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Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity

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HIGHLIGHTS

- Mice maintained on a western diet become obese and display hyperphagia.
- Hyperphagia results from increases in meal size and rate of consumption.
- Intestinal and plasma endocannabinoids increase in western diet-induced obese mice.
- Inhibiting peripheral endocannabinoid signaling normalizes feeding patterns.

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ABSTRACT

The endocannabinoid system in the brain and periphery plays a major role in controlling food intake and energy balance. We reported that tasting dietary fats was met with increased levels of the endocannabinoids, 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide, in the rat upper small intestine, and pharmacological inhibition of this local signaling event dose-dependently blocked sham feeding of fats. We now investigated the contribution of peripheral endocannabinoid signaling in hyperphagia associated with chronic consumption of a western-style diet in mice ([WD] i.e., high fat and sucrose). Feeding patterns were assessed in male C57BL/6Tac mice maintained for 60 days on WD or a standard rodent chow (SD), and the role for peripheral endocannabinoid signaling at CB_1Rs in controlling food intake was investigated via pharmacological interventions. In addition, levels of the endocannabinoids, 2-AG and anandamide, in the upper small intestine and circulation of mice were analyzed via liquid chromatography coupled to tandem mass spectrometry to evaluate diet-related changes in endocannabinoid signaling and the potential impact on food intake. Mice fed WD for 60 days exhibited large increases in body weight, daily caloric intake, average meal size, and rate of feeding when compared to control mice fed SD. Inhibiting peripheral CB_1Rs with the peripherally-restricted neutral cannabinoid CB_1 receptor antagonist, AM6545 (10 mg/kg), significantly reduced intake of WD during a 6 h test, but failed to modify intake of SD in mice. AM6545 normalized intake of WD, average meal size, and rate of feeding to levels found in SD control mice. These results suggest that endogenous activity at peripheral CB_1Rs in WD mice is critical for driving hyperphagia. In support of this hypothesis, levels of 2-AG and anandamide in both, jejunum mucosa and plasma, of ad-libitum fed WD mice increased when compared to SC mice. Furthermore, expression of genes for primary components of the endocannabinoid system (i.e., cannabinoid receptors, and endocannabinoid biosynthetic and degradative enzymes) was dysregulated in WD mice when compared to SC mice. Our results suggest that hyperphagia associated with WD-induced obesity is driven by enhanced endocannabinoid signaling at peripheral CB_1Rs.

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1. Introduction

Significant scientific and clinical evidence suggests one major driver of obesity is chronic consumption of foods that contain large quantities of fats and sugars (i.e., the western diet) [1,2]. Humans, rodents, and possibly other mammals detect dietary fats [3–7] and sugars [8] via receptors in the oral cavity and alimentary tract, which are critical in mediating preferences displayed for these high-energy foods [6,9,10]. Numerous signaling pathways play important roles in the control of food intake, energy balance, and reward [9,11], including endocannabinoid (eCB) signaling at cannabinoid CB_1Rs in the brain [12–23] and periphery [12,24–34].

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Recent evidence suggests that the intake of palatable foods may be controlled by peripheral eCB signaling [9]. For example, tasting emulsions containing mono- (i.e., oleic acid) or di-unsaturated fats (i.e., linoleic acid) – but not carbohydrate (i.e., sucrose) or protein – was met with large increases in eCB levels in the rat upper small intestine [27,29]. Pharmacological inhibition of eCB signaling at peripheral CB_1Rs blocked (*i*) the intake of dietary fats in sham feeding rats [27] and (*ii*) robust preferences for di-unsaturated fats in a sham feeding two-bottle choice test [29] [see [6,35] for description of the sham feeding paradigm in rat, which isolates the cephalic phase of feeding from post-ingestive influence].

In addition to tasting dietary fats, we reported that fasting for up to 24 h is associated with increases in production of the eCB, 2-arachidonoyl-*sn*-glycerol (2-AG), in the upper small intestine of rats through a cholinergic-dependent mechanism that possibly involves the vagus nerve [28]. For these experiments, fasting-induced 2-AG biosynthesis in the jejunum mucosa was blunted in rats that received either full subdiaphragmatic vagotomy or local intraduodenal infusion of the subtype 3 muscarinic acetylcholine receptor (m_3 mAChR) antagonist, DAU5884 [28]. Furthermore, pharmacological inhibition of small intestinal m_3 mAChRs or CB_1Rs blocked fasting-induced refeeding [28]. Thus, gut-brain eCB signaling is a proposed orexigenic signal that may promote feeding under several distinct behavioral and metabolic conditions.

Several studies in humans and rodents suggest that peripheral eCB levels are increased under conditions of obesity [36–42]; however, a role for peripheral eCB signaling in driving hyperphagia associated with a western-style diet (WD) is unknown. In the current study, we investigated the impact of chronic consumption of WD on eCB levels in circulation and the upper small intestinal epithelium of mice, the contribution of WD-induced enhancements in eCB signaling at peripheral CB_1Rs in promoting hyperphagia associated with a WD, and expression of genes encoding key eCB system components in the small intestine.

2. Materials and methods

2.1. Animals

Eight-week old male C57BL/6 mice (Taconic, Oxnard, CA, USA) were group-housed with free access to water and food, unless otherwise noted for food deprivation studies, and maintained on a 12 h light/dark cycle (lights off at 1800 h). Test diets consisted of standard lab rodent chow [(SD) Lab Diet 5001, St. Louis, MO, USA; 13.4% kcal as fat, 56% kcal from carbohydrates, mostly starch], or western-style diet [(WD) Research Diets D12709B, New Brunswick, NJ, USA; 40% kcal as fat, 43% kcal from carbohydrates, mostly sucrose]. Five days prior to experimentation, animals were single-housed in cages with wire mesh inserts to prevent coprophagia during 24 h food deprivation experiments. For studies analyzing feeding behaviors, mice were single-housed in feeding chambers (TSE, Chesterfield, MO, USA) with free access to water and SD or WD (described further below in Feeding behavior). All procedures met the U.S. National Institute of Health guidelines for care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

2.2. Feeding behavior

Animals were acclimated to feeding chambers for five days prior to experimentation, and tested following 60 days on their respective test diet. Feeding behavior was monitored for the subsequent 24 h to assess daily intake patterns, or for 6 h following drug treatments. Feeding parameters included total caloric intake, average meal size, average rate of intake (kcals from food per minute), average number of meals, average meal duration, and average post-meal interval.

2.3. Chemicals and administration schedule

The peripherally restricted Cannabinoid Receptor Type 1 (CB_1) antagonist, AM6545 (Sigma, St. Louis, MO, USA), was administered by IP injection at 10 mg/kg in 2 mL/kg. Vehicle consisted of 7.5% dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA), 7.5% tween 80 (Chem Impex Intl Inc., Wood Dale, IL, USA), 85% sterile saline (warmed in a bath sonicator for 30 min) 30 min prior (16:30 h) to the onset of behavioral analysis in feeding chambers. All control conditions were identical, except without drug present in the vehicle. The pharmacokinetics and half-life of AM6545 are not well-established, thus we chose to evaluate intakes over a period of 6 h from time of administration after the onset of the dark phase. Mice maintained on SD or WD were split into two groups and analyzed across two sessions of behavioral testing such that each subgroup received either vehicle or drug with 72 h between administration.

2.4. Tissue processing

2.4.1. Lipid extraction

Isofluorane was used to anesthetize animals at time of tissue harvest (0900 to 1100 h), following 24 h food deprivation or ad-libitum feeding. Blood was collected by cardiac puncture and stored in EDTA-lined tubes on ice, then plasma was obtained by centrifugation (1500 *g* for 10 min, maintained at 4 °C). Jejunum was rapidly collected, washed with phosphate-buffered saline (PBS) on ice, sliced longitudinally on a stainless steel plate on ice, scraped with a glass slide to obtain mucosa, then snap-frozen in liquid N_2. All samples were stored at −80 °C until processing. Frozen tissues were weighed and subsequently homogenized in 1.0 mL of methanol solution containing the internal standard, [$^2H_5$] 2-AG and [$^2H_4$]-AEA (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted with chloroform (2 mL) and washed with water (1 mL). Lipids were similarly extracted from plasma samples, with the exception of a 0.9% saline wash replacing water (0.1 mL plasma at the expense of saline). Organic phases were collected and separated by open-bed silica gel column chromatography as previously described [28]. Eluate was gently dried under N_2 stream (99.998% pure) and resuspended in 0.1 mL of methanol:chloroform (9:1), with 1 μL injection for analysis by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC/MS/MS).

2.4.2. Measurement of 2-AG and anandamide

Data was collected using an Acquity I Class UPLC system coupled to a Xevo TQ-S Mass Spectrometer (Waters, Milford, MA, USA) with accompanying electrospray ionization (ESI) interface. Lipids were separated on an Acquity UPLC BEH C_{18} column (2.1 × 50 mm i.d., 1.7 μm, Waters) with inline Acquity guard column (UPLC BEH C_{18} VanGuard Pre-column; 2.1 × 5 mm i.d., 1.7 μm, Waters), and eluted by a gradient of methanol in water (0.25% acetic acid, 5 mM ammonium acetate) according to the following gradient at a flow rate of 0.4 mL per min: 80% methanol 0.5 min, 80% to 100% methanol 0.5–2.5 min, 100% methanol 2.5–3 min, 100% - 80% methanol 3–3.1 min). Column temperature was maintained at 40 °C, and samples were maintained in the sample manager at 10 °C. Argon (99.998%) was used as collision gas. MS detection was in positive ion mode and capillary voltage set at 0.1 kV. Cone voltage and collision energy as follows, respectively: 2-AG = 30v, 12v; [$^2H_5$] 2-AG = 25v, 44v; anandamide = 30v, 14v; [$^2H_4$] anandamide = 26v, 16v. Lipids were quantified using a stable isotope dilution method detecting protonated adducts of the molecular ions $[M + H]^+$ in the multiple reaction monitoring (MRM) mode. Acyl migration from 2-AG to 1-AG is known to occur [43], thus all reported values for 2-AG represent the sum of 2-AG and 1-AG. Tissue processing and LCMS analysis from an individual experiment occurred independently of other experiments. Extracted ion chromatograms were used to quantify 2-AG ($m/z = 379.3 > 287.3$) and anandamide ($m/z = 348.3 > 62.0$),

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