Effects of Dietary Biotin on Enhanced Sucrose Intake and Enhanced Gustatory Nerve Responses to Sucrose Seen in Diabetic OLETF Rat

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Summary We used the sucrose preference test and taste nerve recording to investigate the effect of dietary biotin on the abnormal sucrose taste sensitivity and preferences seen during the course of diabetes mellitus. For this, we used Otsuka Long-Evans Tokushima fatty (OLETF) rats. The chorda tympani nerve (CT nerve) response to sucrose (> 1 M) was of greater relative magnitude in OLETF rats than in non-diabetic control (Long-Evans Tokushima Lean, LETO) rats, but the responses to other basic taste stimuli (such as HCl, quinine-HCl and L-glutamic acid) did not differ between the two groups. In behavioral experiments using a two-bottle preference test, solution intake for sucrose (> 50 mM) was higher in OLETF rats than in LETO rats. The neural responses to sucrose (1.5–2 M) in OLETF rats were lower when given a biotin-high diet (BH-OLETF) than when given a biotin-basal diet (BB-OLETF), but this was not true of the other basic tastes. However, there were no significant differences between BH-OLETF and BB-OLETF rats in terms of sucrose solution intake. These findings suggest that the enhanced sugar sensitivity observed in OLETF rats is probably the result of a genetic difference between OLETF and LETO rats, though the discrepancy can be modified by the dietary biotin level.

Key Words biotin, non-insulin-dependent diabetes mellitus (NIDDM), taste sensitivity to sucrose, taste preference, chorda tympani nerve

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Alterations in taste sensitivity and taste preferences occur during the course of diabetes mellitus (1, 2). Schelling et al (1) demonstrated that a specific impairment in the detection of sucrose and glucose, but not of other tastes, could be found in some diabetic patients. This finding suggests the possibility that some alteration in taste receptors may take place in certain diabetic states.

In a behavioral and neurophysiological study using mice, Ninomiya et al (3) found that chorda tympani (CT) nerve responses to four sugars (sucrose, glucose, fructose, and maltose) were of greater relative magnitude and of lower threshold in the diabetic db/db mouse than in the adult lean mouse. However, this was not true of the responses to other basic tastes. In the behavioral experiments in the same study (3), a two-bottle preference test demonstrated that taste preference scores for the four sugars at suprathreshold concentrations, except 1.0M, were higher for db/db mice than the control mice.

The Otsuka Long-Evans Tokushima fatty (OLETF) rat serves as a spontaneously diabetic genetic model for non-insulin-dependent diabetes mellitus (NIDDM). Five gene markers in the same linkage group (Es-2, 3, 8, 9, 10) in OLETF rat are different from those of the lean control (Long-Evans Tokushima Lean, LETO) rat, and cause OLETF rats to exhibit diabetes with hyperglycemia and mild obesity (4). Our recent study (5) demonstrated that the administration of a high concentration of dietary biotin (7.44mg/kg diet) to OLETF rats decreased the plasma glucose level, insulin secretion, and body weight gain without inducing a decrease in food consumption. This observation suggests that biotin may improve the glucose metabolism in this diabetic rat.

In this study, we recorded the responses of the CT nerve to various taste stimuli for OLETF rats, and compared these responses (i) to those seen in LETO rats, and (ii) OLETF rats fed a high concentration of dietary biotin.

MATERIALS AND METHODS

Experimental animals. Male OLETF and LETO rats were obtained from Tokushima Research Laboratories, Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Both strains originated from the same colony of Long-Evans rats. Each rat was individually housed in a stainless-steel cage in an air-conditioned room (24±2°C) with 50% humidity. The room was illuminated from 8:00 to 20:00h. The feeding regimen for the OLETF and LETO rats followed the method of Zhang et al (5) with slight modifications. In brief, all animals (10 OLETF and 5 LETO) were kept on a basal diet (Table 1) from 5 to 13 weeks of age. Then, the OLETF rats were given one of two biotin diets—a basal-biotin diet (BB) or a high-biotin diet (BH)—from 13 to 22 weeks of age. All LETO rats were given the basal-biotin diet for the latter period.

Measurement of plasma glucose levels and insulin levels in OLETF and LETO rats. Before the start of the behavioral and neurophysiological experiments (i.e., at 22 weeks of age), the plasma glucose level in each rat was measured using a
Table 1. Composition of experimental diet.

| Ingredient                        | Amount (%) |
|-----------------------------------|------------|
| Sucrose                           | 60         |
| Raw egg white<sup>1</sup>         | 20         |
| Soybean oil<sup>2</sup>           | 6          |
| Salt mixture<sup>3</sup>          | 6          |
| Cellulose powder                  | 6          |
| Vitamin mixture<sup>4</sup>       | 2          |

<sup>1</sup>Spray-dried egg white protein from Q.P. Corporation, Japan.
<sup>2</sup>Wako Pure Chemical Co., Ltd., Osaka, Japan.
<sup>3</sup>Oriental’s Salt Mixture, Oriental Yeast Co., Ltd., Japan.
<sup>4</sup>One of two kinds of vitamin mixture according to the intended biotin level of diet: biotin-basal or biotin-high<sup>a</sup> level diet.

* Biotin content of experimental diets.

| Biotin-basal diet (BB) | Biotin-high diet (BH) |
|------------------------|-----------------------|
| Biotin (mg/kg diet)    | 1.44<sup>a</sup>      | 7.44<sup>b</sup> (BB + 6) |

<sup>a</sup>One kilogram of the biotin-basal diet contains 200g spray-dried egg white. Two hundred grams of the egg white contains 100mg avidin (1.47¿mol), which binds to 5.89¿mol of biotin (1.44mg).
<sup>b</sup>Biotin-high diet (BH) contains 6mg/kg more biotin than the biotin-basal diet (BB).

standard glucose oxidase method (6) (Glucose C-II test kit, Wako Pure Chemical Co., Ltd., Osaka, Japan). The immunoreactive insulin level in the plasma was determined with the aid of a Shionogi Insulin RIA kit (Shionogi Co., Ltd., Osaka, Japan) which employs the double-antibody method of radioimmunoassay (7).

Behavioral experiment. The design of the behavioral test was derived from the experimental method developed by Torii (8) for assessing taste preference. The experimental groups were divided into BB-OLETF rats (n = 5), BH-OLETF rats (n = 5), and BB-LETO rats (n = 5). The 5 animals in each group were housed together in a stainless-steel, wire-bottom cage from 22 weeks to 26 weeks of age. Two bottles were set up on each cage, and the rats were allowed a free choice of solution. One bottle contained the test solution and the other contained distilled water. The tastant solutions were prepared by the method of Deems and Friedman (9). The tastant used was sucrose (0.005, 0.025, 0.05, 1, 2M), and solutions were prepared by dissolving reagent grade sucrose in distilled water. The five different sucrose solutions were presented in ascending order on consecutive days with the whole series being presented five times (i.e., 25d in all). Solution intake for each rat was taken as the
average of the five daily solution intakes. The average value for the group was then calculated for each sucrose solution.

**Electrophysiological experiment.** In preparation for recording from the CT nerve, each rat was deeply anesthetized with an initial dose of sodium pentobarbital (50 mg/kg) and urethane (150 mg/kg), with supplemental doses throughout the experiment as needed. After a tracheotomy, the rat was positioned on its right side with the head held stationary by a non-traumatic head-holder. Surgical exposure of the CT nerve was achieved via an incision in the cheek below the level of the zygomatic arch, overlying muscles being dissected away to expose the mandible. After disarticulation of the temporo-mandibular joint, the condyloid and coronoid mandibular processes and the zygomatic arch were removed with rongeurs. Beneath the pterygoid muscles, a group of nerves can be seen, the smallest of which is the CT nerve.

The CT nerve was cut as close to the maxilla as possible. Following the removal of the perineural sheath, the nerve was placed on a Pt/Ir electrode under fluorohydrocarbon oil. Extracellular action potentials were recorded differentially, with one electrode being placed at the edge of the wound (10).

In the analysis of whole-nerve responses, the peak height of the integrated response was measured. The relative response magnitude for each stimulus was then calculated, with the magnitude of the response to 0.1 M NaCl being taken as unity (1.0). This relative response was used for the statistical analysis (11). Data were compared using one-way ANOVA followed by a post hoc l.s.d. test when appropriate.

**RESULTS**

**Plasma glucose levels and insulin levels in OLETF and LETO rats**

Figure 1 shows the plasma insulin and glucose levels in OLETF and LETO rats at 22 weeks of age. The plasma glucose levels (mean±SD) were 16.5±1.4 mg/L in BB-OLETF rats, 13.2±1.0 mg/L in BH-OLETF rats, and 12.3±0.4 mg/L in BB-LETO rats (Fig. 1a). There was a significant difference between BB-OLETF and BH-OLETF rats (p<0.01), and between BB-OLETF and BB-LETO rats (p<0.01), but not between BH-OLETF and BB-LETO rats.

As shown in Fig. 1b, the BB-OLETF rats had a significantly higher insulin level (82.8±17.0 μU/mL) than did the BH-OLETF (31.1±8.0 μU/mL) or BB-LETO (31.3±7.1 μU/mL).

**Behavioral experiment**

Figure 2 shows the concentration-intake relationships for sucrose solutions in BB-OLETF, BH-OLETF, and BB-LETO rats. At sucrose concentrations of 50 mM or more, the solution intake per 100 g body weight was significantly greater for BB-OLETF rats than for BB-LETO rats. However, there were no significant differences between these groups at concentrations of 0.025 M or less (Fig. 2a). In

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Fig. 1. Effect of dietary biotin on plasma glucose (a) and plasma insulin (b) levels in OLETF and LETO rats. In OLETF rats: OLETF-BB, biotin-basal diet group; OLETF-BH, biotin-high diet group. In LETO rats: LETO-BB, biotin-basal diet group. Results are expressed as mean±SD: a p<0.01 (n=5) versus control (BB-LETO) rats; b p<0.01 (n=5) versus BH-OLETF rats (one-way ANOVA).

Fig. 2. Concentration-solution intake relationships for sucrose in BB-OLETF and BB-LETO rats (a), and BB-OLETF and BH-OLETF rats (b). Solution intake (mL/100 g B.W./day) data are shown as means±SD. Data were obtained from 5 BB-OLETF, 5 BH-OLETF, and 5 BB-LETO rats. a p<0.05, b p<0.01 versus BB-OLETF (○) rats (one-way ANOVA).

the comparison between BB-OLETF and BH-OLETF rats, there was a significant difference only at 0.025 M, although the actual difference was quite small (Fig. 2b).

Electrophysiological experiment

Figure 3 shows integrated responses of the whole CT nerve to 0.1 M NaCl and
Fig. 3. Typical examples of integrated responses of the whole chorda tympani nerve to 0.1 M NaCl and various sucrose solutions. After each stimulation (shown by the upward arrow), and when the response had begun to plateau or decline, the tongue was rinsed with de-ionized water for 20–30 s. Off-responses (sharp peaks) appearing just after water rinse (shown by the downward arrow) were not counted as part of the response to sucrose. The time constant of the integrator was 5.6 s.

to sucrose solution of various concentrations. The responses to sucrose solutions of 1.0 M or more were of much greater magnitude in BB-OLETF rats than in BB-LETO rats. As can be seen in Table 2, in terms of relative magnitude, the response to 1.0 M sucrose was 1.81 times greater in BB-OLETF rats than in BB-LETO rats; this difference was statistically significant ($p<0.02$). In contrast, no such difference was observed for L-glutamic acid, HCl, or quinine HCl solutions. Moreover, the response to KCl solution was smaller in BB-OLETF rats than in BB-LETO rats ($p<0.05$). In terms of their CT nerve responses, there were no significant differences between BB-OLETF and BH-OLETF rats, as shown in Table
Table 2. Integrated CT nerve responses to various taste stimuli.

| Stimuli       | BB-LETO | BB-OLETF | BH-OLETF |
|--------------|---------|----------|----------|
| 0.1 mM NaCl  | 1.0     | 1.0      | 1.0      |
| 0.01 mM HCl  | 0.62 ± 0.02 | 0.78 ± 0.20 | 0.75 ± 0.11 |
| 0.1 mM KCl   | 0.45 ± 0.08 | 0.28 ± 0.08* | 0.39 ± 0.12 |
| 0.01 mM L-Glu| 0.27 ± 0.05 | 0.35 ± 0.12 | 0.33 ± 0.05 |
| 0.02 mM quinine | 0.41 ± 0.10 | 0.49 ± 0.09* | 0.56 ± 0.19 |
| 1.0 mM sucrose | 0.31 ± 0.08 | 0.55 ± 0.09** | 0.44 ± 0.09 |

Values are means ± SD, the integrated response to each stimulus being expressed relative to the response to 0.1 mM NaCl; 5 adult rats (26 wk of age) were used in each group. *p < 0.05, **p < 0.02 versus BB-LETO rats (control).

Fig. 4. Concentration-response relationships for effect of sucrose on integrated activity of whole chorda tympani (CT) nerve. BB-OLETF and BB-LETO rats (a), and BB-OLETF and BH-OLETF rat (b). Relative responses are shown as mean ± SD. Data were obtained from 5 BB-OLETF, 5 BH-OLETF, and 5 BB-LETO rats. *p < 0.05, †p < 0.02, ‡p < 0.01 versus BB-LETO rats (one-way ANOVA).

2 (in which the only sucrose concentration shown is 1.0 mM).

Concentration-response relationships for the effects of sucrose on CT nerve activity in BB-OLETF, BH-OLETF, and BB-LETO rats are shown in Fig. 4. At the higher concentrations tested (over 0.5 mM), the responses to sucrose were significantly larger (in terms of relative magnitude) in BB-OLETF rats than in BB-LETO rats (p < 0.02). However, there were no significant differences between these groups at concentrations of 0.5 mM or less.

As shown in Fig. 4b, the response to sucrose tended to be lower in BH-OLETF rats than in BB-OLETF rats, although the difference reached statistical significance only at concentrations over 1.0 mM; one-way ANOVA, p < 0.02 (1.5 mM), p < 0.05 (2.0 mM).
DISCUSSION

The diabetic signs of the OLETF rat

In this study, we observed two novel signs of diabetes in genetically diabetic (OLETF) rats. The first sign of diabetes was seen when recording the whole CT nerve responses of OLETF rats: they showed greater relative responses to 1–2M sucrose than did the nondiabetic (LETO) rats. However, the OLETF rats did not differ from the LETO rats in their responses to other taste substances (HCl, quinine-HCl, or glutamic acid), and the former's response to KCl was actually reduced. This suggests a specific increase in the response to sucrose in the OLETF rat. This result is consistent with Ninomiya's neurophysiological data (3), although we observed a difference between BB-OLETF and BB-LETO rats in terms of response magnitude only at the higher sucrose concentrations (1 M or more) (Fig. 3a).

The second sign of diabetes was that OLETF rats had a greater intake of sucrose solution (0.05 M or more) than did LETO rats. A strong taste preference for sugars has already been reported in other diabetic models, such as alloxan- or streptozotocin-induced diabetic rats and db/db mice (3). In contrast to the findings of a previous study (3), we did not observe a raised preference for low-sucrose concentrations in OLETF rats.

Thus, our neurophysiological and behavioral observations indicate that the OLETF rat differs from the LETO rat in its sensitivity to sucrose, but only to highly concentrated sucrose solutions. It is conceivable that this behavioral result may be due, at least in part, to the greater response of the CT nerve to sucrose solution seen in OLETF rats.

In contrast to this study, previous human studies have either shown that diabetic subjects possess rather higher taste thresholds for glucose (1) and sucrose (12) than nondiabetic subjects, or they have shown no difference between such patients (2). It is known that, in terms of its clinical and pathological features, the disease suffered by OLETF rats resembles human NIDDM (4). However, there have been no reports of the development of a peripheral neuropathy with impairment of both motor and sensory conduction velocities in OLETF rats, although a peripheral neuropathy is one of the most common degenerative complications of diabetes in humans. Such a difference in pathology between OLETF and human diabetes may underlie the apparent discrepancy between the present neurophysiological results and data from previous psychological studies in humans (1, 2, 12).

The effect of dietary biotin on the signs of diabetes in OLETF rats

In this study, we observed that dietary biotin significantly decreased the plasma glucose level and plasma insulin level in OLETF rats at 22 weeks of age. Kawano et al (4) have demonstrated that late onset hyperglycemia (> 18 weeks of age) occurs in OLETF rats. In addition, biotin deficiency leads to hyper-insulinemia, and administration of biotin can reverse that disturbance (7, 13). The results of this study are thus consistent with these previous findings. Chauhan and Dakshinamurti
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(14) demonstrated that the administration of biotin markedly increased glucokinase activity by an action at the transcription phase. In our recent study on STZ-induced diabetic rats (15), we found that biotin injection increased glucokinase activity in the liver and pancreas, and improved glucose handling, without increasing insulin secretion. Thus, it is possible that a high level of dietary biotin may improve at least some signs of diabetes by enhancing glucokinase activity.

In addition, the administration of high dietary biotin significantly decreased the CT nerve response to at least higher concentrations of sucrose in OLETF rats. This result suggests that biotin can selectively normalize the hypersensitivity of sucrose-sensitive taste cells in OLETF rats. The mechanism by which biotin may cause such a decline in the function of the taste transduction systems remains to be elucidated. However, it may be relevant that the administration of biotin markedly increases cGMP in various tissues in the rat (16, 17). These studies may indicate that biotin modulates the intracellular signal transduction mechanism. Such a change in intracellular transduction on the administration of biotin may underlie the partial reversal of the hyper-reactivity of certain taste cells seen in the OLETF rat.

On the other hand, sucrose solution intake by OLETF rats was either unaffected or only slightly decreased by dietary biotin, suggesting that the strong taste preference for sucrose in OLETF rats was due not only to a stronger CT response to sucrose solution, but also to the presence of other genetic or metabolic factors in OLETF rats.

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