Leptin as a modulator of sweet taste sensitivities in mice

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Edited by Dale Purves, Duke University Medical Center, Durham, NC, and approved July 19, 2000 (received for review February 15, 2000)

Leptin acts as a potent inhibitory factor against obesity by regulating energy expenditure, food intake, and adiposity. The obese diabetic *db*/*db* mouse, which has defects in leptin receptor, displays enhanced neural responses and elevated behavioral preference to sweet stimuli. Here, we show the effects of leptin on the peripheral taste system. An administration of leptin into lean mice suppressed responses of peripheral taste nerves (chorda tympani and glossopharyngeal) to sweet substances (sucrose and saccharin) without affecting responses to sour, salty, and bitter substances. Whole-cell patch-clamp recordings of activities of taste receptor cells isolated from circumvallate papillae (innervated by the glossopharyngeal nerve) demonstrated that leptin activated outward K^+ currents, which resulted in hyperpolarization of taste cells. The *db*/*db* mouse with impaired leptin receptors showed no such leptin suppression. Taste tissue (circumvallate papilla) of lean mice expressed leptin-receptor mRNA and some of the taste cells exhibited immunoreactivities to antibodies of the leptin receptor. Taken together, these observations suggest that the taste organ is a peripheral target for leptin, and that leptin may be a sweet-sensing modulator (suppressor) that may take part in regulation of food intake. Defects in this leptin suppression system in *db*/*db* mice may lead to their enhanced peripheral neural responses and enhanced behavioral preferences for sweet substances.

Sweet-sensing taste receptor cells are very important for animals to be able to detect carbohydrate sources of calories. Previous studies demonstrated that genetically diabetic *db*/*db* mice that exhibit extreme obesity (1, 2) have greater gustatory neural sensitivities (3–5) and higher behavioral preferences for various sweet substances (3) compared with lean control mice. In contrast, these mouse lines did not differ from controls in response to salty, sour, and bitter substances (NaCl, HCl, and quinine). The increase in sweet sensitivity as well as pancreatic β cell activity (6) in *db*/*db* mice has been reported to begin as early as 7 days of age, suggesting that these characteristics may be genetically induced by the action of the *db* locus. Recently, the *ob* gene has been cloned and found to encode the protein leptin (7). Leptin, released from adipocytes, has been found to inhibit food intake and to increase energy expenditure (8–11). Soon after these findings, it was revealed that the *db* gene encodes the leptin receptor (Ob-R) and that the *db*/*db* mouse mutation occurs at the intracellular domain of the Ob-R (12–15), especially the signal-transducing long form of the Ob-R (Ob-Rb). This finding, therefore, raises the possibility that the enhanced sweet sensitivity of taste nerve responses in *db*/*db* mice may be a consequence of defects in the Ob-Rb.

In the present study, we investigated taste responses of receptor cells and peripheral nerves before and after leptin application. We demonstrate that the taste organ is a new peripheral target for leptin, that the Ob-Rb is expressed in a limited population of taste cells of lean mice, and that leptin selectively inhibits sweet responses through activation of outward K^+ currents. Defects in this leptin suppression system in *db*/*db* mice may lead to their enhanced neural and behavioral sensitivities to sweet stimuli.

Materials and Methods

Subjects. Diabetic [8–20 weeks of age, 50–62 g body weight (b.w.)] and nondiabetic (8–20 weeks of age, 23–34 g b.w.) littermates were obtained from mating pairs of the C57BL/KsJ-db mouse strain originally supplied from The Jackson Laboratory. Diabetic mice with *db*/*db* genotype were referred to as *db*/*db* mice, whereas nondiabetic control mice with +/+ genotype are referred to as control mice. We also used adult BALB/c mice (BALB, 8–20 weeks of age, 23–34 g b.w.) as one additional lean control group. For the recordings of neural responses, each mouse was fasted for 24 h before each experiment.

Recording of Taste Responses of the Mouse Chorda Tympani (CT) and Glossopharyngeal (IXth) Nerves. The details of surgical procedures and chemical stimulation were described in previous papers (16–18).

Surgical procedures. Each mouse was anesthetized with an i.p. injection of sodium pentobarbital (40–50 mg/kg of b.w.) and maintained at a surgical level of anesthesia with supplemental injections of sodium pentobarbital. The right CT nerve was exposed at its exit from the lingual nerve by removal of internal pterygoid muscle. The CT nerve then was dissected free from surrounding tissues and cut at the point of its entry to the bulla. The right IXth nerve was exposed by removal of the digastricus muscle and posterior horn of the hyoid bone. The IXth nerve then was dissected free from underlying tissues and cut near its entrance to the posterior lacerated foramen. For whole-nerve recording, the entire nerve was placed on a silver wire electrode. For single-fiber recording (from only the CT nerve of C57BL control mice), a single or a few fibers of the nerve were teased apart with a pair of needles and lifted on a silver wire electrode. An indifferent electrode was positioned nearby in the wound. Neural responses induced by chemical stimulation of the tongue were fed into an amplifier (Iyodenshikogaku K-1) and integrated with time constant of 1.0 s.

Chemical stimulation. For chemical stimulation of the fungiform taste papillae innervated by the CT nerve, the anterior half of the tongue was enclosed in a flow chamber. For chemical stimulation of the vallate and foliate papillae on the posterior part of the tongue, innervated by the IXth nerve, an incision was made on each side of the animal's face from the corner of the mouth, and the papillae were exposed and their trenches opened by slight tension applied through a small suture sewn in the tip

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: Ob-R, leptin receptor; Ob-Rb, signal-transducing long form of the Ob-R; b.w., body weight; CT, chorda tympani; IXth, glossopharyngeal; Sac, sodium saccharin; Suc, sucrose; QHCl, quinine·HCl; CP, circumvallate papilla; ET, epithelial tissue; Ggust, gustducin; RT-PCR, reverse transcription–PCR.

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Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.190066697. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.190066697

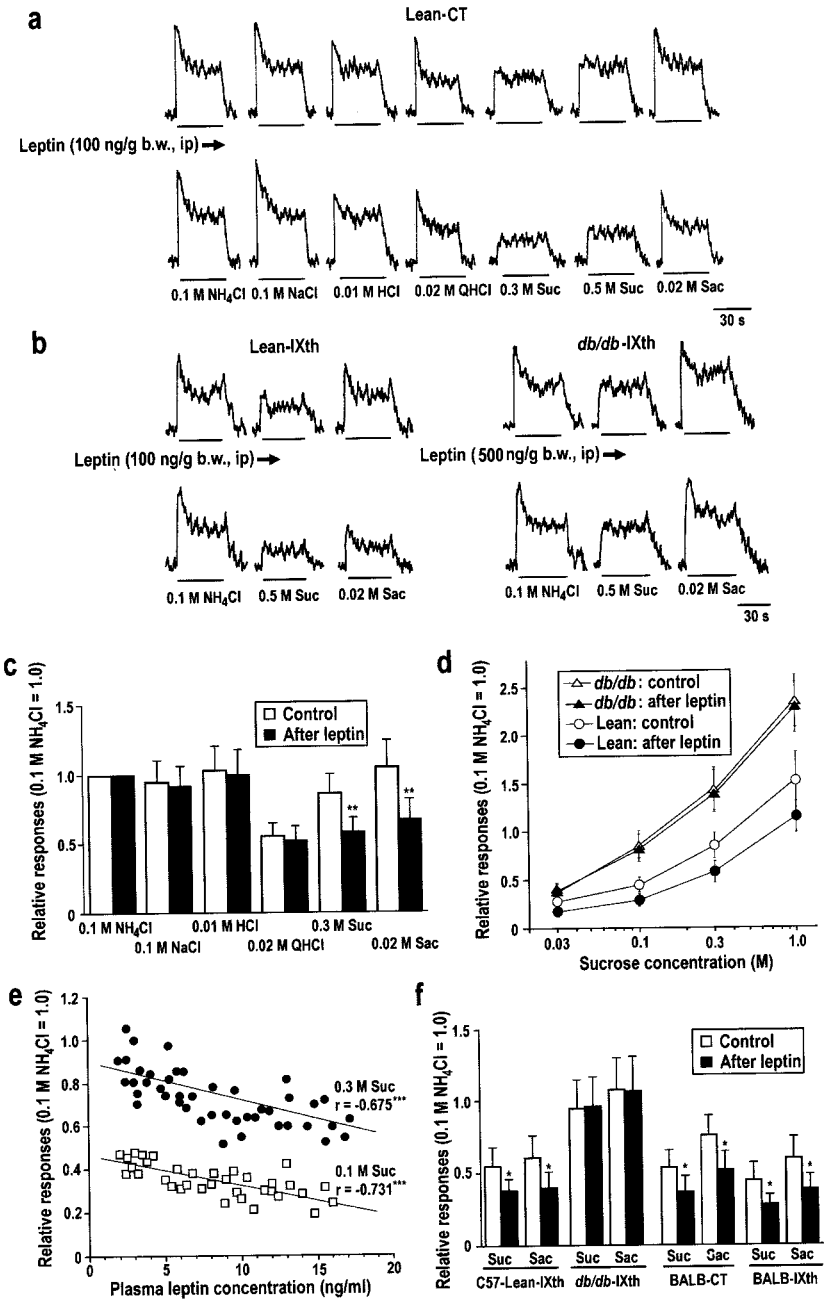

Fig. 1. (*a*) Sample recordings of integrated responses of the CT nerve to 0.1 M NH_4Cl, 0.1 M NaCl, 0.01 M HCl, 0.02 M QHCl, 0.3 M sucrose (Suc), 0.5 M Suc, and 0.02 M Sac before (*Upper*) and after (*Lower*) i.p. injection of leptin in control lean mice. (*b*) Responses of the IXth nerve to 0.1 M NH_4Cl, 0.5 Suc, and 0.02 M Sac before (*Upper*) and after (*Lower*) leptin in lean and *db*/*db* mice. (*c*) Response profiles to six taste stimuli before (Control) and after leptin (100 ng/g of b.w.) (n = 16–20). (*d*) Concentration-response relationships for sucrose before and after leptin in lean control (n = 12) and *db*/*db* mice (n = 8). Doses of leptin were 100 ng/g of b.w. for control and 500 ng/g of b.w. for *db*/*db* mice. (e) Relative responses to 0.1 or 0.3 M Suc of lean mice (n = 43 or 36) were plotted as a function of their plasma leptin levels (ng/ml). ***, *t* test, $P < 0.001$. (*f*) Responses to 0.5 M Suc and 0.02 M Sac before and after leptin (100 ng/g of b.w.) in the IXth nerve of C57BL lean (n = 7) and *db*/*db* (n = 5) mice, and CT (n = 8) and IXth nerve (n = 6) of lean BALB mice. *, *t* test, $P < 0.05$.

of the tongue. Solutions were delivered to each part of the tongue (into the chamber for the anterior part) by gravity flow. Solutions used for chemical stimuli were 0.01–1.0 M sucrose, 0.02 M sodium saccharin (Sac), 0.1 M NaCl, 0.1 M NH_4Cl, 0.01 M HCl, and 0.02 M quinine·HCl (QHCl). The first two chemicals, which taste sweet to humans, previously had been behaviorally categorized as belonging to the same group ("sweet group") in C57BL and BALB strains of mice by using a conditioned taste aversion paradigm (Y.N. and K.N., unpublished observation). These chemicals were dissolved in distilled water at ≈24°C. During chemical stimulation of the tongue, the test solution flowed for 40 s at the same flow rate as the distilled water used for rinsing the tongue (0.5 ml/s). The stability of each preparation was monitored by the periodic application of 0.1 M NH_4Cl. A recording was considered to be stable when the 0.1 M NH_4Cl response magnitudes at the beginning and end of each stimulation series deviated by no more than 15%. Only responses from stable recordings were used for the data analysis.

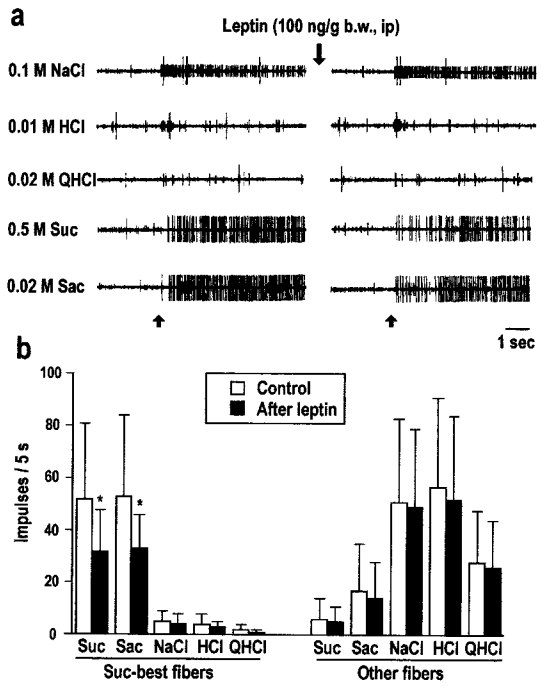

Fig. 2. (*a*) Sample recordings of two single CT fibers (one small and one large) in response to 0.1 M NaCl, 0.01 M HCl, 0.02 M QHCl, 0.5 M Suc, and 0.02 M Sac before and after leptin (100 ng/g of b.w.) in lean C57BL mice. In the large unit (Suc-best fiber), responses to sucrose and saccharin decreased after leptin, whereas the small unit (NaCl-best fiber) showed no such leptin inhibition. (*b*) Response profiles to 0.5 M Suc, 0.02 M Sac, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl before (control) and after 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, $P < 0.05$.

Administration of leptin and measurement of plasma leptin levels. After recording a series of control responses, each mouse was administrated a single i.p. injection of 100–500 ng/g of b.w. of recombinant leptin (Petro Tech E. C., London) dissolved in PBS (pH 7.4, at concentrations of 8–100 μg/ml). The recording of taste responses in each animal was continued until a particular point of time (10, 30, or 60 min after the leptin administration), and then 500 μl of the blood was collected from the cervical vein and immediately mixed with 50 μl of 40 mM EDTA (pH 7.4). Plasma leptin levels were measured by using a mouse leptin RIA kit (Linco Research Immunoassay, St. Charles, MO).

Data analysis of neural activities. In the analysis of whole-nerve responses, the magnitude of the integrated response at 5, 10, 20, and 30 s after stimulus onset was measured and averaged. Relative response magnitude (averaged) for each stimulus was calculated when the response magnitude to 0.1 M NH_4Cl was taken as a unity (1.0) and this value was used for statistical analysis. In the analysis of single-fiber responses, single fibers were identified by uniform spike height, singular wave form, and examination of latencies between contiguous spikes (19, 20). Frequency-time histograms of impulse discharges before, during, and after chemical stimulation of the tongue were made by means of a spike-analysis system (Iyodenshikogaku SAS-1). For data analysis, we used the net average frequency for the first 5 s after the stimulus onset obtained by subtracting the spontaneous frequency for a 5-s period before stimulation.

Recordings of Activities of Taste Receptor Cells. *Isolation of taste cells.* After the tongue was cut out under deep anesthesia with ethyl ether, the mice were then killed by cervical hemorrhage. A mixture of 2 mg/ml collagenase and 0.5 mg/ml dispase was injected between the epithelium and muscle layers of tongue. After 30 min, the tongue epithelium containing a circumvallate papilla was peeled off and further treated with the enzymes for 10 min and divalent cation-free solution containing 2 mM EDTA for an additional 5–10 min. The taste bud cells were finally dissociated from the papilla by gentle agitation using a micropipette.

Recording of taste cell activities. Taste cells were identified by their spindle shape and elongated cell process. The membrane currents and the membrane potentials of single taste cells were recorded by whole-cell configuration of patch-clamp techniques (21) with an Axopatch 200A (Axon Instruments, Foster City, CA). The ionic currents in response to depolarizing voltage steps or ramp wave depolarized from -140 mV to $+80$ mV with a rate of 290 mV/s were recorded under voltage-clamp mode, and the membrane potentials were recorded under current-clamp mode. Changes in these currents and membrane potentials were examined when the taste cells were perfused with a bath solution containing 1–1,000 ng/ml recombinant murine leptin or 5 mM Sac. The standard external (bath) solution contained: 145 mM NaCl, 4.7 mM KCl, 3.3 mM $CaCl_2$, 0.1 mM $MgCl_2$, 2.0 mM Hepes, and 7.8 mM glucose; the divalent cation-free external solution contained: 135 mM NaCl, 10 mM Na-pyruvate, 5 mM KCl, 10 mM Hepes, 2 mM EDTA, and 10 mM glucose; Cl^--substituted external solution contained: 145 mM Na-gluconate, 4.7 mM KCl, 3.3 mM $CaCl_2$, 0.1 mM $MgCl_2$, 2.0 mM Hepes, and 7.8 mM glucose; the standard internal (pipette) solution contained: 145 mM KCl, 10 mM Hepes, 0.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 10 mM EGTA, and 5 mM ATP. All solutions were adjusted to pH 7.4, and the external solutions were equilibrated with 100% O_2. All experiments were carried out at room temperature (≈24°C).

Examination of Ob-R Expression in Taste Receptor Cells. *A reverse transcription–PCR (RT-PCR) protocol.* By using the same procedures that we used for taste-cell isolation, tissues with [circumvallate papilla (CP)] and without [epithelial tissue (ET)] circumvallate papilla were isolated from the tongue of lean control mice (BALB). Total RNA of the tissues was extracted by the acid/phenol method (22). A cDNA was generated by reverse transcription [oligo(dT) primer] with the superscript preamplification system (GIBCO/BRL). Primer sequences for each PCR were as follows. Common region of Ob-R: 5′-CCAGATTCGATATGGCTTAAGTGGA-3′ (+1,786 to +1,810) and 5′-AAGGTTAAAATTCACAAGGGAAGCG-3′ (+2,263 to +2,239) (GenBank accession no. U58861); product size, 478 bp. Ob-Rb: 5′-AATTGTTCCTGGGCACAAGGACTGA-3′ (+2,714 to +2,738) and 5′-TTACTGGAGATGCAGTTGCTGACAG-3′ (+3,126 to +3,102) (GenBank accession no. U58861); product size, 413 bp. Gustducin (Ggust; taste-cell-specific G protein): 5′-AGATGGGAAGTGGAATTAGTTCAGA-3′ (+112 to +136) and 5′-ATCGTGGGCCGCTCTAGGCACC-3′ (+1,180 to +1,156) (GenBank accession no. X65747). β-Actin: 5′-ATCGTGGGCCGCTCTAGGCACC-3′ (+22 to +43) and 5′-CTCTTTGATGTCACGCACGATTTC-3′ (+564 to +541) (GenBank accession no. X03765); product size, 543 bp. Because the sequence data of mouse Ggust was not available from GenBank, PCR primers for mouse Ggust were synthesized from the rat sequence, and the PCR products were confirmed by sequence analysis. PCR was performed on ASTEC PC-701 with the following conditions: 95°C for 3 min (1 cycle); 95°C for 1 min, 65°C for 1 min, 72°C for 1 min and 30 s (40 cycles); and 72°C for 7 min (1 cycle). The PCR solution (pH 8.3) contained 10 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride (Tris·HCl), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM deoxyribonucleoside 5′-triphosphate (dNTP), and 0.05 unit/μl of *Taq* polymerase (Takara Shuzo, Kyoto).

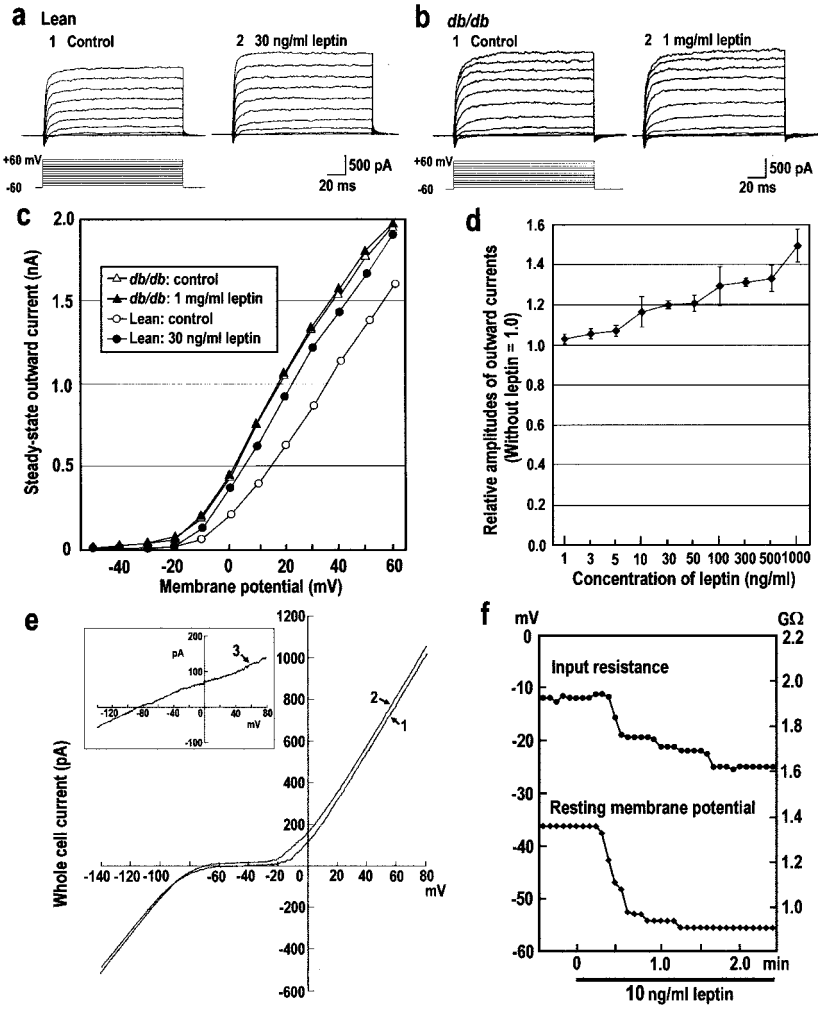

Fig. 3. The influence of extracellular leptin on the taste cell currents in control lean (BALB) mice and diabetic (*db*/*db*) mice. (*a* and *b*) Voltage-dependent whole-cell currents evoked by depolarizing voltage steps up to +60 mV in 10-mV increments from a holding potential of −60 mV in the absence (*1*) and in the presence (*2*) of leptin in BALB mice (*a*) and *db*/*db* mice (*b*). The amplitudes of transient inward and outward currents greatly varied from cell to cell. (*c*) *I–V* curves of amplitudes of steady-state outward currents obtained from the records *a* and *b*. (*d*) Dose–response relationship for the increment ratio of steady-state outward currents as a function of leptin concentration. Amplitudes of steady-state outward currents induced by a voltage step from −60 mV to +40 mV were measured and the amplitudes in the absence of leptin were taken as 1.0. The error bar represents the SEM obtained from three to eight cells. (*e*) The whole-cell currents induced by a depolarizing ramp wave of 290 mV/s in the absence (curve 1) and in the presence (curve 2) of leptin. (*Inset*) *I–V* relation obtained by subtracting curve 1 from curve 2 demonstrating that the reversal potential of leptin-activated current was approximately −87 mV. (*f*) Changes in resting membrane potentials and input resistances induced by 10 ng/ml leptin measured under current clamp mode.

Immunohistochemistry. A small tissue block containing circumvallate papilla was excised from the tongue of BALB mouse and instantaneously frozen with liquid nitrogen. Serial tissue sections with l0-μm thickness were cut in the cryostat (Leica Instruments CM3000, Deerfield, IL). After fixation with 4% formaldehyde, sections were treated with 0.3% hydrogen peroxide in methanol to quench endogenous peroxide activity and with the mixture of 0.5% normal rabbit serum and 1% BSA to block nonspecific binding. After these procedures, the sections were incubated with the primary antibody against the common region of all splicing forms of Ob-R (M-18; Santa Cruz Biotechnology) diluted 1:100 overnight at 4°C, then with biotinized anti-goat IgG (Zymed), and finally in the avidin–biotin complex conjugated with horseradish peroxidase diluted 1:100 (Kit ABC Vectastain; Vector Laboratories). Peroxidase activity was revealed by diaminobenzidine. Some sections from the same tissue were stained by the same procedure except that the primary antibody was not used; these sections were taken as negative controls.

Results

Leptin Selectively Suppressed Taste Nerve Responses to Sweet Stimuli in Nondiabetic Mice. Responses of the CT nerve in lean control mice to 0.1–1.0 M sucrose and 0.02 M Sac started to decrease approximately 10 min after the injection of leptin at a dose of 100 ng/g of b.w., then reached near maximum suppression level (65% of control) approximately 30 min after the injection (Fig. 1*a*). This suppression level maintained until approximately 60 min, then the responses started to recover. Recovery of the responses to near their control levels took more than 2 h after the injection (data not shown). Meanwhile, plasma leptin level increased to approximately 6.0 ng/ml from the control level (approximately 3.5 ng/ml) at 10 min after the injection and reached about 12.0 and 14.0 ng/ml at 30 and 60 min, respectively. Responses to sweet substances measured from 10 to 60 min after the injection were significantly smaller than the responses of same animals before the injection [Fig. 1 *c* (Student's *t* test, $P < 0.05$) and *d* (for concentration–response relationship for su-

crose)]. They also were significantly smaller than the time-matched responses of control animals injected with saline (data not shown), whereas no significant reduction in responses to salty, sour, and bitter-tasting stimuli (0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl) were observed (t test, $P > 0.05$), suggesting a selective suppression of responses to sweet substances by leptin. Such suppression was not found when leptin (1.0 μg/ml) was applied from outside to the tongue of lean mice. In *db/db* mice, taste responses of the CT were not affected (t test, $P > 0.05$) even after the injection of leptin at a dose of 500 ng/g of b.w., as shown in the concentration–response relationships for sucrose (Fig. 1*d*), although plasma leptin level was elevated above 150 ng/ml from the control level of approximately 90 ng/ml before the injection. Relative responses to 0.1 and 0.3 M sucrose in the CT nerve of lean mice were negatively correlated with their plasma leptin levels (Fig. 1*e*, $r = -0.675$ for 0.3 M sucrose and $r = -0.731$ for 0.1 M sucrose; $P < 0.001$), indicating that sucrose responses decrease with increasing plasma leptin levels. Such selective suppression of sweet responses by leptin also was observed in the CT nerve of BALB mice and the IXth nerve of both C57BL and BALB mice [Fig. 1 *b* (Lean-IXth) and *f* (suppressed to 62.2–69.1% of the control)], whereas again no such suppression was found in the IXth nerve responses of the *db/db* mice (Fig. 1 *b* (*db/db*-IXth) and *f*). An analysis of responses of single CT nerve fibers of lean C57BL mice demonstrated that suppression of responses to sucrose and saccharin (approximately 60% of controls) by leptin was observed in a limited population of nerve fibers (9/30; 30.0%) that predominantly responded to sucrose among four basic taste stimuli (Suc-best fibers), but not in other types of fibers (Fig. 2).

Leptin Suppressed Taste Cell Excitabilities Through K^+ Conductance Activation. In the taste cells isolated from circumvallate papillae of lean BALB mice, the effect of leptin applied to extracellular fluid was to enhance the persistent outward currents in response to depolarizing voltage steps, with little effect on the voltage-dependent transient inward currents (Fig. 3 *a* and *c*). As seen in the dose–response curve (Fig. 3*d*), the enhancing effect of leptin first appeared at about 10 ng/ml and increased with increasing concentration. Twenty-two of 134 taste cells (16.4%) demonstrated augmentation of steady-state outward currents with extracellular leptin of 100 ng/ml. Almost all leptin-sensitive cells also showed a diminition of outward currents by bath application with 5 mM Sac, implying that leptin-sensitive cells may be sweet-responsive cells (data not shown). The effects of leptin at concentrations over 10 ng/ml were significantly higher compared with the effect of 1 ng/ml leptin (two sample t test with Welch's correction, $P < 0.05$–0.001). The steep changes in the effect of leptin occurred at a concentration range from 3 ng/ml to 10 ng/ml in taste cells, which is consistent with the change in taste-nerve responses (Fig. 1*d*). This concentration range corresponds to the physiologically variable range of plasma leptin in normal lean mice. The reversal potential of leptin-activated current (Fig. 3*e*, curve 3), which was obtained by subtracting the whole-cell current in the absence of leptin (curve 1) from that in the presence of leptin (curve 2), was about -87 mV, which is close to E_k (-86.4 mV, under the present condition), indicating that the current is primarily a K^+ current. Substitution of NaCl with Na-gluconate in the external solution did not change the reversal potential of leptin-activated currents, which suggests that the current was not carried by Cl^- but by K^+. Fig. 3*f* shows resting membrane potential changes and simultaneous input resistance changes induced by 10 ng/ml leptin under the current clamp mode. In most taste cells that displayed K^+ conductance increase in response to leptin, the resting membrane potentials ranging from -35 to -74 mV were hyperpolarized by 5–21 mV in the presence of 10–50 ng/ml leptin. These results suggest that plasma leptin activates K^+ channels of some taste cells resulting in hyperpolarization of membrane potentials, which in turn leads to inhibition of depolarizing receptor potentials evoked by sweet stimuli.

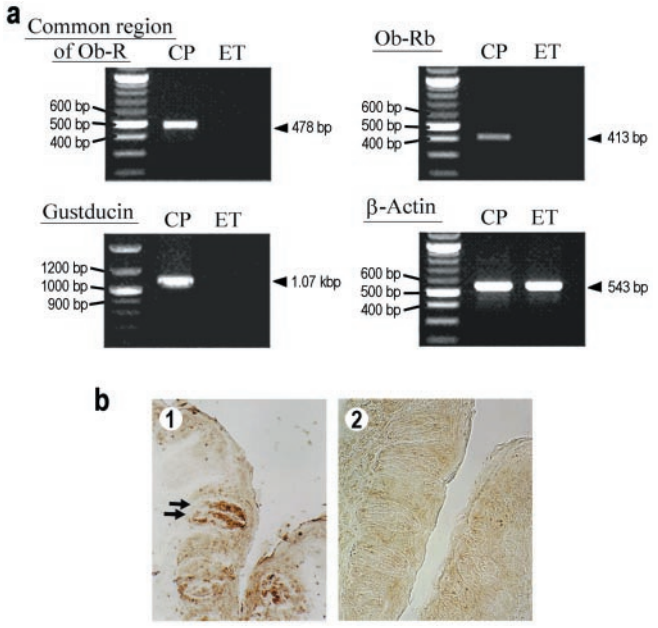

Fig. 4. Expression of leptin receptor (Ob-R) in the taste cells of circumvallate papillae in BALB mice. (*a*) Expression of mRNA for the common region of Ob-R (478 bp), specific for Ob-Rb (413 bp), for Ggust (1.07 kbp), and for β-actin (543 bp) in the circumvallate papillae (CP) and the tongue epithelium (ET) with no taste papillae. Although mRNA for β-actin (543 bp) was detected in both CP and ET, mRNAs for Ob-Rs and Ggust were detected only in CP. (*b*) Immunostaining of Ob-R with antibodies binding to common region of all types of Ob-R in circumvallate papillae. Immunoreactivity was found in some spindle-shaped cells in taste buds (arrows in *1*) but not in control section (*2*), which was stained without the primary antibody.

By contrast, the voltage-dependent currents of *db/db* mouse taste cells were little affected even by 1 mg/ml leptin (Fig. 3 *b* and *c*). All taste cells of *db/db* mice ($n = 22$) showed no increase in whole-cell K^+ conductance with 100 ng/ml leptin in the bath. These results suggest that leptin was unable to act on taste cells through the leptin receptor (Ob-R) in *db/db* mice.

Expression of Ob-R in Taste Tissue. By RT-PCR and immunohistochemical analyses, we investigated expression of Ob-R in taste cells of circumvallate papilla in lean control BALB mice. RT-PCR analysis showed that mRNAs for common region of all types of Ob-R and specific for Ob-Rb were expressed in circumvallate papilla, although they were not expressed in the tongue epithelium possessing no taste buds (Fig. 4*a*). The mRNA for Ggust also was clearly detected only in circumvallate papilla, whereas mRNA for β-actin was expressed in both circumvallate papilla and tongue epithelium. Immunoreactivity to the antibodies was clearly detected in some spindle-shaped cells within circumvallate papilla as indicated by the arrows in Fig. 4*b1*. These lines of evidence suggest that Ob-R, especially Ob-Rb, is expressed in taste cells of circumvallate papilla in BALB mice.

Discussion

It is known that leptin normally is produced by adipose tissue and acts at the hypothalamus to reduce food intake and to increase energy expenditure. The Ob-R, especially Ob-Rb, which is generally believed to be the signal-transducing receptor, expresses not only sites in the central nervous system (23, 24) but also sites in variety of peripheral cells, such as T cells (25),

vascular endothelial cells (26), muscle cells (27), and pancreatic β cells (28). The present results show that a subset of taste receptor cells are affected by leptin and express Ob-R, indicating that taste cells are a peripheral site of leptin action. Similar to pancreatic β cells (29) and hypothalamic neurons (30), leptin increased K^+ conductance of taste cells, which resulted in hyperpolarization and reduction of cell excitability. It is proposed that sugar and nonsugar sweeteners activate separate transduction cascades in the same sweet-responsive cell, one involves cAMP and the other involves inositol trisphosphate (IP_3) in rat circumvallate taste bud cells (31). Sugar increases intracellular cAMP, which activates protein kinase A (PKA) and closes K^+ channels mediated by phosphorylation, which in turn leads to influx of Ca^{2+} through voltage-gated Ca^{2+} channels. On the other hand, artificial sweeteners such as saccharin elevate IP_3 levels, which induces Ca^{2+} release from intracellular stores and an elevation of intracellular Ca^{2+} concentration. Interestingly, the IP_3 pathway is proposed to result in closure of K^+ channels via protein kinase C (PKC)-mediated phosphorylation activated by concomitantly produced diacylglycerol (DAG) (32).‖ Therefore, both second messenger pathways may converge on the same K^+ channels in taste cells. If this is the case, leptin activation of outward K^+ currents may suppress responses to both sucrose and saccharin to a similar extent in sweet-responsive taste cells, although no direct evidence exists at present. This assumption is supported by the finding that when the plasma leptin level was elevated by an injection of recombinant leptin, the taste nerve responses to both sucrose and saccharin were suppressed significantly but there was no suppression of the responses to other taste modalities. The recordings from single taste nerve fibers demonstrated that impulse numbers in response to sucrose and saccharin were selectively suppressed only in the subset of fibers that responded to sweet stimuli best (Suc-best fibers). This finding indicates that sweet information from leptin-suppressive taste cells is transmitted to Suc-best fibers. To verify this hypothesis, however, many of its key questions, such as whether leptin receptors would be expressed in sweet-sensitive receptor cells and whether enhancement of K^+ currents would occur in these same subsets of cells, must be answered by further investigations.

In the present study, we found that responses of taste cells and nerves in *db/db* mice were not influenced by leptin levels at all. This finding is probably attributable to their defects of Ob-R. Their enhanced CT (also IXth nerve) responses to sweeteners may be caused by increase in excitabilities of sweet-responsive receptor cells resulting from lack of this leptin suppression system.

In summary, previous findings of enhanced sweet responses and defects of Ob-R in *db/db* mice led us to study possible effects of leptin on taste responses and to find the existence of leptin suppression system on sweet taste sensitivities in normal lean mice. Probably, leptin influences food intake not only through the central nervous system but also at peripheral level acting as a sweet-sensing modulator.

We thank Dr. G. K. Beauchamp, Dr. Y. Oomura, and Dr. Y. Fukami for critical discussions and valuable comments on the manuscript. This work was supported in part by Grants-in-Aid 947047 (Y.N.) and 9557147 (Y.N.) from the Ministry of Education, Science, Sport, and Culture of Japan, and Grant of Program for Promotion of Basic Research Activities for Innovative Bioscience from Bio-Oriented Technology Research Advancement Institution.

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