Endocannabinoids: An appetite for fat

Vincenzo Di Marzo[1]

Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli, Italy

It has been known for decades that marijuana causes the "munchies," i.e., a hunger for palatable food, and for more than 10 y that endocannabinoids (eCBs), in some ways marijuana's counterpart in the organism, are orexigenic mediators (1). When injected in the hypothalamus (HT) or nucleus accumbens (NAc), two key brain areas for the homeostatic and hedonic control of food intake, these compounds stimulate food consumption by acting at the cannabinoid CB_1 receptor, one of the two G protein-coupled receptors for marijuana's psychotropic and appetite-inducing component, Δ^9-tetrahydrocannabinol (2, 3). Conversely, systemic pharmacological blockade of CB_1 receptors causes anorectic effects in rodents exposed to palatable food, or food-deprived for a few hours, and in obese animals (3–6). The two most studied eCBs, anandamide and 2-arachidonoylglycerol (2-AG), are considered local mediators produced by cells only following stimulation, and in this they differ from other signals controlling food intake, which are released into the bloodstream and/or prestored in vesicles (1). What makes the eCBs similar to most orexigenic signals is the fact that their concentrations in the HT and NAc, but also in the proximal intestine, which transmits to the brain the state of "emptiness" or "fullness" of the gut, increase during food deprivation and decrease immediately after food consumption (3, 7, 8). This mechanism is presumably caused by the opposing effects on eCB levels of hormones such as leptin, on the one hand, and ghrelin and corticosterone, on the other hand, the levels of which also vary during food deprivation and refeeding (1, 6). These food intake- and hormone-sensitive changes in eCB signaling are thought to play a key role in the regulation of (*i*) the release of central neurotransmitters and neuropeptides controlling food intake and (*ii*) the activity of vagal fibers from the duodenum to the brainstem, which signal gastric distension (1). In this scenario, the results of the elegant study by DiPatrizio and coworkers published in PNAS (9), although in full agreement with the general orexigenic function of eCBs and CB_1 receptors, might seem somewhat surprising. In fact, the authors report that a fatty meal elevates eCBs selectively in the rat proximal small intestine, and propose that this effect: (*i*) is induced by the orosensory properties of the meal; (*ii*) is mediated by vagal afferent and efferent terminals, and (*iii*) reinforces fat intake via CB_1 receptor activation (9).

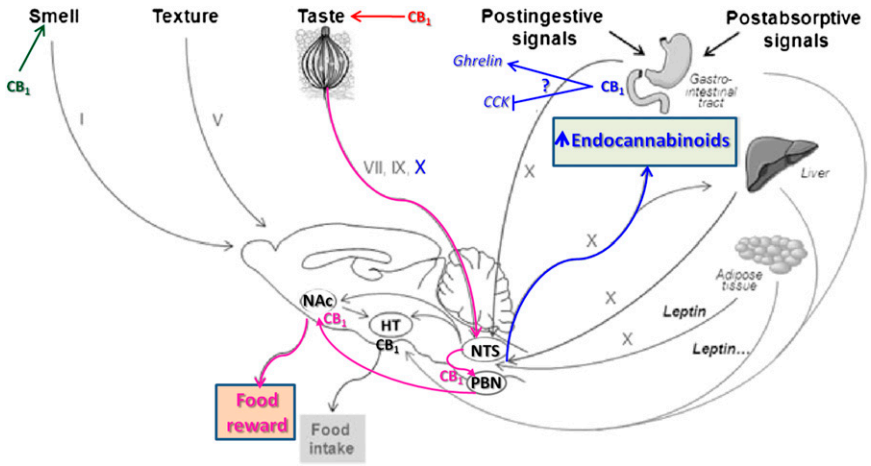

Fig. 1. Effects of CB_1 receptor activation by eCBs on the orosensory properties of food. Adapted from Gaillard et al. (10). Blue, red, or purple arrows and text denote CB_1-mediated actions activated by fat, sucrose, or both, respectively (9, 17, 18). Blunted arrow denotes inhibition. Roman numbers denote cranial and sympathetic nerves. Green arrow and text depict the recently reported CB_1-mediated enhancement of odor sensitivity in *Xenopus laevis* larvae (19).

The authors used a "sham-feeding" protocol, which consists of immediately draining liquid foods from the stomach through a chronically implanted gastric cannula (9). In this way, the stomach does not undergo the distension that follows each meal and the ensuing production of gastric anorexigenic signals such as cholecystokinin (CCK), nor the direct activation of vagal fibers that transmit changes in gastric volume to the hindbrain. Thus, sham feeding produces effects on food intake specifically linked to the orosensory properties of food and blunts homeostatic negative feedbacks on food intake, including the previously observed postingestive reduction of small intestinal eCB levels (7, 8). The authors found that liquid meals containing sucrose or proteins do not alter eCB levels in the proximal intestine, which were, instead, enhanced by a fatty meal, although not in sham-fed rats that had underwent subdiaphragmatic vagotomy. The authors suggest that "cephalic signals elicited by sham feeding of fat, but not other nutrients, selectively mobilize eCBs in the upper gut through a mechanism that is mediated by efferent vagal fibers" (9). This phenomenon results in increased liquid fat consumption via activation of duodenal CB_1 receptors, as intraduodenal injection of the CB_1 antagonist rimonabant, or systemic injection of a more peripherally restricted CB_1 antagonist, reduced fat, more than normal chow, intake in sham-fed rats.

These findings are striking, as they imply the existence of mechanisms through which fat "sensors" in the oropharyngeal cavity can stimulate cranial afferents, and hence vagal efferents in the proximal small intestine, thereby enhancing eCB levels and acting as a "priming trigger" for further intake of fat versus other nutrients. Among such sensors, CD36, TRPC5, GPR40, and GPR120 are expressed in taste buds (10, 11), and it will be interesting to see if any of them is connected with this phenomenon. However, one caveat of the study is that the authors, possibly to habituate the animals to the stress induced by this procedure, performed sham feeding with the test nutrients for 4 d (30 min per day, followed by ad libitum feeding) before analyzing eCB levels in various peripheral and central tissues after a fifth administration of the test meals. Therefore, it is not possible from these data to understand whether fat-induced stimulation of small intestinal eCB levels was the result of just the last test, or rather an adaptive mechanism induced by the fatty meal over the 5 d of the experiment. Indeed, a recent study showed that a high-fat diet administered for 3 d can enhance hypothalamic 2-AG levels in free-feeding mice (12). It would be interesting to replicate the results by

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[1]E-mail: vdimarzo@icmib.na.cnr.it.

DiPatrizio and colleagues (9) in freely feeding rats, and check if, after one trial of food deprivation followed by one brief bout of high-fat chow, the small intestinal levels of eCBs are transiently elevated, to be then reduced during ad libitum feeding with this diet, as shown in a previous study (8). Furthermore, as fasting-induced elevation of duodenal eCB levels is dramatically enhanced in obese rats (8), it would be intriguing to see the mechanism revealed by DiPatrizio et al. (9) undergoing dysregulation in obesity.

The authors also studied the biochemical mechanisms through which liquid fat enhanced the small intestinal levels of eCBs, by measuring the overall activity of the anabolic and catabolic pathways for these mediators, and the levels of some anandamide and 2-AG biosynthetic precursors (reviewed in ref. 1). These experiments allowed the authors to propose that reduction of the activity of the 2-AG hydrolyzing enzyme(s) might underlie the elevation of 2-AG levels, whereas both enhancement of biosynthesis and reduction of hydrolysis would explain the increase of anandamide levels. Although these latter data are perhaps still preliminary, both in view of the fact that several enzymes and biosynthetic precursors participate in the regulation of eCB levels, and because the authors did not measure the mRNA or protein expression levels of any of these enzymes, they are intriguing and provide initial information on whether the stimulation of small intestinal eCB levels is an acute effect or the result of repeated lipid meal sham feeding. In fact, eCB biosynthesis is Ca^{2+}-sensitive and stimulated within minutes after increase of intracellular Ca^{2+}. By contrast, no example of acute inactivation of eCB metabolic enzymes has been reported, and usually it is the expression of these enzymes that undergoes down-regulation by chronic stimuli (1, 13). Thus, one might speculate that small intestinal elevation of anandamide levels was the result of both acute and chronic orosensory stimulation by fats, whereas the effect on 2-AG was mostly caused by chronic stimulation. Depolarization of vagal efferent terminals in the proximal intestine might be directly responsible for the acute effect. Inhibition of leptin signaling at *ob* receptors in CCK1-receptor–expressing vagus afferents of the proximal intestine might, instead, explain the effects on both eCB biosynthesis and degradation, as this hormone enhances the expression of FAAH (13) (the enzyme responsible for anandamide, and to some extent 2-AG, degradation) and reduces eCB production in the HT (6) and adipocytes (14). The occurrence of both these mechanisms (i.e., fat ingestion-induced vagal efferent depolarization or leptin signaling inhibition) in sham-fed rodents needs to be investigated.

The mechanisms through which stimulation of CB_1 receptors in the proximal intestine stimulates fat intake in sham-fed rats also remain to be investigated. The authors hypothesize that these might include influence on "the generation or action of neurohumoral factors that affect satiation (meal size) and satiety (intermeal interval), such as ghrelin" (9). Indeed, ghrelin acts on both homeostatic and hedonic aspects of food intake (15), and systemic CB_1 receptor blockade inhibits its circulating levels in ad libitum-fed rats (16). However, ghrelin is produced by P/D1 cells in the stomach fundus, and locally acting mediators such as the eCBs might not easily reach this tissue from their site of production in the proximal intestine. This latter tissue contains instead the I cells, which produce CCK. Thus, eCBs might inhibit CCK release from I cells, thereby stimulating food intake.

Previous studies showed that eCB activation of CB_1 receptors in the pontine parabrachial nucleus (PBN), a station transmitting to upper limbic structures the gustatory information received from second-order neurons of the nucleus tractus solitarius (NTS), which in turn receives information from oropharyngeal afferents, stimulates the intake of both fat- and sucrose-containing meals (17). Thus, both vagal afferents that transsynaptically (via the NTS) communicate with the PBN, and those connected to the proximal intestine, reinforce the intake of palatable/high-caloric nutrients via different CB_1-mediated mechanisms, although only the former are capable to also strengthen the gustatory/rewarding properties of sucrose (Fig. 1). Moreover, recent findings revealed the presence of CB_1 receptors also in T1r3-expressing taste bud cells, which transduce sucrose sensing, and showed that local CB_1 activation by eCBs enhance the sweet responses of isolated taste bud cells, whereas systemic CB_1 activation selectively enhances the behavioral and chorda tympani gustatory nerve responses elicited by sweeteners (18). However, in this previous study, no evidence was provided to show that sucrose activates eCB signaling in the tongue or chorda tympani (18), whereas the present authors found that liquid fat does not alter eCB levels in the tongue, nor in brain areas involved in food intake (including the PBN, HT, and ventral striatum) (9).

In conclusion, the important work by DiPatrizio et al. (9) fits well with the notion that vertebrates have developed several different mechanisms through which the gustatory and olfactory (19) properties of different palatable foods can be reinforced by eCBs and CB_1 receptors, in ways distinct from, although probably integrated with, the pathways through which this signaling system influences other homeostatic or hedonic aspects of food intake (1). One aspect that should be investigated next is whether the physical nature of the meal—food texture and consistency—also modulates eCB signaling in tissues that control appetite.

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