

Ryusuke Yoshida,<sup>1</sup> Kenshi Noguchi,<sup>1,2</sup> Noriatsu Shigemura,<sup>1</sup> Masafumi Jyotaki,<sup>1</sup> Ichiro Takahashi,<sup>2</sup> Robert F. Margolskee,<sup>3</sup> and Yuzo Ninomiya<sup>1,4</sup>



## Leptin Suppresses Mouse Taste Cell Responses to Sweet Compounds



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**Leptin is known to selectively suppress neural and behavioral responses to sweet-tasting compounds. However, the molecular basis for the effect of leptin on sweet taste is not known. Here, we report that leptin suppresses sweet taste via leptin receptors (Ob-Rb) and  $K_{ATP}$  channels expressed selectively in sweet-sensitive taste cells. Ob-Rb was more often expressed in taste cells that expressed T1R3 (a sweet receptor component) than in those that expressed glutamate-aspartate transporter (a marker for Type I taste cells) or GAD67 (a marker for Type III taste cells). Systemically administered leptin suppressed taste cell responses to sweet but not to bitter or sour compounds. This effect was blocked by a leptin antagonist and was absent in leptin receptor-deficient *db/db* mice and mice with diet-induced obesity. Blocking the  $K_{ATP}$  channel subunit sulfonylurea receptor 1, which was frequently coexpressed with Ob-Rb in T1R3-expressing taste cells, eliminated the effect of leptin on sweet taste. In contrast, activating the  $K_{ATP}$  channel with diazoxide mimicked the sweet-suppressing effect of leptin. These results indicate that leptin acts via Ob-Rb and  $K_{ATP}$  channels that are present in T1R3-expressing taste cells to selectively suppress their responses to sweet compounds.**

Leptin is a hormone from adipose tissue that regulates food intake, energy expenditure, and body weight by activating leptin receptors of the hypothalamus and other peripheral tissues (1,2). Multiple isoforms of leptin receptors produced by alternative splicing of the transcript from the *Lepr* gene have been reported, but a single receptor isoform (Ob-Rb) appears to account for all of the action of leptin (3,4). Mice homozygous for the *db* point mutation of the *Lepr* gene (*db/db* mice) lack functional Ob-Rb leptin receptors, and are

hyperphagic, massively obese, and diabetic (3). In comparison with lean controls, *db/db* mice have increased gustatory neural sensitivity (5–7) and a stronger preference for multiple sweet substances (5), uncovering the sweet suppressive effect of leptin (8). Exogenous administration of recombinant leptin suppresses the responses of peripheral taste nerves (the chorda tympani and glossopharyngeal nerves) of lean mice (8) and diminishes the behavioral responses of normal lean mice and leptin-deficient *ob/ob* mice (9) to sweet compounds without affecting responses to salty-, sour-, and bitter-tasting compounds. RT-PCR, in situ hybridization, and immunohistochemistry have demonstrated that Ob-Rb is expressed in mouse taste cells (TCs) in fungiform papillae (FP) and circumvallate papillae (VP) (8–11). Outward potassium currents of isolated TCs are increased by applied leptin (8). These data suggest that taste receptor cells are peripheral targets for leptin.

However, previous studies have not revealed the molecular mechanism by which leptin suppresses sweet. Earlier studies demonstrated the existence of Ob-Rb in TCs but did not determine whether any particular type of TC expresses Ob-Rb (8–11). TCs are morphologically and functionally classified into three subtypes (Type I, II, and III cells) (12). Type II cells express sweet and umami taste receptor components T1R1, T1R2, and T1R3 or bitter receptors T2Rs (13,14), and respond to sweet, umami, or bitter taste stimuli (15). Type III cells express markers such as GAD67 (16) and respond to sour stimuli or multiple electrolytes (15,17). Type I cells express glutamate-aspartate transporter (GLAST) (18) and may act as supporting cells similar to glial cells in the nervous system. In the current study, we have gone on to determine which types of TCs express Ob-Rb.

<sup>1</sup>Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

<sup>2</sup>Section of Orthodontics and Dentofacial Orthopedics, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

<sup>3</sup>Monell Chemical Senses Center, Philadelphia, PA

<sup>4</sup>Division of Sensory Physiology, Research and Development Center for Taste and Odor Sensing, Kyushu University, Fukuoka, Japan

Corresponding author: Ryusuke Yoshida, yoshida.ryusuke.319@m.kyushu-u.ac.jp, or Yuzo Ninomiya, ninomiya.yuzo.285@m.kyushu-u.ac.jp.

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In our previous study (19), we found that basolateral application of endocannabinoids enhanced the responses of T1R3-expressing TCs to sweet taste stimuli. Using this recording system, we now investigate the effect of leptin on TC responses to tastants. We examined the effects of leptin on taste responses of sweet-, sour-, and bitter-sensitive TCs. We also examined the contribution of Ob-Rb in TCs using genetic and pharmacological approaches. In addition, we investigated whether diet-induced obesity (DIO), which is known to produce leptin resistance (20), affects the effect of leptin on sweet taste.

The suppression by leptin of sweet taste responses may be mediated by an increase in outward potassium current (8); however, the molecular target in TCs that mediates this effect is not known. A recent study found that  $K_{ATP}$  channels that serve as a metabolic sensor in pancreatic islet cells (21) were expressed in T1R3-positive TCs (22). Leptin is reported to activate  $K_{ATP}$  channels in rat CRI-G1 insulin-secreting cells (23), mouse pancreatic  $\beta$ -cells (24), and rat hypothalamic neurons (25). From these observations, we hypothesize that the suppression by leptin of TC responses to sweet may be mediated by the modulation of their  $K_{ATP}$  channels. Here we examine coexpression in TCs of the sulfonylurea receptor 1 (SUR1) subunit of the  $K_{ATP}$  channel with the Ob-Rb leptin receptor. We also examined the effects of the  $K_{ATP}$  channel blocker glibenclamide (Gc) on leptin suppression of sweet taste. In addition, we examined the effect of the  $K_{ATP}$  channel activator diazoxide (Dz) on sweet taste responses of TCs. Our results indicate that leptin suppresses taste responses of sweet-sensitive TCs via activation of Ob-Rb and  $K_{ATP}$  channels expressed in these cells.

## RESEARCH DESIGN AND METHODS

### Animals

All experimental procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Committee for Laboratory Animal Care and Use at Kyushu University (Fukuoka, Japan). Subjects were adult (>8 weeks old) male and female C57BL/6J mice ( $n = 6$  for histology) and transgenic mice expressing green fluorescent protein (GFP) under control of the T1R3 promoter (T1R3-GFP mice,  $n = 82$  for electrophysiology) (26), the gustducin promoter (gustducin-GFP mice,  $n = 5$  for electrophysiology) (27), or the GAD67 promoter (GAD-GFP [ $\Delta$ neo] mice,  $n = 3$  for histology,  $n = 6$  for electrophysiology) (28). For the generation of T1R3-GFP *db/db* mice, *db/+* mice (The Jackson Laboratory, Bar Harbor, ME) were crossed with T1R3-GFP mice to generate T1R3-GFP *db/+* mice. These mice were further crossed with *db/+* mice to breed T1R3-GFP *db/db* mice ( $n = 6$  for electrophysiology). To generate DIO mice, for 12 weeks 4-week-old male T1R3-GFP mice were fed either a high-fat diet (catalog #D12492; Research Diets, New Brunswick, NJ) that contained 60% kcal as fat ( $n = 8$ ) or a normal diet ( $n = 7$  for electrophysiology).

### Immunohistochemistry and In Situ Hybridization

The procedures were modified from those used previously (9,19,29). For in situ hybridization (ISH), digoxigenin (DIG)-UTP-labeled RNA probes for Ob-Rb were prepared as previously described (9). This riboprobe corresponds to nucleotide positions 2,714–3,126 in the intracellular domain of Ob-Rb. For VP, frozen blocks of the dissected tongue containing VP embedded in the OCT compound (Sakura Finetechnical, Tokyo, Japan) were sectioned into 8- $\mu$ m-thick slices. For FP, dissected anterior tongue was injected with 100  $\mu$ L Tyrode solution containing 0.5–1 mg/mL elastase (Elastin Products, Owensville, MO) to peel the lingual epithelium. These sections and peeled lingual epithelia were fixed in 4% paraformaldehyde, treated with Protease K (Life Technologies, Grand Island, NY), and then prehybridized in  $5\times$  SSC/50% formamide for 2 h at room temperature. Hybridization was performed in a hybridization buffer containing 50% formamide,  $5\times$  SSC,  $5\times$  Denhardt solution, 500  $\mu$ g/mL denatured salmon testis DNA, 250  $\mu$ g/mL denatured baker yeast tRNA, 1 mmol/L dithiothreitol, and 20–200 ng/mL anti-sense or sense RNA probe for 18 h at 60°C. After hybridization, preparations were washed with  $5\times$  SSC and  $0.2\times$  SSC at 65°C. Subsequently, preparations were treated with 0.3%  $H_2O_2$  in Tris-NaCl-Tween buffer, 1% blocking reagent (Roche, Mannheim, Germany), and the primary antibody for T1R3 (1:50, goat anti-T1R3 [N20] polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA), SUR1 (1:100, rabbit anti-SUR1 [H-80]; Santa Cruz Biotechnology), GFP (1:100, rabbit anti-GFP [FL] polyclonal; Santa Cruz Biotechnology), or GLAST (1:200; rabbit anti-GLAST; Frontier Institute, Hokkaido, Japan). Then, tissues were incubated with secondary antibodies for DIG (1:500, anti-DIG Fab fragments conjugated with alkaline phosphatase; Roche) and T1R3 (1:500, peroxidase-conjugated donkey anti-goat IgG; Jackson ImmunoResearch Laboratories, West Grove, PA) or GFP or GLAST (1:500, peroxidase-conjugated donkey anti-rabbit IgG; Jackson ImmunoResearch Laboratories); and then tyramide-Alexa Fluor 488 substrate (Molecular Probes TSA Kit; Life Technologies), AP buffer, and HNPP/FastRed AP substrate (HNPP Fluorescent Detection Kit; Roche). For triple staining with T1R3, SUR1, and Ob-Rb, the double Tyramide Signal Amplification labeling method (30) was combined with ISH. Procedures were the same until the treatment with tyramide-Alexa 488 substrate for staining T1R3. Then preparations were incubated with 0.9%  $H_2O_2$  in Tris-NaCl-Tween buffer and secondary antibody for SUR1 (1:500, peroxidase-conjugated donkey anti-rabbit IgG; Jackson ImmunoResearch Laboratories), and then tyramide-Alexa Fluor 647 substrate (Molecular Probes), AP buffer, and HNPP/FastRed AP substrate. The fluorescence-labeled TCs were observed by using a laser-scanning microscope (FV-1000; Olympus, Tokyo, Japan).

### TC Recordings

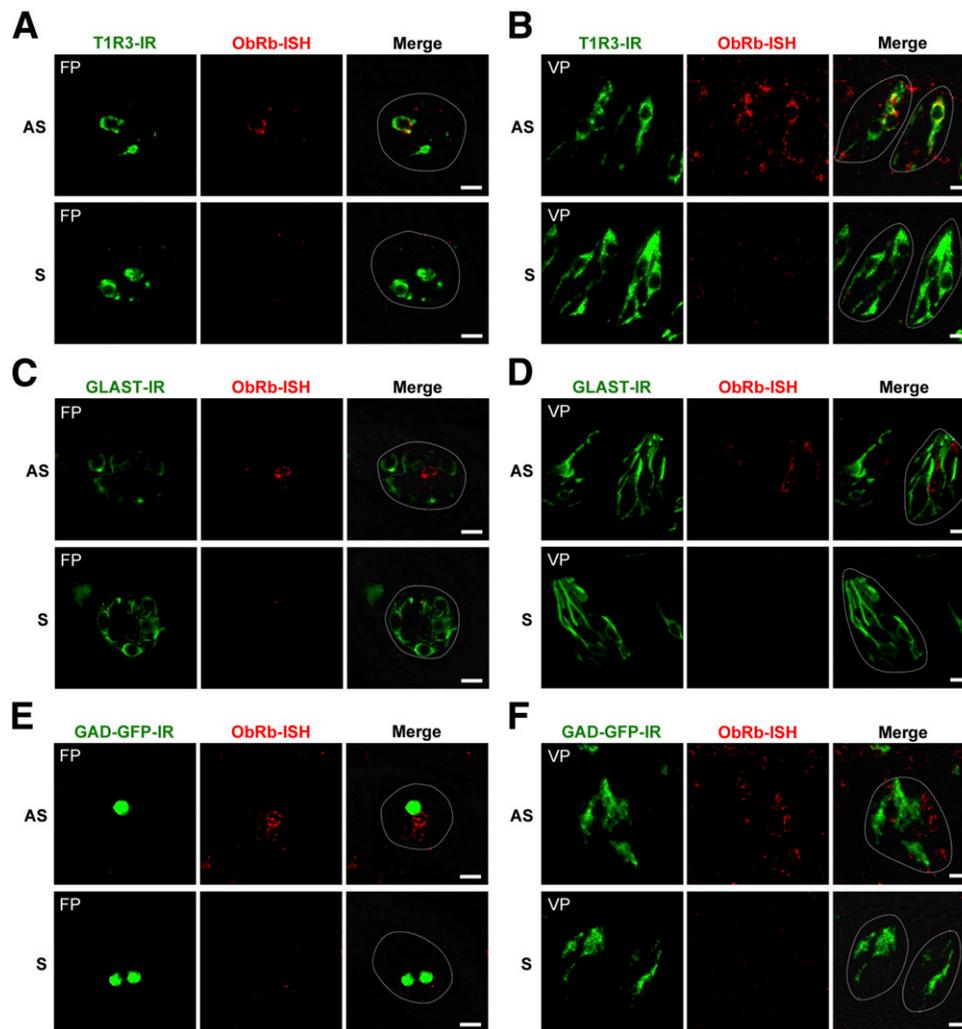
Recording procedures were as used previously (15,19,31). Dissected anterior tongue was injected with 100  $\mu$ L

Tyrode solution containing 0.5–1 mg/mL elastase (Elastin Products) to peel the lingual epithelium. Individual FP taste buds with a piece of surrounding epithelium were excised from this sheet, and the mucosal side was drawn into the orifice of the stimulating pipette. A gentle suction on the stimulating pipette was maintained to perfuse taste solutions and to hold the taste bud in place. Bath solution (Tyrode solution) was continuously flowed into the recording chamber with a peristaltic pump at ~2 mL/min. The receptor membrane was rinsed with distilled water at least 30 s before and after taste stimulation (15–20 s). GFP-expressing TCs were identified by a confocal laser-scanning microscope (FV-1000; Olympus) and were approached by a recording electrode (inner diameter ~1–3  $\mu\text{m}$ , pipette resistances 1.5–3.5 M $\Omega$ ). Electrical signals were recorded by a high-impedance patch-

clamp amplifier (Axopatch 200B; Axon Instruments, Union City, CA) interfaced with a computer by an analog-to-digital board (Digidata 1320A; Axon Instruments).

### Solutions

Tyrode solution contained the following (in mmol/L): NaCl 140, KCl 5, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1, NaHCO<sub>3</sub> 5, HEPES 10, glucose 10, and sodium pyruvate 10, with pH adjusted to 7.4 with NaOH. Saccharin-Na<sup>+</sup> (3–50 mmol/L), HCl (10 mmol/L), and quinine-HCl (10–20 mmol/L) were used as sweet, sour, and bitter tastants, respectively. Recombinant murine leptin (PeproTech, Rocky Hill, NJ); leptin antagonist (LA) L39A/D40A/F41A (Protein Laboratories Rehovot, Rehovot, Israel), which is a specific antagonist for leptin receptor (32); and Gc and Dz (Sigma-Aldrich, St. Louis, MO) were applied from the basolateral side of TCs.



**Figure 1**—Expression of Ob-Rb and TC markers in FP and VP. *A* and *B*: Expression of T1R3 and Ob-Rb in C57BL/6J mice. *C* and *D*: Expression of GLAST and Ob-Rb in C57BL/6J mice. *E* and *F*: Expression of GAD67-GFP and Ob-Rb in GAD67-GFP mice. Expression of T1R3 (T1R3-IR), GAD67-GFP (GAD-GFP-IR), and GLAST (GLAST-IR) was detected by immunohistochemistry (green). Expression of Ob-Rb (ObRb-ISH) was detected by ISH (red). The sense probe serves as a negative control. AS, antisense probe for Ob-Rb; S, sense probe for Ob-Rb. Dotted lines indicate the outline of taste buds. Scale bars = 10  $\mu\text{m}$ . Quantitative data are shown in Table 1.

Gc and Dz were prepared as 100 mmol/L stock solutions in DMSO and diluted to their final concentration with Tyrode solution. Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

### Data Analysis

Action potential waveform analyses were as previously described (31). The number of spikes per unit of time was counted throughout the recording. The mean spontaneous impulse discharge was calculated by averaging the number of spikes over the 10-s period in which distilled water flowed over the taste pore prior to each stimulation. Response magnitude was obtained by counting the total number of impulses for the first 10 s after taste stimulation and subtracting the spontaneous impulse discharge. Data were excluded if the spontaneous impulse discharge rate after the washout of agents (i.e., at the end of the experiment) was significantly different from that before treatment with agents (i.e., at the start of the experiment). Potential baseline differences indicative of washout were noted by comparing six 10-s periods by *t* test for baseline levels. Data were also excluded if taste responses after washout did not recover to  $\geq 70\%$  of the control response.

Statistically significant differences were evaluated by one-way repeated-measures ANOVA followed by post hoc *t* test with Bonferroni correction and Student or paired *t* test. Calculations were performed using the statistical software package SPSS (IBM, Armonk, NY), and differences were considered to be significant at  $P < 0.05$ .

## RESULTS

### Expression of Ob-Rb in Taste Buds

We determined by immunohistochemistry and ISH which types of TCs expressed the Ob-Rb leptin receptor. In C57BL/6J mice,  $\sim 75\text{--}80\%$  of Ob-Rb-expressing TCs also expressed T1R3 (a marker for sweet and umami responding Type II cells) (Fig. 1A and B and Table 1). About 40% of T1R3-expressing TCs in FP or VP expressed Ob-Rb (Fig. 1A and B and Table 1). About 10% of TCs expressing Ob-Rb expressed GLAST (a marker for Type I cells) (18) (Fig. 1C and D and Table 1). Only a small percentage of TCs expressing Ob-Rb expressed GAD67 (a marker for Type III cells, which respond to sour taste or electrolytes) (15) (Fig. 1E and F and Table 1).

**Table 1—Coexpression of Ob-Rb and TC type markers in FP and VP TCs (data from 3 mice)**

	FP		VP	
	<i>n</i>	%	<i>n</i>	%
Ob-Rb/T1R3	57/142	40.1	116/281	41.3
Ob-Rb/GLAST	4/121	3.3	14/280	5.0
Ob-Rb/GAD67	0/42	0	2/227	0.9
T1R3/Ob-Rb	57/73	78.1	116/151	76.8
GLAST/Ob-Rb	4/55	7.3	14/126	11.1
GAD67/Ob-Rb	0/56	0	2/118	1.7

### Leptin Selectively Suppresses Taste Responses of Sweet-Sensitive TCs

Next, we examined the effect of leptin on TC responses to different taste stimuli using an experimental setup that allows separate stimulation of the apical (taste-sensing) and basolateral (leptin-responding) faces of mouse FP TCs (15,19,31). To identify particular types of TCs, we used the following transgenic mice: T1R3-GFP for sweet, GAD67-GFP for sour, and gustducin-GFP mice for bitter (15,19).

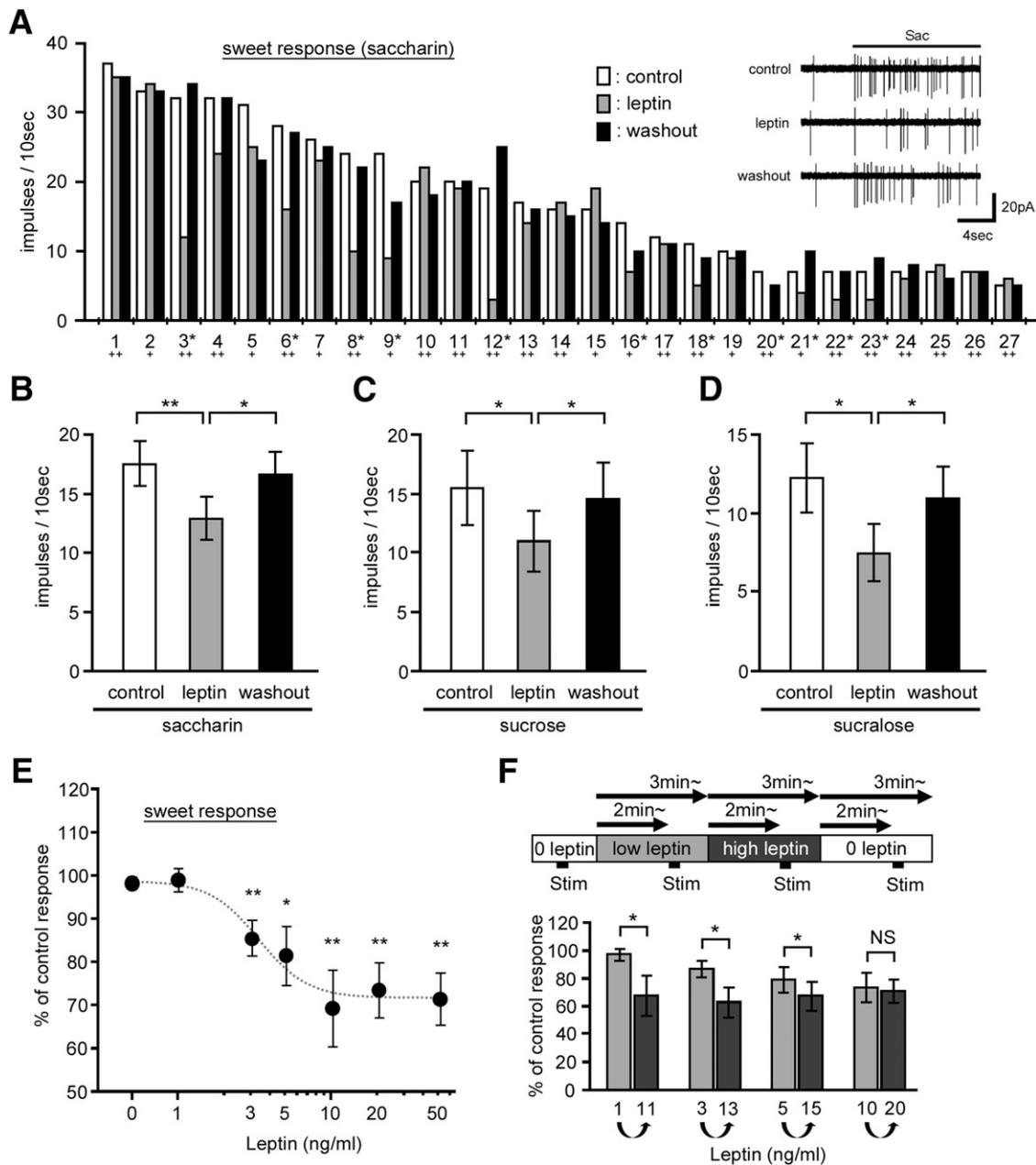
In some T1R3-GFP TCs, responses to saccharin as a prototypical sweet stimulus were suppressed by bath application of 20 ng/mL leptin and recovered after washout (Fig. 2A, inset). In total, 12 of 29 sweet-sensitive T1R3-GFP TCs showed suppression of their responses to saccharin ( $< 60\%$  of control response) by bath application of 20 ng/mL leptin (Fig. 2A). Leptin suppression of the responses of T1R3-GFP TCs to saccharin was statistically significant (Fig. 2B) and dose dependent (Fig. 2E), with effects noted at  $\geq 3$  ng/mL leptin. This effect reached a maximal level ( $\sim 70\%$  of control response) at 10–20 ng/mL leptin. Responses of T1R3-GFP TCs to sucrose and sucralose were also suppressed by 20 ng/mL leptin (Fig. 2C and D).

TCs adapt to basal plasma leptin levels in vivo and their sweet sensitivity may be affected by changes in plasma leptin level (33). Therefore, we investigated whether adaptation to leptin affected TC responses to sweet. In these experiments, each lower concentration (1, 3, 5, or 10 ng/mL) of leptin was first administered for  $> 3$  min, and then the higher concentration (+10 ng/mL; 11, 13, 15, and 20 ng/mL) of leptin was administered. Sweet responses of T1R3-GFP TCs were recorded during the administration of the lower and higher concentrations of leptin (Fig. 2F). Sweet responses of TCs were suppressed by 3–10 ng/mL leptin in a dose-dependent fashion (Fig. 2F), similar to the suppression shown in Fig. 2C. After first adapting to 1, 3, or 5 ng/mL leptin, subsequent increases of 10 ng/mL leptin significantly suppressed the sweet responses of TCs (Fig. 2F). After first adapting to 10 ng/mL leptin, a subsequent increase of 10 ng/mL leptin had no significant effect on the sweet responses of TCs (Fig. 2F). These results suggest that taste sensitivities of sweet TCs may be regulated by plasma leptin levels.

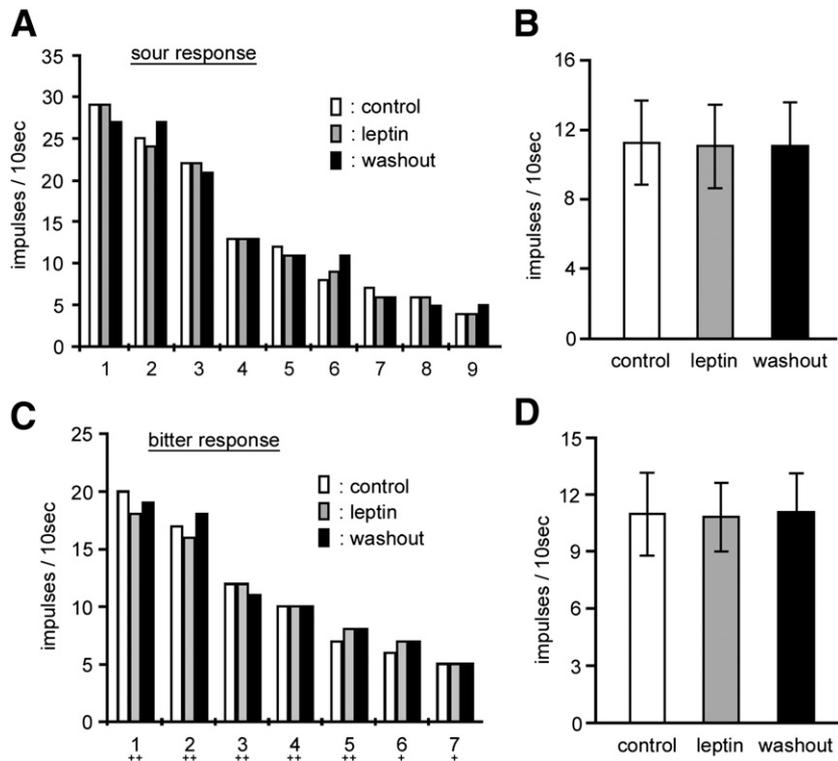
We also examined the effects of leptin on sour- and bitter-sensitive TCs. As shown in Fig. 3, the responses of GAD67-GFP TCs to HCl (sour) (Fig. 3A and B) and those of gustducin-GFP TCs to quinine-HCl (bitter) (Fig. 3C and D) were not affected by bath application of 20 ng/mL leptin. These results indicate that leptin selectively suppresses the taste responses of sweet-sensitive TCs.

### Ob-Rb Mediates Suppression of Sweet by Leptin

TCs are known to express functional Ob-Rb leptin receptors (8–11). Furthermore, intraperitoneal injection of leptin affects neural and behavioral responses to sweeteners of wild-type mice but not of *db/db* mice (8,9). Thus, leptin suppression of sweet is likely to be mediated by Ob-Rb, which we have found to be expressed on sweet-sensitive TCs (Fig. 1 and Table 1). Consistent with these findings, bath application



**Figure 2**—Effect of bath-applied leptin on responses of T1R3-GFP TCs from mouse FP to sweet compounds. **A**: Responses of 29 individual T1R3-GFP TCs to 10 (+) or 20 (++) mmol/L saccharin before (white), during (gray) and after (black) treatment with 20 ng/mL leptin. Asterisks indicate TCs showing inhibition (<60% of control) by leptin of responses to sweet compounds. Inset represents sample recordings of TC responses to sweet compounds before (control), during (leptin) and after (washout) treatment with 20 ng/mL leptin. Effects of leptin on summarized responses of T1R3-GFP TCs to 10 or 20 mmol/L saccharin (**B**) ( $n = 27$ ,  $F$  value = 10.946,  $P < 0.001$ , repeated-measures ANOVA), 200–500 mmol/L sucrose (**C**) ( $n = 8$ ,  $F$  value = 10.134,  $P = 0.002$ , repeated-measures ANOVA), and 3–10 mmol/L sucralose (**D**) ( $n = 8$ ,  $F$  value = 9.301,  $P = 0.003$ , repeated-measures ANOVA). Values are the mean  $\pm$  SE. \* $P < 0.05$ , post hoc  $t$  test. **E**: Dose-dependent effect of leptin on responses of T1R3-GFP TCs to sweet compounds. Each response was normalized to the control response before the application of leptin. Values are the mean  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$ , Student  $t$  test (vs. 0 ng/mL leptin,  $n = 10$ –27). **F**: Leptin suppresses the responses of T1R3-GFP TCs to sweet compounds. The top panel shows the experimental protocol. After recording control responses (0 ng/mL leptin) to stimulation with sweet compounds (10 or 20 mmol/L saccharin), low concentrations of leptin (1, 3, 5, or 10 ng/mL, respectively) were administered for 2 min and responses to sweet compounds were recorded. Then higher concentrations of leptin (11, 13, 15, or 20 ng/mL, respectively) were administered, and responses to sweet compounds were recorded. The lower panel shows a summary of the effects of an increase in 10 ng/mL leptin on responses of TCs to sweet compounds after adaptation to several different concentrations of leptin ( $n = 7$ –8). Each response was normalized to the control response before application of leptin. Values are the mean  $\pm$  SE. NS, not significant difference; Stim, stimulation. \* $P < 0.05$ , paired  $t$  test.



**Figure 3**—Effect of bath-applied leptin on the responses of mouse fungiform TCs to sour and bitter compounds. **A:** Responses of nine individual GAD-GFP TCs to 10 mmol/L HCl before (white), during (gray), and after (black) treatment with 20 ng/mL leptin. Bath-applied leptin did not affect the responses of sour-sensitive TCs. **B:** Effects of leptin on summated responses of GAD-GFP TCs on the sour stimulus 10 mmol/L HCl.  $n = 9$ ,  $F$  value = 0.108,  $P = 0.898$ , repeated-measures ANOVA. **C:** Responses of seven individual gustducin-GFP TCs to 10 (+) or 20 (++) mmol/L quinine-HCl before (white), during (gray), and after (black) treatment with 20 ng/mL leptin. Bath-applied leptin did not affect the responses of bitter-sensitive TCs. **D:** Effects of leptin on summated responses of gustducin-GFP TCs to bitter (10 or 20 mmol/L quinine-HCl).  $n = 7$ ,  $F$  value = 0.3,  $P = 0.746$ , repeated-measures ANOVA. Values are the mean  $\pm$  SE.

of 20 ng/mL leptin did not affect the responses of sweet-sensitive TCs to saccharin in *db/db* mice (Fig. 4A–C). In addition, leptin suppression of the sweet responses of T1R3-GFP TCs was inhibited by the addition of LA (100 ng/mL; Fig. 4D–F). Altogether, these data indicate that leptin acts on Ob-Rb to suppress the taste responses of sweet-sensitive TCs.

#### Suppression of Sweet by Leptin in Obese Mice

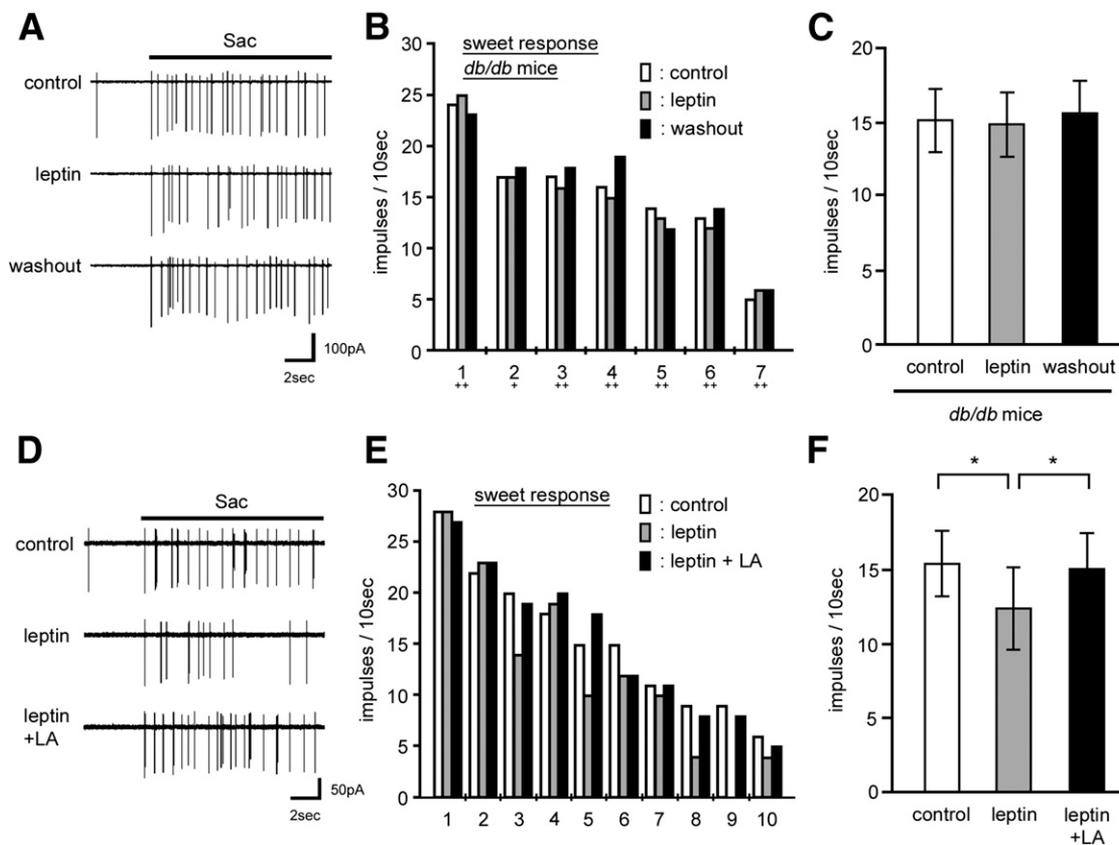
DIO is known to produce leptin resistance (20); therefore, the suppression of sweet responses by leptin might be reduced in DIO mice. To test this possibility, we examined the effect of leptin on responses to sweet compounds by T1R3-GFP TCs isolated from DIO mice. T1R3-GFP mice fed the high-fat diet for 12 weeks became obese and displayed elevated circulating leptin levels (weight  $37.2 \pm 3.1$  g, plasma leptin concentration  $68.1 \pm 16.0$  ng/mL,  $n = 9$ ) compared with those fed the normal diet for 12 weeks (weight  $24.5 \pm 1.4$  g, plasma leptin  $3.73 \pm 0.84$  ng/mL,  $n = 7$ ). When these high fat-fed DIO mice received bath-applied leptin (20 ng/mL), only three of nine T1R3-GFP-positive TCs tested showed weak suppression of their response to saccharin (Fig. 5A and B). In total, the responses of T1R3-GFP-positive cells in DIO mice to saccharin were not significantly different before or after bath application of

20 ng/mL leptin (Fig. 5C). In contrast, leptin significantly suppressed responses to saccharin in T1R3-GFP mice that were fed a normal diet (Fig. 5D). These results indicate that DIO caused sweet-sensitive T1R3-GFP-positive TCs to become resistant to leptin.

#### Leptin Acts on TC $K_{ATP}$ Channels To Suppress Sweet

Leptin suppression of sweet is associated with an increase in outward potassium currents (8). T1R3-positive TCs are known to express  $K_{ATP}$  channel subunits (22), and leptin activates  $K_{ATP}$  channels in pancreatic  $\beta$ -cells (24) and hypothalamic neurons (25). Therefore, we sought to determine whether the  $K_{ATP}$  channel mediates the suppression of sweet taste by leptin. We used immunohistochemistry and ISH to examine the expression of T1R3, the  $K_{ATP}$  channel subunit SUR1, and the Ob-Rb leptin receptor in TCs (Fig. 6A and B). A subset of T1R3-expressing TCs in FP and VP expressed SUR1 (~80–90%) and Ob-Rb (~40%) (Table 2). About 40% of T1R3-positive TCs expressed both SUR1 and Ob-Rb (Table 2), indicating that SUR1 and Ob-Rb were frequently coexpressed in T1R3-expressing TCs.

Next, we examined the effect of the  $K_{ATP}$  channel blocker Gc on leptin suppression of sweet. The suppression of T1R3-GFP TC responses to saccharin caused by bath application of 20 ng/mL leptin was effectively blocked by the



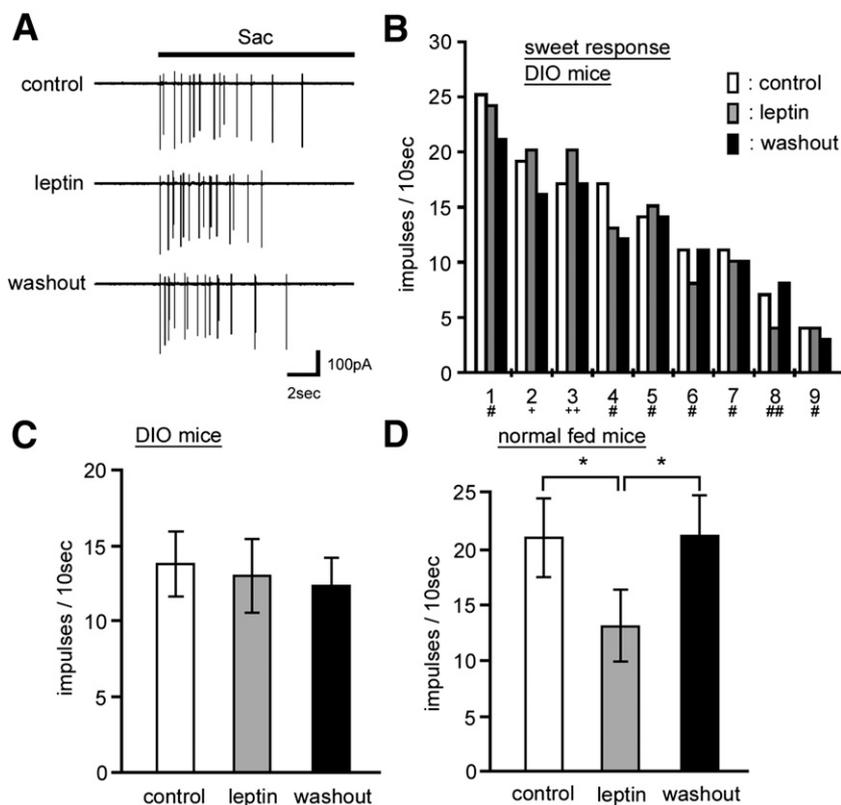
**Figure 4**—Suppression of responses to sweet compounds by leptin is mediated by Ob-Rb. **A**: Sample recordings of TC responses of a *db/db* mouse to sweet compounds before (control), during (leptin), and after (washout) treatment with 20 ng/mL leptin. **B**: Responses of seven individual T1R3-GFP TCs in *db/db* mice to 3 (+) or 20 (++) mmol/L saccharin before (white), during (gray), and after (black) treatment with 20 ng/mL leptin. **C**: Effects of leptin (20 ng/mL) on summated responses to sweet compounds of T1R3-GFP TCs in *db/db* mice.  $n = 7$ ,  $F$  value = 1.043,  $P = 0.382$ , repeated-measures ANOVA. **D**: Sample recordings of TC responses to sweet compounds before (control) and during treatment with 20 ng/mL leptin (leptin) and 20 ng/mL leptin plus 100 ng/mL LA (mutant L39A/D40A/F41A, leptin + LA). **E**: Responses of 11 individual T1R3-GFP TCs to 20 mmol/L saccharin before treatment (white), during treatment with 20 ng/mL leptin (gray), and during treatment with 20 ng/mL leptin + 100 ng/mL LA (black). **F**: LA (100 ng/mL) blocked the ability of leptin (20 ng/mL) to suppress responses to sweet compounds in sweet-sensitive T1R3-GFP TCs. Each response was normalized to the control response before the application of leptin.  $n = 10$ ,  $F$  value = 6.252,  $P = 0.009$ , repeated-measures ANOVA. \* $P < 0.05$ , post hoc  $t$  test. Values are the mean  $\pm$  SE.

addition to the bath solution of 30  $\mu$ mol/L Gc (Fig. 6C). In total, we tested T1R3-GFP TCs with 20 ng/mL leptin plus 30  $\mu$ mol/L Gc and found that leptin suppression of sweet responses was blocked significantly by the addition of Gc (Fig. 6D) in a dose-dependent manner (Fig. 6E). Significant suppression of sweet responses by leptin was impaired by  $\geq 10$   $\mu$ mol/L Gc. It is important to note that 30  $\mu$ mol/L Gc increased the spontaneous firing rate of T1R3-GFP TCs in the presence of 20 ng/mL leptin ( $2.14 \pm 1.12$  [30  $\mu$ mol/L],  $-0.02 \pm 0.09$  [10  $\mu$ mol/L],  $-0.31 \pm 0.12$  [3  $\mu$ mol/L],  $-0.05 \pm 0.12$  spikes/10 s [1  $\mu$ mol/L],  $n = 7-8$ ). We also examined the effect on sweet taste responses of T1R3-GFP TCs of the  $K_{ATP}$  channel activator Dz. Responses to saccharin were suppressed by bath application of 40  $\mu$ mol/L Dz and were recovered after washout of Dz (Fig. 6F). We tested T1R3-GFP TCs with 40  $\mu$ mol/L Dz and found that Dz significantly suppressed their responses to saccharin (Fig. 6G). These results suggest that leptin suppression of sweet responses

in T1R3-expressing TCs is mediated by the activation of  $K_{ATP}$  channels.

## DISCUSSION

In the current study, we sought to uncover the mechanism whereby leptin acts on the peripheral taste system to suppress responses to sweet compounds. We found that Ob-Rb leptin receptors are expressed in  $\sim 40\%$  of T1R3-expressing TCs and that leptin affects only taste responses from sweet-responsive TCs. The cells that coexpress T1R3 and Ob-Rb are likely to be the target of the selective action of leptin on sweet taste. Our results may also explain the enhanced preference for sweet compounds in knockout mice lacking vasoactive intestinal peptide. Vasoactive intestinal peptide knockout mice have decreased expression of Ob-Rb in their taste buds but not in the hypothalamus (11). Less Ob-Rb in T1R3-positive TCs would lead to a diminished ability of leptin to suppress responses from these cells (i.e., these mice should have enhanced sweet taste



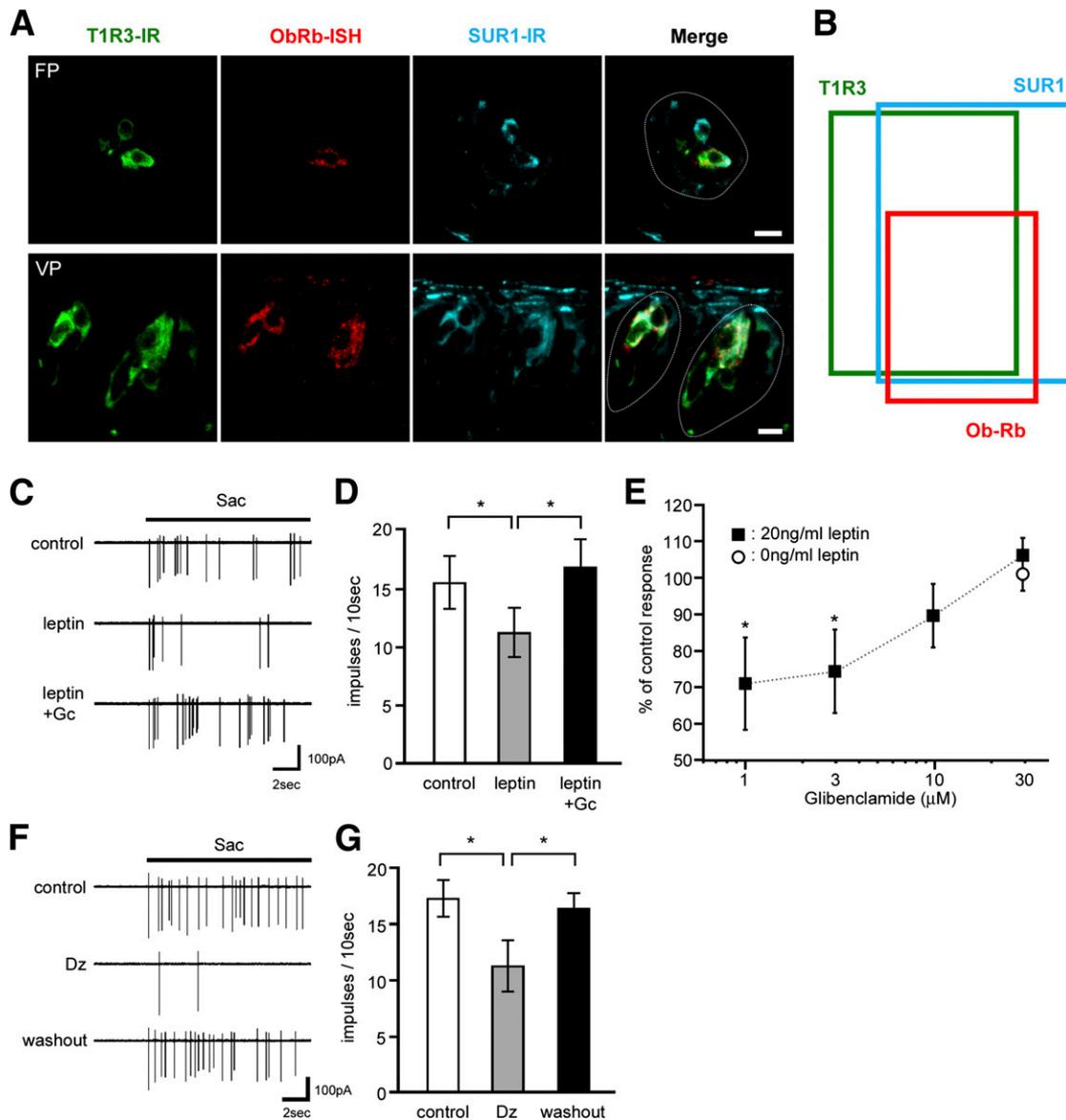
**Figure 5**—Effect of bath-applied leptin on responses to sweet compounds of fungiform TCs from obese mice. *A*: Sample recordings of TC responses of a DIO mouse before (control), during (leptin), and after (washout) treatment with 20 ng/mL leptin. *B*: Responses of nine individual sweet-sensitive T1R3-GFP TCs in DIO mice to 3 (+), 10 (++) , 20 (#), and 50 (##) mmol/L saccharin (Sac) before (white), during (gray), and after (black) treatment with 20 ng/mL leptin. *C*: Effects of leptin on summated sweet responses of T1R3-GFP TCs from DIO mice.  $n = 9$ ,  $F$  value = 1.688,  $P = 0.216$ , repeated-measures ANOVA. *D*: Effects of leptin on summated responses of T1R3-GFP TCs from mice fed the normal diet for 12 weeks (normal fed mice). Individual responses are shown in Fig. 2A (cell numbers 1, 3, 6, 8, 12, 13, 15, 23, and 26).  $n = 9$ ,  $F$  value = 7.638,  $P = 0.005$ , repeated-measures ANOVA. \* $P < 0.05$ , post hoc  $t$  test. Values are the mean  $\pm$  SE.

responses). Another group, Lu et al. (34), found that leptin increased temperature-dependent gustatory nerve responses to sucrose in mice. Differences in results between our group and Lu et al. (34) may be due to methodological differences, including the temperature at which experiments were performed and the choice of rinse solutions for stimuli.

Our results show that sweet-sensitive TCs are the target cells of the action of leptin to selectively suppress sweet taste. That leptin only affected responses from  $\sim 50\%$  of sweet-sensitive cells is consistent with our finding that only  $\sim 40\%$  of T1R3-expressing TCs also expressed Ob-Rb. Genetic and pharmacological disruption of Ob-Rb impaired the ability of leptin to suppress taste responses of sweet-sensitive TCs, indicating that leptin is acting through Ob-Rb on sweet-sensitive TCs. That taste responses of sour-sensitive GAD-GFP TCs and bitter-sensitive gustducin-GFP TCs were not affected by leptin is consistent with our previous results (8,9) and with our finding that GAD67-expressing Type III cells do not express Ob-Rb. We have not examined the coexpression of Ob-Rb with bitter taste receptor T2Rs, and the effects of leptin on salt and umami were not examined in this

study. We think that it is unlikely that leptin affects the responses of salt-sensitive TCs because leptin is known not to affect neural and behavioral responses to salt stimuli (8,9). Predicting the effect of leptin on umami-sensitive TCs is complicated. Umami-sensitive TCs and nerve fibers are classified into S1, S2, M1, and M2 types according to their response profiles (35–37). S1-type cells at least are plausible targets of leptin because they respond to umami and sweet stimuli, and may express all T1R subunits (37) including T1R3 (which we found to be coexpressed with Ob-Rb).

We previously found that the injection of 0.1  $\mu\text{g}/\text{kg}$  leptin into lean mice increased plasma leptin levels from 3.5 to 10 ng/mL and generated the maximum suppression of sweet taste responses ( $\sim 65\%$  of control), while further increases in plasma leptin levels did not elicit further suppression (8). Here we found that the effective concentration range at which leptin suppressed sweet responses of TCs was between 1 and 10 ng/mL, and the suppression of sweet reached a maximal level at 10–20 ng/mL plasma leptin. Even if TCs were adapted to low concentrations of leptin (e.g.,  $\leq 5$  ng/mL), further increases in leptin concentration suppressed sweet. In humans, plasma leptin



**Figure 6**—The  $K_{ATP}$  channel underlies leptin suppression of responses to sweet compounds. **A**: Triple staining for T1R3 (green, immunohistochemistry), Ob-Rb (red, ISH), and SUR1 (blue, immunohistochemistry) in FP (top panels) and VP (bottom panels) of C57BL/6J mice. Scale bars = 10  $\mu$ m. Dotted lines indicate the outline of taste buds. Quantitative data are shown in Table 2. **B**: Schematic diagram of the pattern of coexpression among T1R3, SUR1, and Ob-Rb in mouse FP and VP TCs. **C**: Sample recordings from a T1R3-GFP TC showing the effect of the  $K_{ATP}$  channel inhibitor Gc. The response of this cell to 3 mmol/L saccharin (Sac) (control) was suppressed by bath application of 20 ng/mL leptin (leptin). Bath-applied leptin along with 30  $\mu$ mol/L Gc (leptin + Gc) led to recovery of responses to saccharin. **D**: Summated effects of 30  $\mu$ mol/L Gc on leptin suppression of responses to sweet compounds in T1R3-GFP TCs.  $n = 8$ ,  $F$  value = 10.652,  $P = 0.002$ , repeated-measures ANOVA.  $*P < 0.05$ , post hoc  $t$  test. Values are means  $\pm$  SE. **E**: Dose-dependent effect of Gc on leptin (20 ng/mL) suppression of sweet responses (black rectangles,  $n = 7-8$ ). Suppression of sweet responses by leptin was affected significantly by Gc concentrations  $\geq 10$   $\mu$ mol/L. The white circle indicates sweet responses of TCs after bath application of 30  $\mu$ mol/L Gc ( $n = 8$ ) without any leptin present. Each response was normalized to the control response before application of leptin. Asterisks indicate statistically significant differences from control response (significant effect of leptin). Values are the mean  $\pm$  SE.  $*P < 0.05$ , Student  $t$  test. **F**: Sample recordings from a T1R3-GFP cell showing the effect of the  $K_{ATP}$  channel activator Dz on responses to saccharin. This cell responded to 50 mmol/L saccharin (control). Such responses were suppressed by bath application of 40  $\mu$ mol/L Dz and recovered after washout of Dz (washout). **G**: Effects of 40  $\mu$ mol/L Dz on summated sweet responses of T1R3-GFP TCs. Dz reversibly suppressed sweet responses of TCs.  $n = 8$ ,  $F$  value = 6.163,  $P = 0.012$ , repeated-measures ANOVA.  $*P < 0.05$ , post hoc  $t$  test. Values are the mean  $\pm$  SE.

levels may specifically affect sweet taste sensitivity in healthy nonobese subjects (33), and a decrease in circulating leptin levels is associated with a decrease in sweet taste thresholds during weight loss in obese females (38). Thus, plasma leptin is likely to be a key regulator of sweet

taste sensitivity in mice and humans through its actions on taste receptor cells.

It had been shown that 10 ng/mL leptin significantly increased nuclear phosphorylated signal transducers and activator of transcription 3 in cultured vagal afferent

**Table 2—Coexpression of T1R3, SUR1, and Ob-Rb in FP and VP TCs (data from 3 mice)**

	FP		VP	
	<i>n</i>	%	<i>n</i>	%
SUR1/T1R3	71/85	83.5	217/252	86.1
Ob-Rb/T1R3	33/85	38.8	104/252	41.3
SUR1/Ob-Rb	35/44	79.5	111/122	91.0
T1R3/Ob-Rb	33/44	75.0	104/122	85.2
T1R3/SUR1	71/93	76.3	217/258	84.1
Ob-Rb/SUR1	35/93	37.6	111/258	43.0
SUR1 + Ob-Rb/T1R3	33/85	38.8	102/252	40.5
SUR1 + T1R3/Ob-Rb	33/44	75.0	102/122	83.6
T1R3 + Ob-Rb/SUR1	33/93	35.5	102/258	39.5

neurons of rats on a low-fat diet, but this was not so in DIO rats on a high-fat diet, indicating that vagal afferent neurons of DIO rats become leptin resistant (39). Analogously, we found that sweet-sensitive TCs in DIO mice became leptin resistant. The plasma leptin level is highly correlated with BMI in both rodents and humans (40); therefore, obese subjects have higher plasma leptin levels than lean subjects. During the course of obesity, basal plasma leptin levels would gradually rise along with a decrease in the functional suppression of sweet taste sensitivity by plasma leptin. Then long-term adaptation to high concentrations (e.g.,  $\geq 10$  ng/mL) of leptin may elicit leptin resistance in TCs. In vagal afferent neurons of DIO rats, the expression of suppressor of cytokine signaling-3 was increased (39). Similarly, the expression of suppressor of cytokine signaling-3 may be increased in TCs of DIO mice, contributing to the development of leptin resistance.

In rodent models of DIO, compared with lean control animals, it has been reported that sweet preference is increased (41), decreased (42), or both (increased at high concentration and decreased at low concentration) (43). The present results are consistent with increased sweet preference. In humans, the relationship between sweet perception and obesity also has been investigated, but with no clear consensus (44,45). During the course of obesity, great changes in multiple biological processes, including the leptin system, are known to occur. Thus, obesity-induced changes in other factors such as the endocannabinoid system (19,46) may also contribute to alterations of sweet taste sensitivity in obese subjects.

Our data indicate that leptin suppresses sweet taste by activating  $K_{ATP}$  channels in T1R3 TCs. This is consistent with previous findings that leptin activates  $K_{ATP}$  channels in rat CRI-G1 insulin-secreting cells (23), mouse pancreatic  $\beta$ -cells (24), and rat hypothalamic neurons (25). However, the intracellular signaling pathways linking the activation of Ob-Rb to an increase in  $K_{ATP}$  channel activity in sweet-sensitive TCs is at present unclear. It was reported (47,48) that phosphoinositide 3-kinase plays an important role in the increased activity of  $K_{ATP}$  channels in CRI-G1 cells and

hypothalamic neurons. Another study (24) found that leptin inhibits phosphatase and tensin homolog deleted from chromosome 10, leading to the activation of  $K_{ATP}$  channels. In addition, recent studies (49,50) reported that leptin promotes  $K_{ATP}$  channel trafficking to plasma membrane via AMPK and cAMP-dependent protein kinase in pancreatic  $\beta$ -cells. One or more of these signaling components may also be involved in leptin suppression of sweet responses in TCs.

Leptin suppresses peripheral sweet taste sensitivities by acting on sweet-sensitive TCs. In addition, leptin influences behavioral responses to sugars and artificial sweeteners by acting on the central nervous system (51–53). Thus, sensory and central systems are likely to have coevolved complementary strategies to modulate behavior. Such effects of leptin on peripheral and central systems may contribute to the antiobesity effect of leptin.

In summary, our findings indicate that leptin suppresses taste responses of sweet-sensitive TCs via their Ob-Rb receptors and subsequent activation of  $K_{ATP}$  channels expressed in these TCs. The concentration of leptin to effectively suppress sweet was 1–10 ng/mL, a physiologically relevant range, suggesting that plasma leptin is likely to be a key regulator of sweet taste sensitivity via its actions on TCs. That leptin did not suppress sweet taste in *db/db* and DIO mice implies that this regulation is impaired by the absence of functional leptin receptors or the diet-induced development of leptin resistance. Sweet sensing is essential for the detection of calorie-rich carbohydrates. The regulation of sweet taste sensitivity by leptin may contribute to ingesting the proper amount of carbohydrate-derived calories in healthy subjects. Pathological states that alter leptin and its receptor, such as metabolic syndrome and diabetes, may disturb the normal regulation of sweet taste sensitivity, which in turn would alter food intake and glucose homeostasis in animals and humans. We speculate that  $K_{ATP}$  channels present in TCs provide an alternative target to regulate sweet taste sensitivity; therefore, food intake and glucose homeostasis can be maintained even though the leptin system has been impaired.

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