The percentage of people with obesity is currently climbing ever higher on a worldwide scale. Excessive consumption of oil-laden and fatty foods greatly contributes to the prevalence of obesity and associated diseases. It is thought that high endocannabinoid levels are one of the reasons why obese people prefer a high-fat diet (HFD) to a normal diet in comparison with non-obese people. Understanding the mechanism of the preference for HFD and reversing it could reduce the prevalence of obesity.

The 2-arachidonoylglycerol (2-AG) is an endocannabinoid that binds to two cannabinoid receptors, CB1 and CB2 (1, 2). These endocannabinoids have been shown to mediate a wide range of biological effects such as reward-related feeding (2 – 4). It has been reported that plasma endocannabinoid 2-AG levels are elevated in obese men (5). These studies suggest that high endocannabinoid levels in obese people may be associated not only with feeding behavior, but also with a preference for HFD.

Endocannabinoids promote astroglial differentiation through the activation of CB1 receptors (6) and mediate neuron-astrocyte communication (7). Astrocytes contribute to the development of rewarding effects (8), and it is known that obesity induces functional astrocytes in the hypothalamus (9). Therefore, astrocytic CB1 receptors in the hypothalamus may be associated with a preference for HFD. However, the role of astrocytic CB1 receptors in the formation of a HFD preference has not been investigated.

The aim of this research is to demonstrate that there is a relationship between the endogenous cannabinoid system and astrocytes for the preference of HFD. In this study, we found a 2-week HFD intake produced a preference for HFD using the conditioned place preference (CPP) test and an increase in the expression of glial fibrillary acid protein (GFAP), a marker of reactive astrocytes. Furthermore, we investigated the pharmacological effects of specific CB1- and CB2-receptor antagonists on the preference for HFD in the CPP test and the expression of GFAP in the hypothalamus. These results suggested that HFD intake led to the development of a preference for HFD via astrocytic CB1 receptors in the hypothalamus.

Abstract. Endocannabinoids have been shown to activate reward-related feeding and to promote astrocytic differentiation. We investigated whether high-fat diet (HFD) intake produced a preference for HFD via an endocannabinoid-dependent mechanism. In the conditioned place preference test, the 2-week HFD–intake group showed preference for HFD and had increased expression of a marker for reactive astrocytes, glial fibrillary acid protein (GFAP), in the hypothalamus. The cannabinoid CB1–receptor antagonist O-2050 reduced the preference for HFD and expression of GFAP in the hypothalamus. These results suggested that HFD intake led to the development of a preference for HFD via astrocytic CB1 receptors in the hypothalamus.

Keywords: preference for high-fat diet, endocannabinoid, astrocyte
The CPP test has been used to evaluate rewarding effects. In this study, the CPP test was carried out to evaluate preferences after a 2-week HFD intake. A test chamber consisting of two compartments of equal size (15 × 15 × 15 cm) was used for the CPP test. The covering of the walls of the dark box was black and that of the light box was white. Both boxes were equipped with a grid floor, and a hatched transparent sheet was placed on the grid of the dark box. Six days were required to complete the following cycle of the CPP test: day 1, measurement of baseline preference to the light and dark boxes; days 2 – 5, conditioning in each box; and day 6, measurement of changes in preference by conditioning. On days 1 and 6, mice were not treated and spent 15 min in each box. On days 2 – 5, mice were confined and offered HFD or ND in the light or dark box, respectively, for 30 min. Mice were placed in the connecting zone on days 1 and 6. The preference scores were expressed as the change in the time (s) spent in the HFD-paired box before and after conditioning.

The expression of GFAP protein was evaluated by Western blotting following sample extraction and SDS-PAGE as described in our previous study (10). Tissue samples were homogenized at 4°C for 1 min in lysis buffer with a protease inhibitor cocktail. Tissue extracts were centrifuged at 15,000 rpm at 4°C for 30 min. The same procedure was followed for the supernatant.

SDS sample buffer was added to aliquots of tissue extracts containing 15 μg of total protein. Proteins were separated by SDS-PAGE (12% – 15% gel). Blotting was performed using a semi-dry method (Bio-Rad, Hercules, CA, USA). The blots were blocked with 5% non-fat dry milk at 4°C, and incubated with anti-GFAP polyclonal antibodies (Ventana, AZ, USA; 1:200) in TBS-T (Tris-buffered saline in 0.1% Tween 20), followed by goat anti-rabbit IgG AP conjugate (1:1000) in TBS-T and bovine anti-goat IgG AP conjugate (1:1000) in TBS-T. The blots were visualized by AP color reagents. The signal intensity of the blots was measured by an image analysis system (NIH Image, version 1.63).

O-2050 (Tocris, Ellisville, MO, USA) was dissolved in 1% Tween 80. AM630 (Tocris) was dissolved in 5% Tween 80 and 5% dimethyl sulfoxide (DMSO). O-2050 (10 mg/kg) and AM630 (3 mg/kg) were administered i.p. for 2 weeks before conducting the CPP test. Time spent in the HFD-paired box was significantly increased by conditioning to voluntary feeding of HFD after 2-week HFD intake, but not by 2-week ND intake (P < 0.05, Student’s t-test; Fig. 1A). Furthermore, the expression of GFAP was significantly increased in the hypothalamus after 2-week HFD intake (P < 0.05, Student’s t-test; Fig. 1B). The 2-week HFD intake led to a preference for HFD over ND in the HFD or ND choice test and significantly increased the levels of serum-cholesterol, serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), and liver weight. Body weight was gradually increased after 2-week HFD intake (data not shown). Therefore, we considered that 2-week HFD–intake mice was an obesity model animal.

A CB1-receptor antagonist O-2050, but not a CB2-receptor antagonist AM630, reduced the preference for HFD after 2-week HFD intake, but not by 2-week ND intake (P < 0.05, Student’s t-test; Fig. 2A). Furthermore, the expression of GFAP was significantly increased in the hypothalamus after 2-week HFD intake (P < 0.05, Student’s t-test; Fig. 2B). The 2-week HFD intake led to a preference for HFD over ND in the HFD or ND choice test and significantly increased the levels of serum-cholesterol, serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), and liver weight. Body weight was gradually increased after 2-week HFD intake (data not shown). Therefore, we considered that 2-week HFD–intake mice was an obesity model animal.

A CB1-receptor antagonist O-2050, but not a CB2-receptor antagonist AM630, reduced the preference for HFD after 2-week HFD intake, but not by 2-week ND intake (P < 0.05, Student’s t-test; Fig. 2A). Furthermore, O-2050, but not AM630, significantly reduced the expression of GFAP in the hypothalamus (F(2,12) = 9.623, P < 0.01, Tukey’s test; Fig. 2B).

The CPP test has been used to evaluate the reward of food and drug (11 – 13). In the present study, the 2-week HFD intake significantly increased the time spent in the HFD-paired box compared with that spent in a ND-paired box in the CPP test. Furthermore, the 2-week HFD intake...
led to a preference for HFD compared with ND in the HFD or ND choice test. These results suggested that HFD intake produced a preference for HFD. Moreover, the CB1-receptor antagonist O-2050, but not the CB2-receptor antagonist AM630, significantly reduced the duration of time spent in the HFD-paired box. It has been reported that the reward-related preference for nicotine and ethanol has been related to CB1 receptors, and blockade of CB1 receptors suppressed the intake of sweet foods more than that of standard food (2, 3, 14). Thus, these facts suggested that 2-week HFD intake led to the development for a preference for HFD, which was mediated via the CB1 receptors.

It has been reported that the involvement of astrocytes have been implicated in the development of the rewarding effects and the drug dependence (8). Moreover, obesity has been known to be associated with the induction of functional astrocytic leptin receptors in the hypothalamus, which is associated with feeding behavior (9). Therefore, we investigated whether 2-week HFD intake could increase the expression of GFAP in the hypothalamus. In the present study, mice that received a 2-week HFD significantly increased the expression of GFAP in the hypothalamus, suggesting that a preference for HFD is related to astrocytic signaling in the hypothalamus. Furthermore, the CB1-receptor antagonist O-2050, but not the CB2-receptor antagonist AM630, significantly reduced the amount of GFAP immunoreactivity in the hypothalamus after 2-week HFD intake. These results were the first to demonstrate that the preference for HFD developed via the activation of astrocytic CB1 receptors in the hypothalamus. Recent studies show that the levels of endocannabinoids such as 2-AG in the hypothalamus was increased when the motivation to eat was high (15). Furthermore, several studies have demonstrated that cannabinoids promote astroglial differentiation via CB1-receptor activation in vitro and mediate neuron–astrocyte communication (6, 7). In this study, the levels of 2-AG in the hypothalamus was increased by 2-week HFD intake (data not shown). The endocannabinoid 2-AG is full agonist of CB1 receptors. Therefore, it was thought that 2-AG differentiated hypothalamic astrocytes in response to 2-week HFD intake. This evidence implies that the preference for HFD is mediated by the endocannabinoid system through increased levels of 2-AG, stimulating astrocytic CB1 receptors in the hypothalamus and might be associated with synaptogenesis through neuron-astrocyte communication.

In the present study, our results demonstrated for the first time that intake of HFD led to the development of a preference for HFD through the activation of CB1 receptors and increased the proliferation of astrocytes in the hypothalamus. Our findings indicate that the endocannabinoid system and astrocytes are targets for the treatment of obesity.

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