Modulation of sweet taste sensitivity by orexigenic and anorexigenic factors

Masafumi Jyotaki, Noriatsu Shigemura and Yuzo Ninomiya

Section of Oral Neuroscience, Kyushu University, Graduate School of Dental Sciences, Fukuoka 812-8582, Japan

Abstract. The present study summarized recent findings on roles of leptin and endocannabinoids as modulators of the peripheral components of sweet taste. The positive effect of endocannabinoids on sweet sensitivity was opposed to that of leptin which suppresses sweet sensitivity. Leptin and endocannabinoids, therefore, not only regulate food intake via central nervous systems but also may modulate palatability of foods by altering peripheral sweet taste responses via their cognate receptors. Orexigenic and anorexigenic factors such as endocannabinoids and leptin may affect energy homeostasis by regulating taste sensitivity.

Key words: Sweet taste, Leptin, Endocannabinoids, Modulation, Energy homeostasis

SENSORY information of taste is important for evaluating the quality of food components. In general, sweet, salty, umami, sour and bitter are considered to be basic taste qualities. Each of these may be responsible for the detection of nutritious and poisonous contents; sweet for carbohydrate sources of calories, salty for minerals, umami for protein and amino acids contents, sour for ripeness of fruits and spoiled foods, bitter for harmful compounds. The detection of these taste qualities begins with the taste receptors on the apical membrane of taste receptor cells. Recent molecular genetic studies have proposed candidate receptors for the 5 basic tastes. Sweet, bitter and umami tastes are mediated by G protein-coupled receptors (GPCRs), taste receptor type 1 family (T1Rs: sweet and umami) and type 2 family (T2Rs: bitter). T1R3 combines with T1R2 to form a sweet taste receptor and with T1R1 to form an umami taste receptor [1, 2]. T2Rs are a family of ~25 highly divergent GPCRs and some of them have been identified their specific bitter ligands [3, 4]. Salty and sour tastes are mediated by channel-type receptors; epithelial sodium ion channel (ENaC) for salty [5], and acid-sensing ion channels (ASICs) [6], hyperpolarization-activated cyclic nucleotide-gated potassium channels (HCNs) [7], and polycystic kidney disease 1L3 and 2L1 heterodimer (PKD1L3+PKD2L1) [8] for sour (Fig. 1). It has also been shown that each of these receptors is expressed in separate population of taste bud cells and genetic elimination of taste receptor (or receptor cells) leads to severe loss of sensitivity to a specific taste quality [1, 4, 9, 10], suggesting that different taste bud cells define the different taste modalities and that activation of a single type of taste receptor cells is sufficient to encode taste quality (Fig.1).

There is growing evidence that taste function can be modulated by hormones or other factors that act on receptors present in the peripheral gustatory system. Leptin, an anorexigenic mediator that reduces food intake by acting on hypothalamic receptors [11], selectively suppresses sweet taste responses and these effects may be mediated by leptin receptor, Ob-Rb, expressed in sweet-sensitive receptor cells [12-14]. Glucagon-like peptide-1 (GLP-1), an incretin that influences glucose transport, metabolism and homeostasis [15], normally acts to maintain or enhance sweet taste sensitivity by its paracrine activity [16]. More recently, we found that sweet-sensitive cells also express receptors for endocannabinoids, orexigenic mediators that induce appetite and stimulate food intake via endocannabinoid (CB1) receptors mainly in hypothalamus [17]. In the peripheral taste system, endo-
cannabinoids also oppose the action of leptin and enhance sweet taste responses in mice [18]. Thus, leptin and endocannabinoids, therefore, not only regulate food intake via central nervous systems but also modulate palatability of foods by altering peripheral sweet taste sensitivity. This paper summarized the data from recent studies on the reciprocal modulation of peripheral sweet taste sensitivity by leptin and endocannabinoids and its role in regulating energy homeostasis.

Leptin inhibition on sweet taste responses in mice

Leptin is a hormone primarily produced in adipose cells. It regulates food intake, energy expenditure and body weight mainly via activation of the hypothalamic leptin receptor. Leptin is thought to promote weight loss, at least in rodents, by suppressing appetite and stimulating metabolism [19]. Mutant mice that have defects in either leptin or the leptin receptor, such as ob/ob and db/db mice, are hyperphagic, massively obese and diabetic [19, 20]. The functional leptin receptor (Ob-Rb) is abundantly expressed in several hypothalamic nuclei, which are major target sites for the hormone. However, Ob-Rb is also expressed in peripheral organs, such as lymph nodes, liver, lung, uterus, adipose tissue, kidney and pancreas [21]. Recently, we have demonstrated that the taste organ is another peripheral target for leptin. The hormone directly acts on taste receptor cells via Ob-Rb expressed in these cells and it specifically inhibits peripheral gustatory neural and behavioral responses to sweet substances without affecting responses to sour, salty and bitter substances in lean mice. Such selective inhibition of sweet taste responses by leptin was not observed in leptin receptor-deficient db/db mice [12, 13, 22]. Instead, the db/db mice showed enhanced gustatory neural responses to sweet compounds. In lean mice, the strength of suppressive effects by leptin was at most about 30% of control responses, and the effect may saturate when plasma leptin concentration reaches about 15 - 20 ng/mL [12, 22]. It was also found that potassium outward currents of isolated taste cells in response to depolarizing voltage steps were increased during bath application of leptin to the cells [12]. Increase of potassium outward current may lead to reduction of cell excitability and impulse frequencies (Fig.3). Indeed, we have recently found that impulse frequencies of a single taste bud cell in response to sweet stimuli were significantly reduced during bath application of 20 ng/mL leptin to the basolateral membrane of the cell and recovered after wash-out of leptin. Such leptin inhibition of sweet taste responses was observed in about a half of sweet-sensitive cells (Yoshida et al., unpublished observation).

Diurnal variation of plasma leptin level and sweet taste sensitivity in human

It has been shown in both rats and humans that there is a diurnal pattern in circulating leptin levels [23, 24]. In humans, leptin levels start rising before noon and peak between 23:00 h and 01:00 h, after which the levels decline until morning [25]. This diurnal pattern of plasma leptin was reported to show meal-related shifts. For example, when meals were shifted by 6.5 h without changing the light or sleep cycles in humans, the plasma leptin levels were similarly shifted by 5-7 h [26]. The nocturnal rise of leptin does not occur if the subjects are fasted [27]. Therefore, if leptin acts as a modulator for sweet taste sensitivity, and it shows diurnal variation, then it follows that the threshold for sweet taste may show correlated diurnal variation.

To examine this possibility, we measured recognition thresholds of non-obese subjects (BMI < 25) for various taste stimuli and plasma leptin levels at several time-points during the day under normal meal conditions with 3 meals, and restricted meal conditions with 1 or 2 meals per day [14]. In the normal feeding condition, leptin concentrations started rising before

Fig. 1 Five basic taste qualities are encoded by a single type of taste receptor cells and taste neurons.
noon and peaked in the night. This rise in leptin occurred later in the 2 and 1 meal conditions resulting in a phase shift of diurnal variation. With regard to taste recognition thresholds, similar to plasma leptin levels, significant time-dependent increases in thresholds for sucrose, glucose and saccharin were observed in the normal meal condition [14]. That is, subjects needed higher concentrations of these sweeteners to detect the stimulus quality when they were tested in the evening compared to the morning. There was also a phase shift in 1 or 2 meal conditions eliminating the time dependent changes in sweetener recognition threshold. Diurnal variations in sweetener thresholds were significantly different among 3 meal conditions. This diurnal variation is sweet-taste selective: it was not observed in thresholds for other taste stimuli (NaCl, citric acid, quinine and mono-sodium glutamate) [14]. By contrast, the diurnal variations for sweet recognition thresholds disappeared in the over-weight and obese subjects (BMI > 25: Shigemura et al., unpublished observation). Mean plasma leptin level of the over-weight and obese subjects at morning was already ~20 ng/mL, which may be around the saturation level of leptin effect in mice (about 15 - 20 ng/mL) [12].
showed meal-related changes with increases evident after each meal in the 3 different feeding conditions. Moreover, increases in blood glucose after the first meal in the 1 and 2 meal conditions were higher than that in the normal, 3 meal feeding condition. Similar tendency was observed in case of plasma insulin levels. The diurnal variation for sweet thresholds in normal feeding condition (3 meals) was independent of the meal timing and thereby blood sugar and plasma levels, suggesting that the lack of diurnal variation for sweet recognition thresholds in the over-weight and obese subjects may be due to their higher basal plasma leptin levels.

**Association between sweet taste sensitivity and post-ingestive insulin responses**

Our human study also demonstrated that blood glucose and plasma insulin levels of non-obese subjects showed meal-related changes with increases evident after each meal in the 3 different feeding conditions. Moreover, increases in blood glucose after the first meal in the 1 and 2 meal conditions were higher than that in the normal, 3 meal feeding condition. Similar tendency was observed in case of plasma insulin levels. The diurnal variation for sweet thresholds in normal feeding condition (3 meals) was independent of the meal timing and thereby blood sugar and plasma levels.
insulin levels [14]. However, it is interesting to note that post-ingestive rises of blood glucose and insulin levels of individuals after meal were negatively correlated with leptin levels and recognition thresholds for sucrose before meal [14]. This suggests that greater post-ingestive rises of blood glucose and insulin levels may be associated with lower leptin levels and higher sweet sensitivities before meal.

With respect to this potential linkage, a previous study showed that oral stimulation with sucrose elicits an increase in activities of the pancreatic branch of the vagal nerve in rats, whereas no such response was observed upon a stimulation with NaCl [28]. The vagal nerve response to sucrose occurs about 5 min after onset of the stimulation and lasts for at least 30 min. Since so-called cephalic-phase insulin release (CPIR) is known to occur as early as 1-4 min after food ingestion [29], the late response observed in the vagal effector nerve to oral stimulation with sucrose should not relate to the CPIR but may be involved in the factors relate to post-ingestive insulin release. In addition, recent studies demonstrated that enteroendocrine cells in the gastrointestinal tract express sweet receptors (T1R2+T1R3) and leptin receptors [30, 31], and release GLP-1 in response to sugars and non-nutritive sweeteners. This leads to an increase in expression of Na+/glucose co-transporter, SGLT1, followed by increased glucose absorption in enterocytes [32]. Also, sweet receptors (T1R2+T1R3) and their downstream signaling molecules, gustducin (Ggust) and phospholipase C-beta 2 (PLCβ2) are found to be expressed in pancreatic beta cells and several artificial sweeteners, such as sucralose, saccharin Na and acesulfame-K are shown to stimulate insulin secretion from the mouse pancreatic islet via sweet receptors on the cell membrane [33]. Pancreatic beta cells are also shown to express leptin receptors [34]. Our recent study demonstrated that enteroendocrine STC-1 cells, like taste cells, responded to sweet compounds and other taste stimuli with rapid increases of intracellular Ca2+ concentration, and Ca2+ responses to sweet compounds were suppressed by leptin in a concentration-dependent manner (Jyotaki et al., unpublished observation). This suggests a possibility that enteroendocrine cells and pancreatic beta cells may also possess comparable sweet sensitivities with diurnal variations parallel with plasma leptin levels and meal-related phase shifts. If this is the case, post-ingestive rises in glucose and insulin levels may be influenced by sweet sensitivities of both taste and gut and pancreatic beta cells [14].

**Endocannabinoid effects on the peripheral taste organ: enhancement of gustatory neural and behavioral responses to sweet compounds in mice**

Endocannabinoids such as anandamide [N-arachidonylethanolamine (AEA)] and 2-arachidonoyl glycerol (2-AG) are known orexigenic mediators that act via CB1 receptors in hypothalamus and limbic forebrain to induce appetite [35, 36] and stimulate food intake [17]. In 2001, Di Marzo and his colleagues found that defective leptin signaling is associated with elevated hypothalamic levels of endocannabinoids in obese db/db and ob/ob mice and Zucker rats [37]. Furthermore, acute leptin treatment of normal rats and ob/ob mice reduces AEA and 2-AG in the hypothalamus. CB1 receptor antagonist SR141716 reduces food intake in wild-type mice but not CB1-knockout (KO) mice [38]. These findings indicate that endocannabinoids in the hypothalamus appear to be under negative control by leptin and contribute to overeating in the development of obesity. If this is also the case in peripheral taste system, it is possible that enhancement of sweet taste responses observed in db/db mice might be due not only to lack of inhibitory effect of leptin but also to the effect of endocannabinoids. To investigate this possibility, therefore, we examined whether endocannabinoids affect peripheral sweet taste reception [18].

We first examined gustatory nerve responses of the wild-type mice to various taste stimuli before and after administration of AEA or 2-AG. The responses were obtained from the chorda tympani nerve, innervating the anterior tongue, and the glossopharyngeal nerve, innervating the posterior tongue. The administration of AEA and 2-AG increases responses of both nerves to sweeteners (sucrose, saccharin Na, glucose and SC45647) in a concentration-dependent manner without affecting responses to salty (NaCl), sour (HCl), bitter (quinine) and umami (monosodium L-glutamate) compounds [18]. After i.p. injection of AEA or 2-AG, increased responses to sweet compounds (~150% of control for 500 mM sucrose) were observed at 10-30 min postinjection and then recovered to the control level at 60-120 min postinjection. Consistently, the endocannabinoids selectively increase behavioral lick responses to sucrose-quinine mixtures with similar
postinjection time course, whereas no such effect was observed in lick rates for salty, sour, bitter and umami compounds. By contrast, CB1-KO mice showed no such enhancement of responses to sweet compounds in both gustatory nerve and behavioral response measurements [18]. Collectively, both gustatory neural and behavioral experiments indicate that administration of endocannabinoids selectively enhances sweet taste responses and the endocannabinoid effect is mediated by their receptor, CB1 receptor.

**Expression of CB1 receptors and sweet response enhancement in taste cells**

Next, to determine whether the endocannabinoid effect occurred at the taste cell level, we compared responses (action potentials) of single taste cells to sweet compounds before and after application of AEA or 2-AG to the cells. To identify taste cells expressing T1R3, a component of both sweet and umami receptors, we used transgenic mice that express green fluorescent protein (GFP) from the T1R3 promoter [39] and recorded taste responses from these cells (Fig. 2A). T1R3-GFP taste cells responded to sweet compounds (sucrose, glucose, saccharin Na and SC45647) and as shown in Fig. 2A, responses to saccharin were enhanced by basolateral treatment of 2-AG. About 60% of cells (27 of 47) showed enhancement of responses to sweeteners (>120% of control) after application of 1 µM/mL AEA or 2-AG to the basolateral side of taste cell membrane. The enhancing effects of AEA and 2-AG on sweet responses of taste cells in wild-type mice saturated at ~1 µM/mL (Fig. 2C). The half max effective concentration (EC50) for enhancing sweet responses of wild-type taste cells by AEA (0.112 µg/mL) was about 6-fold greater than that of 2-AG (0.017 µg/mL). The effective concentrations of the endocannabinoids are within physiological ranges found in various tissues [40]. In CB1-KO mice, sweet responses of taste cells were not affected by 1 µg/mL AEA (Fig. 2B, open rectangle) or 2-AG (Fig. 2B, open circle). A pharmacological blocker of CB1 receptors, AM251, suppressed the sweet enhancing effect of 1 µg/mL 2-AG (Fig. 2C), however, the CB2 receptor blocker AM630 did not (Fig. 2D). These data indicate that endocannabinoids act on CB1 receptors to enhance sweet taste responses of taste cells [18].

Our immunohistochemical study showed that in wild type mice about 70% of taste cells expressing CB1 receptors co-expressed T1R3; ~60% of taste cells expressing T1R3 also expressed CB1 receptors. In CB1-KO mice CB1 immunoreactivity in taste cells was absent [18]. In the central nervous system, CB1 receptors are expressed in presynaptic cells and underlie modulation (inhibition) of transmitter release from presynaptic cells [41]. In the peripheral taste organ, CB1 immunoreactivity was observed in fewer than 12% of GAD67-expressing taste cells which in mice are thought to be presynaptic cells [18, 42]. GAD67-expressing presynaptic cells are reported to be primarily sensitive to sour taste stimuli [43, 44]. Endocannabinoids did not affect sour taste responses, indicating that presynaptic cells are not the major target for endocannabinoids in the taste organ. Instead, the majority of taste cells expressing CB1 receptors are sweet-sensitive cells expressing T1r3: endocannabinoids act to enhance sweet taste responses through these taste receptor cells known to lack well elaborated synapses.

To date, various peptides such as leptin [12-14], cholecystokinin (CCK) [45, 46], vasoactive intestinal peptide (VIP) [46], neuropeptide Y (NPY) [47] and GLP-1 [16] are reported to be involved in the modulation of peripheral taste sensitivity. Among them, leptin, and GLP-1 are shown to be modulators for sweet taste. Leptin specifically suppresses sweet taste responses and these effects may be mediated by leptin receptors (Ob-Rb) on taste cells [12-14]. GLP-1 signaling increases sweet and sour taste sensitivity and these effects may be mediated by GLP-1 receptors on adjacent intragemmal afferent nerve fibers [16]. Our study showed that endocannabinoids selectively enhance sweet taste sensitivity via CB1 receptors on the taste cells. Both endocannabinoids and GLP-1 enhance sweet taste but their specificity (sweet vs. sweet and sour) and targets (taste cells vs. afferent fibers) differ, suggesting that these modulators have different roles in modulating sweet taste. Circulating endocannabinoid levels inversely correlate with plasma levels of leptin in healthy human subjects [48]. Both endocannabinoids and leptin affect responses of taste cells via their cognate receptors. Therefore, endocannabinoids and leptin may reciprocally regulate peripheral sweet taste sensitivity.

Systemic administration of exogenous cannabinoids or endocannabinoids in rodents causes hyperphagia [49] and increases the preference for palatable substances such as sucrose solution or food pellets
These effects are mediated by the CB₁ receptor: pre-treatment with the CB₁ receptor antagonist SR141716A inhibited hyperphagia and reduced consumption of both bland and palatable foods [49-51]. The natural ‘liking’ reactions of rats to sweet compounds were amplified by endogenous cannabinoid signals in nucleus accumbens [52]. Thus, endocannabinoids may be related to hedonic aspects of sweet taste. Our findings provide evidence that the peripheral taste organ is also a target of cannabinoids. Increases in taste cell responses, nerve responses and lick responses to sucrose especially at its higher (more palatable) concentrations found in this study are in line with the previous findings mentioned above [18]. This modulation of peripheral sweet taste sensitivities by endocannabinoids may play a significant role in regulating feeding behavior.

Acknowledgements

This work was supported by Grant-in-Aids 18109013, 1807704 (Y.N.) for Scientific Research from Japan Society for the Promotion of Science.

References


47. Leptin, endocannabinoid and sweet taste

lacking the type 3 inositol 1,4,5-trisphosphate receptor. 


