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Ion channels and second messengers involved in transduction and modulation of sweet taste in mouse taste cells*

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Absract: Leptin, a hormone released from the adipose tissue, inhibits food intake and increases energy expenditure. We have found a novel function of leptin as a modulator of sweet taste sensitivity in mice. In lean normal mice, the gustatory nerve responses to sweet stimuli were selectively suppressed depending on plasma leptin level after an intraperitoneal injection of recombinant leptin. Patch-clamp studies using isolated taste cells of lean mice showed that extracellular leptin enhanced K^+ currents of sweet-responsive taste cells, which led to membrane hyperpolarization and a reduction of sweetener-induced depolarization. Reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization analyses demonstrated specific expression of mRNA of the long-form functional leptin receptor (Ob-Rb) in taste tissue and cells of lean mice. The genetically diabetic *db/db* mice, which have defects in Ob-Rb, demonstrated neither a suppression of gustatory neural responses to sweeteners nor an increment of whole-cell K^+ conductance of taste cells even with high doses of leptin. These results suggest that Ob-Rb is specifically expressed in sweet-responsive taste cells of lean mice and that leptin suppresses sweetener-induced depolarization via activation of K^+ channels, leading to a decrease in impulses of sweet-best fibers. The enhanced sweet responses of *db/db* mice may result from the lack of inhibitory modulation by leptin.

INTRODUCTION

Leptin, the product of the obese (*ob*)-gene, is a newly discovered hormone that is released from adipose tissue [1]. In mice, the *ob*-gene is known to be located on chromosome 6 on which several genes influencing bitter sensitivities are also located [2]. Leptin acts on the receptors in the hypothalamus and causes body weight loss by the effects of suppressing food intake and increasing energy expenditure (Fig. 1). Soon after the discovery of leptin, it was found that the *db* gene encodes leptin receptor [3], and genetically diabetic *db/db* mice have defects at the intracellular domain of the leptin receptor,

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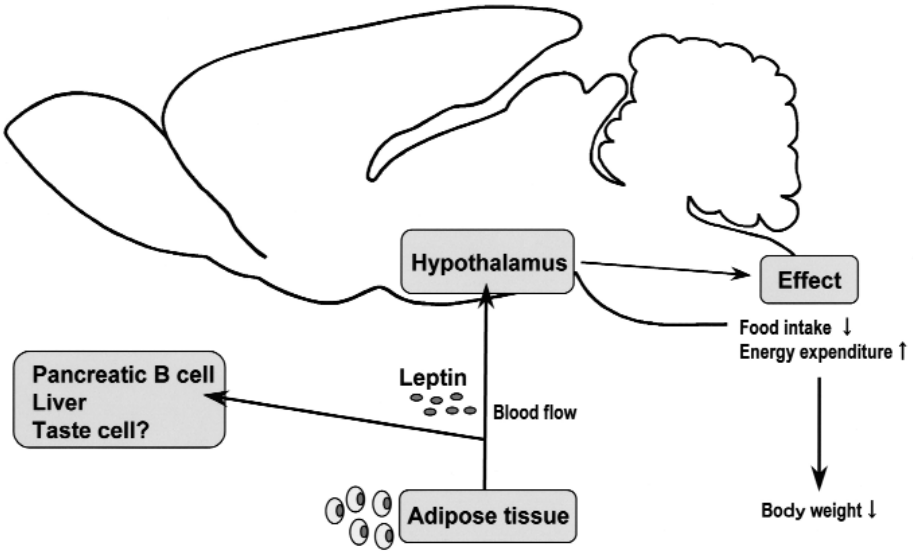

Fig. 1 Principal action of leptin in the central nervous system. Expression of leptin receptor is also detected in some peripheral tissues.

specifically its signal-transducing long form, Ob-Rb [4,5]. The expression of Ob-Rs has been detected also in peripheral tissues such as liver, skeletal muscle, kidney, and pancreatic B cells [6]. It has been reported that leptin suppresses glucose-induced insulin release by causing activation of an ATP-suppressible K^+ channel in pancreatic B cells [7,8].

In addition, we have recently found a novel function of leptin as a modulator of sweet sensitivity of the peripheral taste system in mice, that is, selective suppression of the responses of taste cells and gustatory nerves to sweet substances [9]. Sweet taste conveys an important signal for animals to identify calorically rich foods. In the present paper, therefore, we will describe the studies that have provided the evidence for the suppressive effect of leptin on sweet taste sensitivity and refer to the possible mechanisms involved in sweet taste transduction and the suppressive modulation of it by leptin.

BACKGROUND FOR FINDING LEPTIN EFFECT ON PERIPHERAL TASTE SYSTEM: ENHANCED SWEET SENSITIVITIES OF THE *db/db* MOUSE

First, we will briefly mention previous studies that led us to investigate leptin effects on peripheral taste responses in mice. About 10 years ago, Ninomiya and colleagues found that genetically diabetic *db/db* mice have greater sensitivities to sweet substances [10–12]. As seen in the records of the integrated responses of the chorda tympani nerve, the responses to sucrose and saccharin were much greater in the *db/db* mouse than in the lean normal mouse (+/+), while the responses to salty, sour, and bitter stimuli were not clearly different between these two mice (Fig. 2A). This result indicated that the responses to sweet substances are specifically enhanced in the *db/db* mouse. Concentration–response curves for sugar stimuli showed that the thresholds for these sugars were also lower in *db/db* mice than in lean normal mice (Fig. 2B).

In addition, it was found that the enhanced sweet sensitivity in *db/db* mice appears already at 7 days of age earlier than the appearance of diabetes. The responses of streptozotocin-induced diabetic mice, moreover, did not clearly differ from those of control mice. These results suggest that diabetic status itself would not be the major factor influencing gustatory neural responses, and the enhanced sweet sensitivity in *db/db* mice may be genetically induced by the action of the *db* locus.

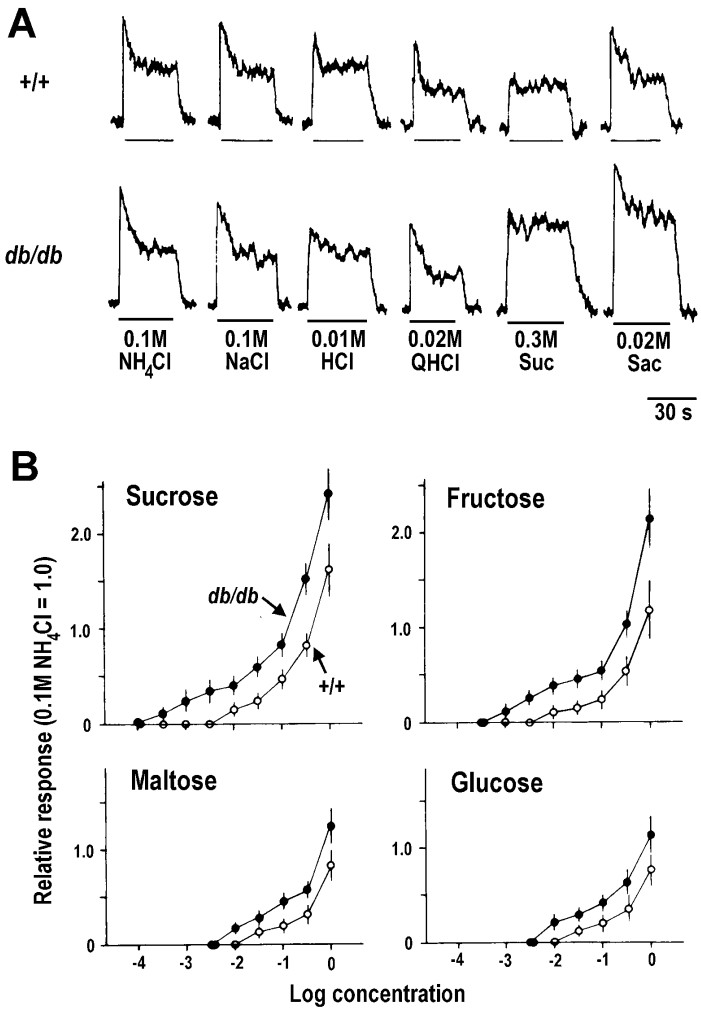

Fig. 2 Enhanced taste nerve responses to sweet substances in *db/db* mice. (A) Sample recordings of integrated responses of the chorda tympani nerve of normal (+/+) and diabetic (*db/db*) mice to six taste stimuli. QHCl, quinine HCl; Suc, sucrose; Sac, saccharin Na. (B) Concentration–response relationships of the chorda tympani nerve for sugar stimuli. Modified from Ninomiya et al. [12] and Ninomiya et al. [10].

The *db* locus had been mapped on chromosome 4 in mice. At present, it is known that chromosome 4 contains several genes affecting sweet sensitivities, such as *dpa* associated with perception of D-phenylalanine, *Sac* controlling preferences for saccharin and some other sweet substances, and T1R1-3 encoding putative sweet taste receptors [13–15]. It had been also known that *db/db* mice show hypersensitivity to glucose in pancreatic B cells before development of their major symptoms.

Together with the finding that *db/db* mice have defects at the intracellular domain of the Ob-Rb, these findings raised the possibility that the enhanced sweet taste sensitivity as well as glucose hypersensitivity of pancreatic B cells in *db/db* mice may result from their defects in the leptin receptor, Ob-Rb.

Therefore, we examined this possibility by comparing taste responses of peripheral nerves and receptor cells before and after leptin application in lean normal mice (C57BL and BALB). Besides these

experiments, we examined the expression of Ob-Rb in taste bud cells and the effect of leptin on sweet perception behaviorally.

SPECIFIC SUPPRESSION OF TASTE NERVE RESPONSES TO SWEET STIMULI BY LEPTIN

Whole taste nerve responses

In lean normal mice, a single intraperitoneal (i.p.) injection of recombinant leptin, 100 ng/g body wt, into anesthetized animals increased plasma leptin levels from about 2.5 ng/ml to around 12 ng/ml at 30 min and around 15 ng/ml at 30 to 60 min after the injection. As seen in the neural response records (Fig. 3A), during such increases in plasma leptin levels, the responses of the chorda tympani nerve, innervating the anterior two-thirds part of the tongue, to sucrose and saccharin slightly decreased, while no changes were observed in responses to other taste stimuli. The responses of the glossopharyngeal nerve, innervating the posterior one-third of the tongue, to sucrose and saccharin were also suppressed with little effects on another taste qualities after a leptin injection. These results indicate that leptin specifically inhibits neural responses to sweet stimuli. Leptin reduced the responses to these sweet stimuli to about 70 % of the controls. On the other hand, *db/db* mice did not show such effects by leptin even after an injection of 500 ng/g body wt. Concentration–response relationships for sucrose also demonstrated the suppression of sucrose response and the raised threshold after a leptin injection in lean normal mice, whereas the responses of *db/db* mice were hardly suppressed (Fig. 3B).

Single taste fiber responses

We next examined the type of taste nerve fibers affected by leptin. As seen in the record of a sucrose-best fiber (large impulse fiber in Fig. 4A), which responded to sweet stimulus best, impulses in response to sucrose and saccharin were selectively decreased after an intraperitoneal injection of leptin. Figure 4B

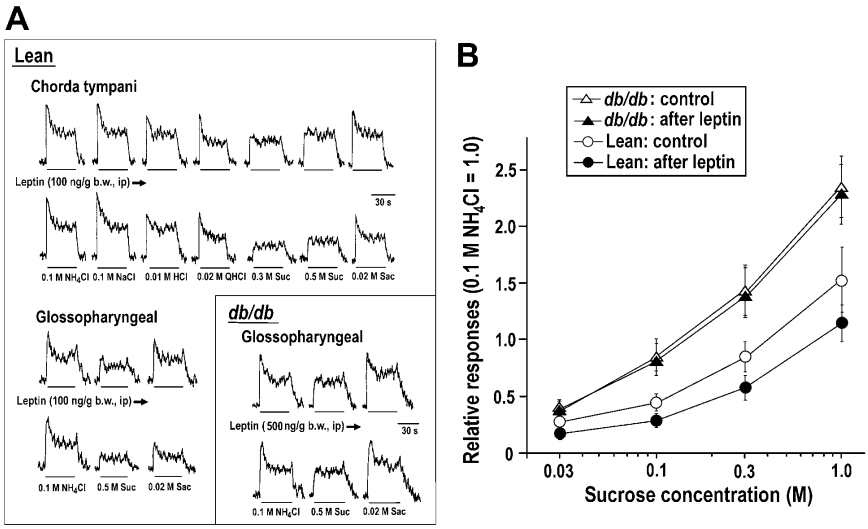

Fig. 3 Gustatory nerve responses before and after an i.p. injection of recombinant leptin in lean normal mice and *db/db* mice. (A) Sample recordings of integrated responses of the chorda tympani nerve and the glossopharyngeal nerve. (B) Concentration–response relationships for sucrose. Modified from Kawai et al. [9].

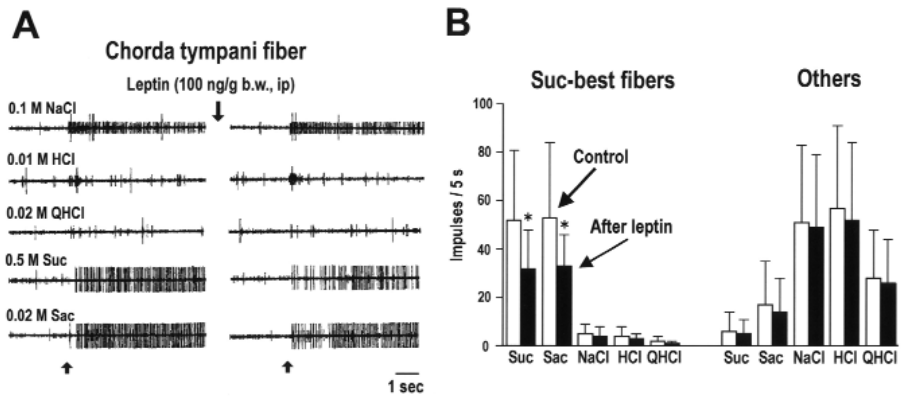

Fig. 4 Changes in responses of single fibers of the chorda tympani nerve after an i.p. injection of leptin. (A) Sample recordings of two single chorda tympani fibers. (B) Response profiles of five taste stimuli before (open bars) and after (filled bars) leptin in two groups of fibers, classified whether they would respond best to sucrose among four basic taste stimuli (Suc-best fibers, $n = 9$) or not (other fibers, $n = 21$). *: t-test, $p < 0.05$. Modified from Kawai et al. [9].

summarizes the effect of leptin on the responses of two classes of nerve fibers, sucrose-best fibers and other fibers. Leptin selectively suppressed sweet responses of sucrose-best fibers, while the responses of other fibers were not influenced by leptin, suggesting that leptin selectively suppresses sweet information transmitted to the central nervous system.

EFFECTS OF LEPTIN AND SACCHARIN ON ELECTRICAL ACTIVITIES OF TASTE RECEPTOR CELLS

To investigate if this suppressive effect on neural sweet responses resulted from the events occurring at receptor cell level, we examined the effects of leptin on taste receptor cells by use of the whole-cell patch-clamp method. Taste receptor cells were isolated from the circumvallate and foliate papillae by enzymatic treatment and gentle agitation.

Figure 5A demonstrates whole-cell currents recorded from taste receptor cells of lean control (left traces) and *db/db* (right traces) mice in response to depolarizing voltage steps, with and without leptin in the bath solution. Under the voltage-clamp condition, lean mouse taste cells demonstrated slight enhancement of outward currents after 30 ng/ml leptin application to the bath. Since these outward currents were totally abolished by the extracellular application with tetraethylammonium (TEA), these currents were thought to be K^+ outflux. In contrast, taste cells of *db/db* mice showed no change in the currents in the presence of leptin even at a dose of 1 mg/ml. The dose–response curve of leptin obtained from leptin-sensitive cells in lean normal mice indicates that the threshold concentration of leptin would be less than 10 ng/ml (Fig. 5B), which is quite consistent with that for taste nerve responses as mentioned above.

Whole-cell currents in response to a depolarizing ramp wave (290 mV/s) were also larger in the presence of 100 ng/ml leptin than in normal external solution. Leptin-activated current reversed at approximately –80 mV, which is close to the K^+ equilibrium potential, confirming that this current is K^+ current (Fig. 6A). Substitution of NaCl with Na-gluconate in the external solution, furthermore, did not change the reversal potential of leptin-activated currents, indicating that the current was not carried by Cl^- but by K^+. In the presence of 10 mM saccharin, the whole-cell current of the same cell was smaller than in normal external solutions. This saccharin-suppressed current also reversed at

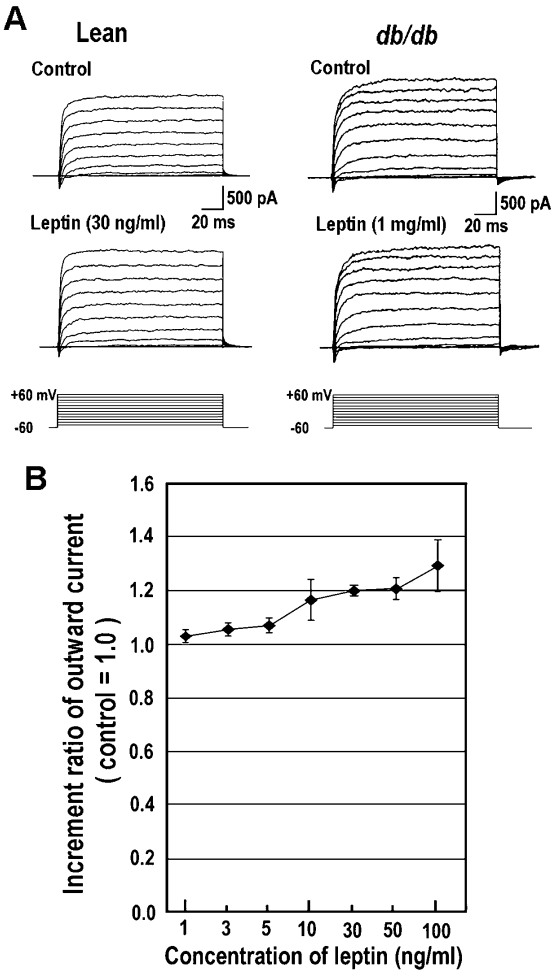

Fig. 5 Influence of extracellular leptin on the taste cell currents in lean normal mice and *db/db* mice. (A) Whole-cell currents evoked by depolarizing voltage steps from a holding potential of –60 mV. (B) Changes in the increment ratio of steady-state outward currents as a function of leptin concentration. The amplitudes in the absence of leptin were taken as 1.0. The error bar represents the standard error of the mean (SEM) obtained from 3–8 cells. Modified from Kawai et al. [9].

about –80 mV. These results indicate that leptin activates K^+ currents and saccharin reversely suppresses K^+ currents in a same cell.

Under current-clamp mode, bath application of 10 ng/ml leptin induced hyperpolarization of resting membrane potential and a simultaneous decrease of input resistance in lean mouse taste cells (Fig. 6B). Furthermore, 5–10 mM of saccharin depolarized taste cells, and this saccharin-induced depolarization was significantly suppressed in the presence of leptin.

In summary, about 17 % of taste cells showed an increase in K^+ conductance with extracellular leptin of 100 ng/ml, and almost all leptin-sensitive cells also responded to 5 mM saccharin with K^+ conductance decrease. This implies that almost all leptin-sensitive cells may be sweet-responsive cells and that leptin induces increase of K^+ conductance in sweet-responsive cells, leading to hyperpolarization of the cells and suppression of sweetener-induced depolarization.

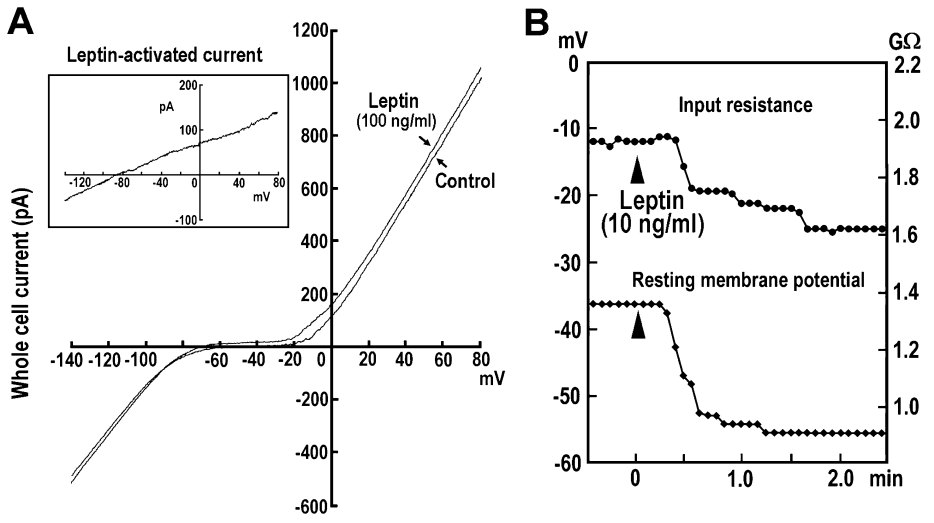

Fig. 6 (A) Whole-cell currents induced by a depolarizing ramp wave in the absence and in the presence of leptin in the bath solution. (B) Change in resting membrane potential and simultaneous input resistance in a taste cell of the lean normal mouse. Modified from Kawai et al. [9].

EXPRESSION OF LEPTIN RECEPTOR IN TASTE BUD CELLS

We next investigated whether taste cells actually express leptin receptor by means of immunohistochemistry, RT-PCR, and in situ hybridization. Leptin receptor has five types of splice variants (Ob-Ra – Ob-Re), and the long form Ob-Rb is essential for intracellular signal transduction.

Some taste bud cells of circumvallate papillae demonstrated immunoreactivity for Ob-Rs. RT-PCR analysis showed that RT-PCR products for both functional leptin receptor (Ob-Rb) and taste cell specific G-protein, gustducin, were observed in the tissues containing the fungiform and circumvallate papillae but not in epithelial tissue without taste papilla. RT-PCR product of *db/db* mice for Ob-Rb was a little bit larger than that of lean mice, because the product includes so called *db*-insertion containing a stop codon which makes the intracellular domain of Ob-Rb shorter than the normal one. In situ hybridization analysis clearly showed expression of Ob-Rb, gustducin, and T1R3 mRNAs in some cells of circumvallate taste buds. As compared with that of gustducin or T1R3, expression of Ob-Rb was weak. From these analyses, it was strongly suggested that a subset of taste bud cells expresses functional leptin receptor Ob-Rb.

BEHAVIORAL STUDY OF LEPTIN EFFECT ON INTAKE OF SWEET SOLUTION

We further examined leptin effects on behavioral responses of lean normal mice, using a conditioned taste aversion paradigm. Lean mice were conditioned to avoid sucrose by an intraperitoneal injection of LiCl immediately after drinking sucrose solution because of induced gastrointestinal disorder. Once they were conditioned to avoid sucrose, they showed a decreased number of licks for sucrose and saccharin solutions. Under such conditions, we measured the numbers of licks to test stimuli, such as 0.1 M NaCl, 0.01 M HCl, 0.3 mM QHCl, 0.01–1.0 M sucrose, and 1–20 mM saccharin, before and after an intraperitoneal injection of leptin or saline. Saline injection did not alter the number of licks to any taste stimulus tested, whereas leptin increased licks of animals to low concentrations of sucrose and saccharin. If the strength of the behavioral aversion for a taste stimulus is reflected by the strength of the perceived sweet taste intensity, this increase in licks for aversive sucrose and saccharin solutions

after leptin may be caused by reduced perception to these sweet stimuli, which might result from suppressed responses of taste cells to these sweeteners.

CHANGES OF TASTE CELL CURRENTS INDUCED BY cAMP, A PUTATIVE SECOND MESSENGER INVOLVED IN SWEET TASTE TRANSDUCTION

With regard to transducton mechanisms for sweet taste, it has been proposed that sugar stimuli induce elevation of intracellular adenosine 3', 5'-cyclic monophosphate (cAMP) after binding to a G protein-coupled receptor [16–19]. Furthermore, gustducin is suggested to be involved in transduction for bitter and/or sweet tastes [20].

Therefore, we investigated whether there is a relationship between intracellular cAMP change and gustducin action. To examine this issue, the effect of membrane-permeable cAMP analog (8-br-cAMP) added to the bath solution on taste cell currents was recorded using the perforated patch-clamp technique, and successively the expression of gustducin in the electrically recorded cells was examined by means of immunocytochemistry.

A prominent effect of 0.25–1.0 mM 8-br-cAMP on taste cells was suppression of voltage-dependent K^+ currents without suppression of inward transient Na^+ currents. Whole-cell currents induced by a depolarizing ramp wave were also decreased in the presence of 8-br-cAMP, and the reversal potentials of the suppressed currents were close to the K^+ equilibrium potential, suggesting that intracellular cAMP elevation reduces K^+ conductance. About 40 % of taste cells demonstrated a decrease in K^+ conductance in response to cAMP increase, and some of those cells also responded to 5–10 mM saccharin in the same manner. No clear relationship, however, was found between the type of response to 8-br-cAMP and gustducin expression.

SUMMARY AND DISCUSSION

The present study has demonstrated that leptin in blood may bind to Ob-Rb expressed in sweet-responsive taste cells and activate K^+ conductance of the cell in a dose-dependent manner leading to membrane hyperpolarization. This hyperpolarization counteracts the depolarization induced by sweet substances such as sucrose and saccharin. The attenuation of depolarization may result in a decrease of transmitter release from the taste cell and a consequent decrease in impulse number of sweet-best fibers which may exclusively transmit sweet information to the central nervous system (CNS) (Fig. 7). Further evidence for a suppressive effect of leptin on sweet responses was obtained behaviorally by using a conditioned taste aversion paradigm. After the lean normal mice were conditioned to avoid sucrose, one group administered a leptin injection showed elevated consumption of aversive sweet solution compared with the other group with a saline injection. This increased consumption may be caused by decreases in responses of taste cells to sweeteners. On the contrary, *db/db* mice with defects in Ob-Rb never showed suppression of gustatory neural responses to sweet stimuli or activation of whole cell K^+ conductance even at an extremely high dose of leptin. It is likely that the enhanced gustatory neural responses to sweeteners in *db/db* mice are due to a lack of this inhibitory modulation by leptin.

Figure 7 also illustrates possible transduction mechanisms for sweet taste, that is, intracellular signal cascades from receptor binding of sweet substances to membrane depolarization. It has been proposed that sugars and nonsugar sweeteners such as saccharin activate separate transduction cascades in the same sweet-responsive cell, one involves cAMP and the other involves inositol triphosphate (IP_3) and diacylglycerol (DAG) [21]. Some previous electrophysiological and biochemical studies [22–28] have suggested that sweet substances such as sugars induce intracellular cAMP elevation probably mediated by Gs, which leads to K^+ channel closure via phosphorylation by the action of cAMP-dependent protein kinase. The present patch-clamp study supports this idea because about 40 % of taste cells displayed a reduction of K^+ conductance in response to membrane-permeable cAMP analog, and some of them also responded to saccharin in the same manner. Since no clear relationships between this type

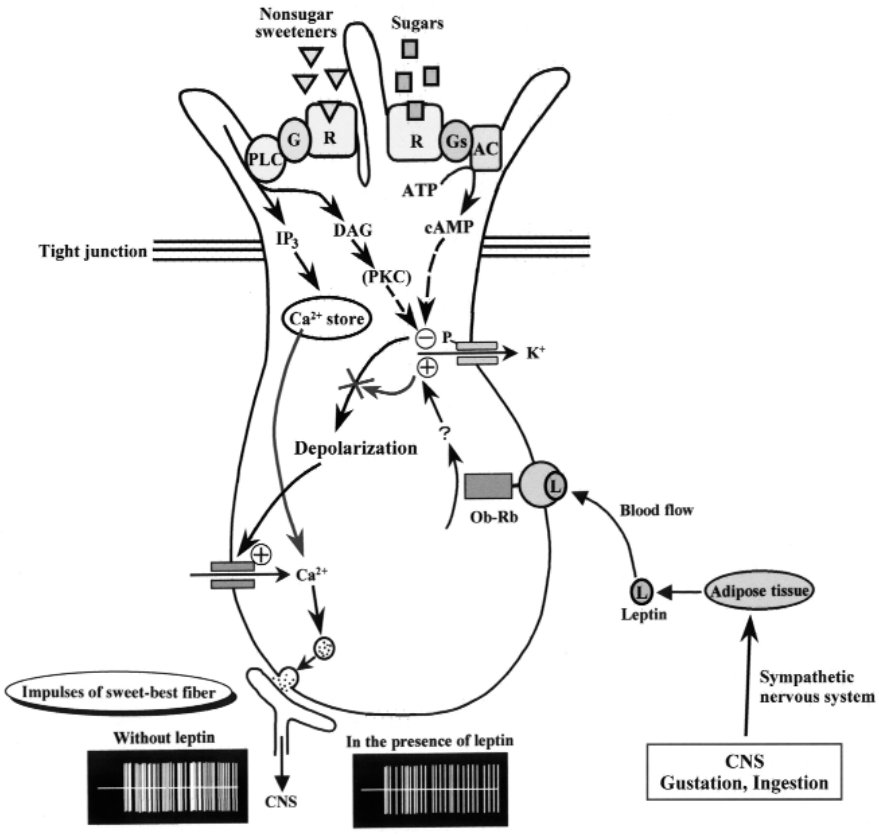

Fig. 7 Putative scheme of the intracellular mechanisms for sweet taste transduction and for leptin modulation on responses to sweeteners. For details, see text.

of response to cAMP increase and gustducin expression were observed, gustducin may not be involved in this response. The recent study by loose-patch recording from hamster fungiform papillae [29] has strongly suggested that the transduction mechanism of synthetic sweeteners may utilize the IP_3 and DAG pathway including the action of DAG-dependent protein kinase (PKC) because their responses were suppressed by a PKC inhibitor. The IP_3 and DAG pathway is also thought to result in blocking K^+ channels via phosphorylation leading to membrane depolarization. Therefore, one of the key events required for cellular activation by sugars and artificial sweeteners is a decrease in resting K^+ conductance and cell depolarization. The depolarization may activate voltage-dependent Ca^{2+} channels, which have been found in murine taste cells [30], and, consequently, induce intracellular Ca^{2+} increase and transmitter release. The possibility that both pathways coexist in the same taste cell raises an issue of what intracellular interaction between them occurs, which needs further investigation.

The effect of leptin also converges on the resting K^+ conductance, exerting suppressive modulation on sweetener-induced responses as mentioned above. To elucidate what intracellular mechanism and type of K^+ channel is activated after leptin binding to Ob-Rb, further electrophysiological and pharmacological studies are required. It has been reported that release of leptin from adipose tissue is stimulated by ingestion and gustatory information, which is mediated by the function of vagous nerves [31–36]. Increased plasma leptin acts on the hypothalamus centrally and sweet-responsive taste cells peripherally and suppresses appetite and sweet sensitivity, respectively. Both effects might cause decrease in food intake as negative feedback.

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