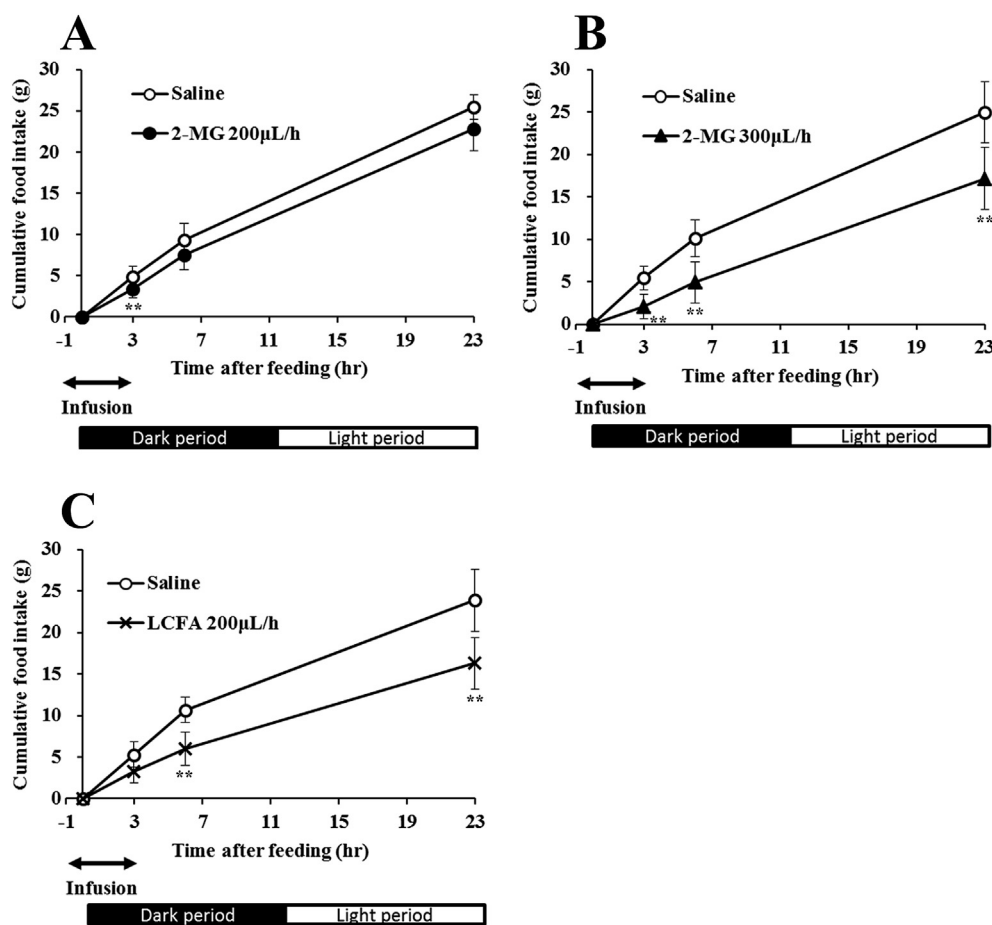


Fig. 1. The effects of intrajejunal 2-MG and LCFA infusion on food intake in rats. (A) Cumulative food intake in rats after jejunal infusion of 2-MG or saline at a rate of 200 $\mu\text{L}/\text{h}$ for 4 h ($n = 8/\text{group}$) (B) Cumulative food intake in rats after jejunal infusion of 2-MG or saline at a rate of 300 $\mu\text{L}/\text{h}$ for 4 h ($n = 6/\text{group}$) (C) Cumulative food intake in rats after jejunal infusion of LCFA or saline at a rate of 200 $\mu\text{L}/\text{h}$ for 4 h ($n = 6/\text{group}$). ** $P < 0.01$ vs. saline treatment (paired t-test).

Table 1

Incidence of diarrhea after intrajejunal infusion of 2-MG and LCFA in rats. 2-MG, LCFA or saline was intrajejunally infused for 4 h in rats. The feeding was restarted 1 h after starting the infusion. Stools were observed 23 h after starting the feeding. * $P < 0.05$ vs. saline treatment (Fisher's exact test).

Treatment	Saline	2-MG	LCFA
Number of animals observed diarrhea	0	0	4
Total number of animals	6	6	6
Significance	—	—	*

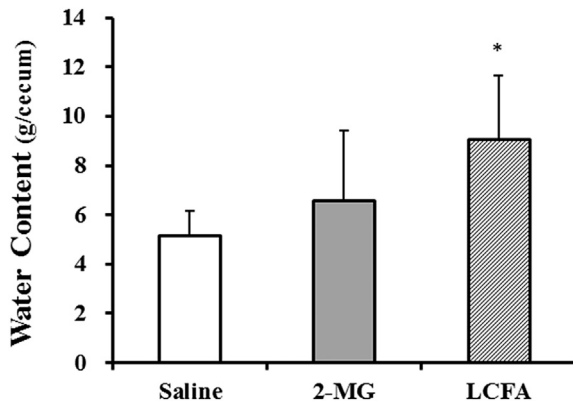


Fig. 2. The effects of intrajejunal 2-MG, LCFA and saline infusion on cecal water contents in rats ($n = 5-6$ /group). * $P < 0.05$ vs. saline treatment (Tukey multiple comparisons).

immunoassay (EIA) using assay kits (Millipore). Both 2-MG and LCFA tended to increase portal plasma GLP-1 levels (26 and 29 pM in the saline treated group ($n = 2$), 107, 76 and 190 pM in the 2-MG treated group ($n = 3$) and 218, 134 and 115 pM in the LCFA treated group ($n = 3$)). Therefore, increases in GLP-1 levels may partly contribute to the effect of 2-MG on food intake. However, the exact mechanisms for the satiety effect of 2-MG and decreases in food intake are still unclear. Further investigations are needed to clarify the contribution of gastrointestinal peptide secretion (including PYY and CCK) and/or the vagal afferent pathway for this effect of 2-MG.

In experiment 1, when food weight was measured, we noticed that some diarrheal stools were observed in LCFA treated rat but not in 2-MG treated rats. We therefore postulated that the sensitivity for gastrointestinal symptoms, such as diarrhea, is different between LCFA and 2-MG. We then compared the effect of intrajejunal infusion of 2-MG and LCFA on diarrhea (experiment 2). As shown in Table 1, in LCFA treated rats, diarrheal stools were observed in 4 out of 6 rats 23 h after starting feeding and this change was statistically significant. In contrast, in 2-MG treated rats, diarrheal stools were not observed in all rats. LCFA reportedly inhibited water and electrolyte absorption in the ileum, which may lead to steatorrhea and diarrhea in humans (11). Therefore, diarrhea induced by LCFA is interpreted that secretory diarrhea. Indeed, intrajejunal infusion of LCFA induced watery diarrhea in experiment 2. However, even though the efficacy of 2-MG on food intake reduction was almost the same as compared to LCFA, 2-MG did not induce diarrhea. To investigate into the mechanism of secretory diarrhea induction, we evaluated the water content in cecal contents in 2-MG (300 μ L/h) and LCFA (300 μ L/h) treated rats at 3 h after starting feeding (diarrhea was observed from 3 to 23 h after starting feeding in LCFA treated group in experiment 2). As shown in Fig. 2, the intrajejunal infusion of LCFA significantly increased cecal water contents. The values in LCFA treated group were 2 times

higher as compared to that in saline treated group. In contrast, the intrajejunal infusion of 2-MG did not change cecal water contents. From these, there is a probability that the difference of reactivity on fluid secretion between 2-MG and LCFA contributes their diarrhea inducibility. Although in-depth investigations are needed to confirm these mechanisms, the difference of diarrhea inducibility by both triglyceride digestive products is quite an interesting phenomenon.

The deletion of monoacylglycerol acyltransferase 2 (MGAT2), which converts 2-MG to diacylglycerol, is known to reduce food intake in high fat diet fed mice (12). In addition, a MGAT inhibitor increased intestinal monoacylglycerol content after lipid loading and decreased food intake only when fed a high fat diet (13). Moreover, transgenic mice that overexpress monoacylglycerol lipase (MGL), which converts monoacylglycerols to LCFA and glycerol specifically in small intestine, were hyperphagic (14).

In conclusion, we demonstrated that jejunal infusion of 2-MG reduced food intake in rats. These results may contribute to understanding the phenomenon in MGAT2 deficient mice, MGL transgenic mice and MGAT inhibitors. In addition, 2-MG did not induce diarrhea at the condition that it comparably reduced food intake as compared to LCFA, suggesting that 2-MG may become a unique and useful fat substitute in clinical, which stimulate satiety without inducing diarrhea.

Conflicts of interest

All the authors indicated no potential conflicts of interest.

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