Leptin increases temperature-dependent chorda tympani nerve responses to sucrose in mice

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Leptin receptors are present in taste buds and previous research indicates that leptin administration modified electrophysiological and behavioral responses to sweet taste. It is now known that sweet taste is temperature dependent. We examined the influence of (1) stimulus temperature on chorda tympani (CT) nerve responses to sucrose, saccharin and NH4Cl; and (2) leptin administration on CT nerve responses to sucrose, saccharin and other basic taste stimuli at 35 °C that maximized sweet-taste sensitivity in C57BL/6 mice. We found that the CT nerve responded with greater magnitude to sucrose and saccharin as stimulus temperature increased from 23 to 35 °C and then declined at higher temperatures. In contrast, the CT nerve responses to NH4Cl increased in magnitude as temperature increased from 23 to 44 °C. We also showed that leptin selectively increased the CT nerve responses to sucrose at 35 °C in both fasted and free-fed mice. The responses of mice treated with the saline vehicle did not change. Our findings are consistent with the notion that leptin binds with its receptors in fungiform taste buds and alters the message conveyed by sugar-responsive neurons to the brain.

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1. Introduction

The adipose-derived hormone leptin is well known for the key role it plays in suppressing consumption and increasing energy expenditure by its action on leptin receptors in critical hypothalamic nuclei [1,2]. Less well studied is the role of leptin in altering taste function. Kawai, et al. [3] were the first to study the influence of leptin on taste. They showed that intraperitoneal administration of leptin into fasted mice reduced the integrated responses of the chorda tympani (CT) and glossopharyngeal (GL) nerves to ≈24 °C sucrose and saccharin, but not to NaCl, HCl, or QHCl. They showed further in patch-clamp studies of isolated taste receptors cells from circumvallate papillae that leptin activated outward potassium currents and hyperpolarization occur only in taste cells with the machinery for sweet taste transduction.

Sweet-taste transduction involves two G protein-coupled receptors, T1R2 and T1R3, which dimerize to form the sweet receptor responsive to a broad range of sugars and artificial sweeteners [6,7]. There is also a T1R-independent mechanism for sweet-taste transduction involving glucose transporters [8,9]. Many studies, including our own, have shown that sweet taste sensitivity is temperature dependent [10,11]. It has been shown that a member of the transient receptor potential (TRP) superfamily of ion channels, TRPM5, underlies the temperature-dependent nature of sweet taste [12–14]. TRPM5 is a Ca2⁺- and voltage-activated nonselective cation channel that is a working component downstream from the G-protein-coupled sweet taste receptor, T1R2 and T1R3 [6,15–18], and is thought to amplify the taste signal by facilitating taste cell depolarization. In fact, TRPM5 knockout (KO) mice have greatly reduced, but not abolished responses of the CT nerve to sweet compounds, suggesting the existence of TRPM5-dependent and TRPM5-independent transduction pathways for sweet taste [7,19]. In addition, TRPM5 is a heat-activated channel whose activity has been shown to increase between 15° and 35 °C [14,20].

To date, the only electrophysiological study that has investigated the influence of leptin on taste nerve responses was done using chemical stimuli delivered at room temperature (≈24 °C) to the lingual taste buds [3]. This study was performed under less than optimal conditions before the influential role of temperature and TRPM5 on sweet taste was thoroughly appreciated. We have had a long-standing interest in how temperature influences neural responses to chemical stimulation of lingual receptors [10,21–23]. Because of this interest, we have adopted a stimulus delivery system with which we can control the temperature of the stimulus solutions over a wide range...
temperature range. Accordingly, we conducted the present study to serve two main purposes. Our first purpose was to confirm and extend prior work investigating the influence of temperature on sweet taste using our stimulus delivery system in mice. We examined the influence of stimulating temperature on the CT nerve responses to sucrose, saccharin, and NH4Cl from 23 to 44 °C in C57BL/6 mice. Our second purpose was to extend prior work investigating the influence of leptin administration on the CT nerve responses to sucrose, saccharin and other basic taste stimuli at 35 °C that maximized sweet-taste sensitivity.

2. Materials and general methods

2.1. Subjects

Adult male C57BL/6 (Jackson Laboratory, Bar Harbor, ME) mice weighing 26–32 g at the start of the experiments were housed individually in transparent plastic cages in a temperature-controlled colony room (22 °C–24 °C) and maintained on a 12:12-h light–dark cycle with lights on at 7:00 am. All the animals were habituated to the animal facility for at least 1 week before CT recording or behavioral testing. All mice had ad libitum access to Purina Rat Chow (no. 5001) and de-ionized water unless otherwise indicated. The Institutional Animal Care and Use Committee at Florida State University approved all procedures.

2.2. Electrophysiological recordings of taste responses of the mouse chorda tympani

The mice were anesthetized with intraperitoneal administration of ketamine (30 mg/kg body weight) followed by urethane (1.2 g/kg). Supplemental urethane injections were given to maintain a deep level of anesthesia without reflex response to foot pinch. The mice were tracheotomized with PE50 tubing, and secured in a non-traumatic head holder (Model 926B Mouse Nose/Tooth Bar Assembly, David Kopf Instruments, Tujunga, CA). Using a mandibular approach, the right CT branch of the facial nerve was exposed and transected where it enters the tympanic bulla. The CT nerve was desheathed, and placed on a platinum wire electrode (positive polarity) and the entire cavity was then filled with high quality paraffin oil (VWR) to isolate the nerve signal from ground and maintain nerve integrity. An indifferent electrode (negative polarity) was attached to the skin overlying the cranium with a tinned-copper alligator clip. Neural activity was differentially amplified (X10,000; A-M Systems, Sequim, WA, bandpass 300–5000 Hz), observed with an oscilloscope, digitized with waveform hardware and software (Spike 2; Cambridge Electronic Design, Cambridge, England), and stored on a computer for off-line analysis.

2.3. Chemical stimuli and solution delivery

The tongue was slightly extended and held in place by withdrawing the thread, sutured to the tongue’s ventral surface, and securing it to the grounding table. Solutions were presented to the anterior tongue at a constant flow rate (50 μl/s) and controlled temperature [24,25] by an air-pressure 32-channel commercial fluid-delivery system and heated perfusion cube (OctaFlow Multi-function Multi-valve Perfusion System, ALA Scientific Instruments, Farmingdale, NY). All solutions were made from reagent-grade chemicals and dissolved in a dilute salt mixture (0.015 M NaCl, 0.022 M KCl, 0.003 M CaCl2, and 0.0006 MgCl2) of artificial saliva [26]. We recorded CT nerve responses to 10-s applications of each taste stimulus. Each stimulus was followed by a rinse of artificial saliva (AS) for 60–90 s to ensure that nerve activity returned to stable baseline levels. Amplified nerve activity was monitored on-line, digitized using Spike 2, and integrated with a root mean square (RMS) calculation with a time constant of 200 ms. The average baseline neural activity immediately preceding each chemical stimulus presentation was subtracted from the integrated response resulting from the 10-s stimulus to calculate the area under the curve (AUC).

3. Experiment 1

In this experiment, we tested the influence of temperature on CT nerve responses to a sucrose concentration series and to a single concentration of sodium saccharin and NH4Cl.

3.1. Subjects and taste protocol

Six mice were used to investigate the effects of different temperatures on CT nerve responses to sweet stimuli. For each mouse, we recorded CT nerve responses to a sucrose concentration series (0.1, 0.3, 0.6 and 1.0 M sucrose) and to 0.02 M sodium saccharin and 0.1 M NH4Cl at eight different temperatures in 3 °C intervals from 23 to 44 °C. To control for individual differences among preparations, we also applied 0.6 M NaCl periodically during the recording session. The AUC responses to the sweet stimuli and NH4Cl were normalized to the average response to 0.6 M NaCl for each animal. We used 0.6 M NaCl for normalization in this experiment because our preliminary tests indicated that CT nerve responses to NaCl stimuli were least affected by differences in stimulus temperature. The response to 0.6 M NaCl was also used to evaluate the stability of the preparation. A recording was considered stable when the responses to 0.6 M NaCl at the beginning and at the end of each stimulation series deviated by no more than 15%. Only responses from stable recordings were used for data analysis.

3.2. Data analysis

All data are presented as group means±SEM. Differences between the responses at different temperatures were performed using two-way and one-way repeated measure ANOVA followed by post hoc pairwise comparisons using contrast coefficients. All analyses were performed using Statistical Program for Social Sciences software (SPSS 13.0) with statistical significance accepted when P<0.05.

3.3. Results

A two-way repeated measures ANOVA showed that the CT nerve responses to sucrose varied significantly as a function of stimulus concentration [F(1.25,189.75) = 136.90, P<0.001] and stimulus temperature [F(1.61,189.39) = 4.67, P<0.01]. As depicted in Fig. 1A, the average sucrose response was maximum at 35 °C across all stimulus concentrations. There was a relatively sharp decline in response magnitude at both lower and higher stimulus temperatures for the three strongest sucrose concentrations and a more modest decline for the weakest sucrose concentration. This is evident from the graphs as well as from post hoc comparison tests. For 0.1 M sucrose, the response magnitude at 35 °C was significantly greater than that at 23 °C (P<0.01), 26 °C (P<0.01), 41 °C (P<0.01) and 44 °C (P<0.05). For 0.3 M sucrose, the response at 35 °C was significantly greater than that at 23 °C, 26 °C, 29 °C, 32 °C, 38 °C, 41 °C and 44 °C (all P-values<0.05). Similarly for 0.6 M sucrose, the average response at 35 °C was significantly greater than that at 23 °C, 26 °C, 29 °C, 32 °C, 38 °C, 41 °C and 44 °C (all P-values<0.05). For 1.0 M sucrose, the average response at 35 °C was significantly greater than that at 23 °C, 26 °C, 41 °C and 44 °C (all P-values<0.05).

As shown in Fig. 1B, temperature had a similar effect on CT nerve responses to 0.02 M saccharin as sucrose. The average response to sodium saccharin was maximum at 35 °C and response magnitude declined at lower and higher temperatures [F(1.49,45.51) = 5.28, P<0.05]. Post hoc comparison tests showed that the average response
at 35 °C was significantly greater than that at 23 °C, 26 °C, 29 °C, 41 °C, and 44 °C (all P-values <0.05).

As shown in Fig. 1C, temperature had a completely different pattern of influence on responses to 0.1 M NH₄Cl than on responses to sucrose and sodium saccharin. The responses of the CT nerve to NH₄Cl increased progressively as temperature increased from the lowest to the highest temperature \([F(2.38,44.62)=34.83, P<0.001]\). Post hoc comparisons revealed that the average response to NH₄Cl at 35 °C was significantly greater than that at 23 °C \([F(1,10)=38.56, P<0.01]\), 26 °C \([F(1,10)=49.87, P<0.001]\), 29 °C \([F(1,10)=16.71, P<0.01]\), and 32 °C \([F(1,10)=48.39, P<0.001]\), but less than that at 41 °C \([F(1,10)=19.30, P<0.01]\) and 44 °C \([F(1,10)=40.27, P<0.01]\).

4. Experiment 2

In this experiment, we tested the influence of peripheral leptin administration on CT nerve responses to chemical stimulation of the tongue at 35 °C.

4.1. Subjects

Twenty-eight mice were divided into four equal groups of 7 animals each. Two free-fed groups were maintained on unrestricted laboratory chow and water before CT nerve recording and during testing received intraperitoneal administration of either leptin (free-fed leptin) or vehicle (free-fed saline). Two fasted groups were given unrestricted access to water and deprived of food for 24 h before CT nerve recording and during testing received either leptin (fasted leptin) or vehicle (fasted saline). Leptin (The National Hormone and Peptide Program, NHPP) was dissolved in PBS (pH 8.0) at a concentration of 200 mg/dl. Mice in the leptin groups (free-fed and fasted) received recombinant leptin at a dose of 100 ng/g body weight while mice in the saline groups received saline.

4.2. Taste stimuli protocol

Chorda tympani recording and chemical stimulation procedures followed the same methods as outlined in Experiment 1. In this...
study, we recorded CT nerve responses to a sucrose concentration series (0.1, 0.3, 0.6 and 1.0 M sucrose), a NaCl concentration series (0.1, 0.3, 0.6 and 1.0 M NaCl) and to 0.01 M citric acid, 0.02 M sodium saccharin, 0.02 M QHCl, and 0.1 M monosodium glutamate (MSG). To control for individual differences among preparations, we also applied 0.1 M NH₄Cl periodically during the recording session. The AUC responses to all stimuli were normalized to the average response to 0.1 M NH₄Cl for each animal. Taste solutions and rinse with AS were applied at 35 °C. The complete stimulus protocol was presented twice, once before and 30 min after the mice were treated with either leptin or saline.

4.3. Effect of leptin on food intake

We determined whether the leptin dose used for CT nerve recording also altered food intake in mice.

4.4. Subjects

Twelve naïve male mice were divided into two equal groups of 6 animals each. The mice in both groups had unrestricted access to water and laboratory chow and food intake and body weight were measured daily at 6:00 PM. The animals were adapted to this schedule for four consecutive days. After the measurement period on day 4, mice in the leptin group received intraperitoneal administration of leptin at a dose 100 ng/g body weight, while mice in the saline group received saline. Twenty-four hours after the injection, daily food intake was measured again.

4.5. Data analysis

All data are presented as the mean±SEM. We analyzed the differences in the CT responses before and after the drug injection using a two-way, repeated measures ANOVA followed by post hoc pairwise comparisons using contrast coefficients. Mauchly’s test of sphericity was used to assess homogeneity of variance between treatment groups [27], and if sphericity was violated, Greenhouse–Geisser corrections were applied. The differences in food intake between leptin- and saline-treated groups on day 5 were compared by an independent- t test. All analyses were performed using the Statistical Program for Social Sciences software (SPSS 13.0) with statistical significance accepted with \( P<0.05 \).

4.6. Results

For the free-fed leptin group, a two-way repeated measures ANOVA with Greenhouse–Geisser correction revealed that CT responses to sucrose varied significantly as a function of stimulus concentration \( [ F(2.2,44.8)=103.77, P<0.001 ] \) and treatment condition \( [ F(1,46)=13.88, P<0.01 ] \). As shown in Fig. 2A, responses to sucrose increased predictably with concentration. In addition, CT nerve responses to sucrose were overall greater 30 min after leptin treatment than before treatment. Post hoc analyses showed that CT responses to 0.1 M \( [ F(1,12)=14.85, P<0.01 ] \) and 0.3 M \( [ F(1,12)=25.14, P<0.01 ] \) were greater following leptin. Similar to sucrose, CT nerve responses to NaCl responses also varied significantly with stimulus concentration \( [ F(1,64.5)=179.37, P<0.001 ] \). However in contrast to sucrose, the responses to all NaCl concentrations, citric acid, saccharin, QHCl and MSG (C) in free-fed C57BL mice before and after leptin treatment. On the left side are raw electrophysiological traces from the CT nerve and on the right the average responses from 7 mice/group. **Significantly greater after leptin treatment; \( P<0.01 \).
acid, saccharin, QHCl and MSG remained unchanged after leptin treatment (see Fig. 2B and C).

For the free-fed saline group, there was a similar pattern of CT nerve responses to all the chemical stimuli—high responsiveness to sucrose and NaCl that increased with stimulus concentration, and modest responses to the other stimuli. As expected, the CT responses to all stimuli were similar before and after saline treatment (data not shown).

For the fasted leptin group, a two-way repeated measures ANOVA with Greenhouse-Geisser correction found that the CT responses to sucrose varied significantly as a function of stimulus concentration \( F(1.8,45.2) = 93.50, P < 0.001 \) and treatment condition \( F(1,46) = 25.40, P < 0.01 \). As shown in Fig. 3A, responses to sucrose increased with stimulus concentration and responses to sucrose were greater 30 min after than before leptin. Post hoc analyses showed that the CT responses to 0.3 M \( F(1,12) = 38.71, P < 0.001 \), 0.6 M \( F(1,12) = 17.50, P < 0.01 \) and 1.0 M \( F(1,12) = 38.89, P < 0.001 \) sucrose were significantly greater following leptin. As shown in Fig. 3B, the CT responses to NaCl also varied significantly as a function of stimulus concentration \( F(1,3,45.7) = 195.71, P < 0.001 \) and were similar before and after leptin treatment. Similarly, the responses to citric acid, sodium saccharin, QHCl, and MSG remain unchanged after leptin treatment (see Fig. 3C).

For the fasted saline group, there was a similar pattern of CT nerve responses to all the chemical stimuli—high responsiveness to sucrose and NaCl that increased with stimulus concentration, and modest responses to the other stimuli. As expected, the CT responses to all stimuli were similar before and after saline treatment (data not shown).

As can be seen in Fig. 4A, the average food intake values for the two groups of mice were similar for the 4 days preceding leptin or saline treatment. We calculated the average daily food intake over the 4 days preceding the drug injection for the two groups. An independent-t test indicated that there was no difference in baseline food intake between leptin and saline groups. However, after the injection on day 4, the average 24-h food intake of the saline group increased while that of the leptin group decreased. This resulted in a significant group difference in food intake \( t = -3.14, P < 0.05 \). In fact, 6/6 saline-injected mice increased their food intake on day 5 compared to day 4 of testing, whereas 5/6 leptin-injected mice decreased their food intake.

![Fig. 3](image1.png)

![Fig. 4](image2.png)
5. Discussion

In Experiment 1, we found that the CT nerve responses to the four different sucrose concentrations peaked at 35 °C and declined at both higher and lower temperatures. This was most apparent for 0.6 M sucrose with the response to it at 35 °C being significantly greater than that to all other temperatures. For the 3 other sucrose concentrations, the CT responses at intermediate temperatures (32–38 °C) were similar to each other and significantly reduced at the lowest (23 and 26 °C) and highest (41 and 44 °C) temperatures. This overall pattern shown in the present study is consistent with prior research showing sweet taste's dependence on temperature [28,29]. We adopted 0.6 M NaCl responses as our standard, because as shown in a previous study as well as based on our preliminary studies, NaCl responses were least affected by temperature [30]. This is most likely due to the temperature insensitivity of the epithelial sodium channel (ENaC), which mediates a significant portion of the NaCl response in the rat CT [10,31].

The temperature dependence on taste varied with the modality of the stimuli. In contrast to sucrose with peak responses at 35 °C, the CT responses to NH$_4$Cl increased with increasing temperature with the weakest response at 23 °C and largest response at 44 °C. As far as we know, this is the first study to show the influence of temperature on CT responses to NH$_4$Cl in mice, a finding consistent with results from a previous study in rats [22,29]. Despite its common use as a standard stimulus in peripheral nerve recordings, temperature's influence on CT responses to NH$_4$Cl may make it a questionable choice as a standard for studies involving the study of taste-temperature interaction.

The most dominant finding of Experiment 2 was that acute leptin administration enhanced CT responses to several sucrose concentrations at 35 °C in both free-fed and fasted mice, without affecting the responses to other basic taste stimuli. The responses to a broad range of NaCl concentrations, citric acid, saccharin, quinine, and MSG were unaffected by leptin administration. It is important to note that the leptin dose (100 ng/g body weight) used in our CT studies suppressed the 24-h food intake of 6 fasted mice compared to 6 fasted vehicle-treated controls, and leptin's effect on CT responses was more pronounced in fasted than free-fed mice. Finally, the CT responses to all stimuli before leptin administration were the same between fasted and free-fed mice. This finding indicates that food restriction alone does not alter CT responses to chemical stimulation of fungiform taste buds.

The present results on leptin were opposite to the results from a previous study showing that leptin reduced CT nerve responses to sucrose and saccharin [3]. There were several methodological differences between the two studies that may have played a role in the outcome. For example, the two studies used different strains of C57 mice. The previous study used BABL/c and C57BL/KsJ-db mice, while we used C57BL6 [3]. Additionally, the two studies differed in anesthetic (sodium pentobarbital vs urethane), stimulation procedure (gravity flow system with tongue enclosed in a flow chamber vs OctaFlow system over an open tongue), and response measure (amplitude of integrated response at single time point vs area under curve over entire stimulation period). The two studies also differed in two other ways known to directly influence to sweet taste sensitivity. In the previous study, water was the solvent and rinse solution for stimuli delivered at 24 °C, whereas in the present study artificial saliva was the solvent and rinse solution for stimuli delivered at 35 °C. Artificial saliva and temperature have both been shown to increase CT nerve responses to sucrose [28,32]. While all of the methodological differences outlined above may have contributed to the discrepant leptin finding, we suspect that stimulus temperature may be the most important factor.

Many studies, including our own, have shown that sweet taste sensitivity is temperature dependent [10,11]. After the original study of leptin on CT nerve responses [3], subsequent research showed that the temperature sensitive heat-activated channel TRPM5 co-expressed with sweet taste-signaling molecules [33–35]. In addition, TRPM5 mediated the temperature-dependent nature of sweet taste [13,14]. It was concluded that sweet taste signaling relies on both TRPM5-dependent and TRPM5-independent transduction pathways [7,19]. The activity of TRPM5 increased between 15° and 35 °C [14,20], thus, it was not surprising that we found the CT nerve responses to increased magnitude as the temperature of sucrose increased from 23 °C to 35 °C. The CT nerve responses to sucrose were significantly lower, however, at 41 °C and 44 °C indicating that these temperatures were beyond the optimal range for TRPM5 activity. The previous study investigated leptin's influence on sweet taste when TRPM5 was relatively inactive (TRPM5-independent) at 24 °C [3], while in the present study sweet taste sensitivity was studied under optimal TRPM5-dependent conditions at 35 °C. Although we don't know exactly how leptin enhances the CT responses to 35 °C sucrose, it likely involves the TRPM5-dependent pathway. Based on work from recent studies, we suspect that leptin binds to leptin receptors on TRC [4,5] thereby increasing cation conductance leading to depolarization [36–40] and increased intracellular calcium concentration [41], which then promotes TRPM5-dependent sweet taste signaling.

As mentioned earlier, we used artificial saliva containing all the major ions normally found in saliva as the rinse solution and solvent for all the chemical stimuli. Saliva is a critical factor impacting taste sensitivity [42,43]. The ionic conditions surrounding the taste cells are essential in taste transduction. Matsumi and Yamamoto showed that under artificial saliva-adapted conditions, the CT nerve responses to sucrose were significantly greater than those obtained when the tongue was adapted to distilled water [32]. Similarly, Breza, et al. found a threefold higher number of sucrose-sensitive neurons in rats adapted to 35 °C artificial saliva compared to rats adapted to room temperature deionized water [44]. Accordingly, artificial saliva is especially important for maintaining chemical sensitivity and response stability to sucrose in mice during long recording periods both before and 30 min after leptin or saline administration.

In conclusion, our study found that leptin administration increased the CT nerve responses to 35 °C sucrose stimuli in both free-fed and fasted mice, which was also proved to be the optimum temperature for sweet taste in mice. This finding contrasts with results from an earlier report that found that leptin decreased CT responses to 24 °C sucrose [3]. There were many methodological differences between the studies, but stimulus temperature and adapting rinse are two factors critically important to sweet taste signaling and most likely contributed to the dissimilar findings. The fact that the two studies used different strains of mice is another likely factor that may have played a role, as well. With respect to the present findings, we propose that leptin binds to leptin receptors on TRC and augments the activity of the TRPM5 channel at 35 °C. In doing so, leptin enhances the intensity signal conveyed by sugar-responsive neurons to the brain, presumably making sucrose taste more sweet and energy-rich. Consequently, intake declines in the service of energy balance and prevention of overfeeding.

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