Enhanced Responses of the Chorda Tympani Nerve to Sugars in the Ventromedial Hypothalamic Obese Rat

Yasutake Shimizu,1,2 Mifumi Yamazaki,1 Keiji Nakanishi,1 Maki Sakurai,1 Atsushi Sanada,1 Tadashi Takewaki,1,2 and Keiichi Tonosaki3

1Department of Veterinary Physiology, Faculty of Agriculture, and 2Department of Basic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193; and 3Department of Oral Physiology, School of Dentistry, Meikai University, Saitama 350p-0283, Japan

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Shimizu, Yasutake, Mifumi Yamazaki, Keiji Nakanishi, Maki Sakurai, Atsushi Sanada, Tadashi Takewaki, and Keiichi Tonosaki. Enhanced responses of the chorda tympani nerve to sugars in the ventromedial hypothalamic obese rat. J Neurophysiol 90: 128–133, 2003. First published March 12, 2003; 10.1152/jn.01170.2002. Sweet taste sensitivity in obese rats with lesions of the ventromedial hypothalamus (VMH) was studied by examining chorda tympani nerve responses to various taste stimuli including sugars. In the early progressive phase of obesity (2 wk after creating VMH lesions), there was no significant difference in the nerve responses to any taste stimulus between sham-operated and VMH-lesioned rats. In contrast, in the late phase of obesity (15–18 wk after VMH lesions), the magnitude of responses to sugars (except for fructose) was prominently greater than that in age-matched controls. High-fat diet-induced obese rats and streptozotocin-diabetic rats also showed greater chorda tympani nerve responses to sugars as was observed in VMH-lesioned obese rats, indicating that VMH lesions might not be specifically related to the enhanced gustatory neural responses to sugars. Although it has been demonstrated that the enhanced responses of the chorda tympani nerve to sugars in genetically diabetic db/db mice is largely attributable to the lack of the direct suppressive effect of leptin on the taste receptor cells, plasma leptin levels were not correlated with the changes in chorda tympani responsiveness to sugars in these models of obesity and diabetes. Accordingly, our results suggest that some chronic factors, including high blood glucose, inefficiency of insulin action, or leptin resistance may be related to the enhancement of chorda tympani nerve responses to sugars.

INTRODUCTION

Destruction of the ventromedial area of hypothalamus (VMH) results in a chronic and persistent elevation of food intake, a hyperphagia that is hyperreactive to the stimulus properties of foods (Corbit and Stellar 1964; Graff and Stellar 1962; Weingarten 1982). A striking characteristic of VMH lesioned rats is their hyperreactivity to the stimulus properties of foods (Corbit and Stellar 1964; Graff and Stellar 1962; Weingarten 1982). When given a relatively palatable diet including standard laboratory chow, they eat far more than normal animals. In contrast, if the palatability of the standard diet is reduced by the addition of quinine-HCl (QHC), the hyperphagic animals reduce their food intake markedly, while normal animals maintain a relatively constant food intake. These feeding characteristics have been represented as “finickiness.”

Although alteration in taste sensitivity and preference in VMH-lesioned rats have been well documented at the behavioral level, possible changes in peripheral taste nerve responsiveness accounting for the behavioral alterations are not known. In genetically diabetic db/db mice, it has been demonstrated that responses of the chorda tympani (CT) nerve to sugars are greater than those of lean control mice (Ninomiya et al. 1999, 2000), but leptin receptors in taste receptor cells would be unaffected by VMH destruction itself. Therefore it is of interest whether taste nerve responsiveness to sugars is elevated or not in VMH-lesioned rats to know the direct effects of leptin on taste-sensing mechanism more precisely.

In the present study, we recorded responses of the CT nerves of the VMH-lesioned rats to various taste stimuli including sugars. Our results showed that the VMH-lesioned rats possess greater sensitivities to sugars except for fructose but not to other taste stimuli than control rats. Additional experiments using dietary obese and streptozotocin (STZ)-induced diabetic rats indicate that some common metabolic impairments might be related to the enhanced gustatory neural responses to sugars in addition to the direct effects of leptin.

METHODS

Subjects

We used female Wistar rats, weighing 180–230 g (6–7 wk old) at the time of delivery from Japan SLC (Shizuoka, Japan). They were housed individually in plastic cages at 22 ± 2°C (mean ± SD) with a 12:12-h light-dark cycle (light on 0700–1900 h) and given free access to laboratory chow (LABO MR Stock, Nihon-Nosan, Yokohama, Japan). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Measurements

Blood samples were obtained after a 6-h fast (9:00 AM to 3:00 PM). Plasma glucose levels were determined by the glucose oxidase method (Glucose B-test, Wako Pure Pharmaceutical, Osaka, Japan). Plasma insulin and leptin concentrations were determined by ELISA kits (Morinaga, Yokohama, Japan).

Statistical analysis

All values were presented as means ± SE. Statistical significance was examined by an ANOVA, with post hoc testing by means of Duncan’s multiple range test. Comparisons between groups were made by Student’s t-test.

RESULTS

CT nerve responses to various taste stimuli in VMH-lesioned rats

Figure 1 shows integrated responses of the CT nerve to various taste stimuli in sham-operated and VMH-lesioned rats. In the dynamic phase (2 wk after creating VMH lesions), there was no significant difference in the nerve responses to any taste stimulus between sham-operated and VMH-lesioned rats (Fig. 1A). In contrast, the magnitude of response to sucrose in the static phase (15–18 wk after VMH lesions) was prominently greater than that in age-matched control (Fig. 1B). Magnitudes of responses to NaCl, HCl, QHCl, and MSG were unchanged even in the static phase (Fig. 1B).

In addition to sucrose, maltose, glucose, and fructose were also used as taste stimuli to see whether the enhancement of the CT nerve responsiveness is specific for sucrose or generalized for other sugars. As shown in Fig. 2A, the nerve responses to the four sugars were similar in both VMH-lesioned rats in the dynamic phase and their age-match sham-operated animals. In the static phase, relative magnitudes of responses to maltose and glucose, in addition to sucrose, were significantly larger than that in control animals, although no such difference was evident in response to fructose (Fig. 2B).
Plasma glucose, insulin, and leptin concentrations in dynamic and static phase of VMH-lesioned rats

Table 1 shows plasma glucose, insulin, and leptin concentrations in the dynamic and the static phases of VMH-lesioned rats. Plasma glucose levels did not significantly change in the dynamic phase, whereas they prominently increased in the static phase. VMH-lesioned rats in the dynamic phase had a 3-fold higher plasma insulin concentration and a 4.5-fold higher plasma leptin concentration than those in controls. These levels were further increased in the static phase. In sham-operated rats, plasma glucose and insulin levels were comparable at 2 and 15–18 wk after operation, but plasma leptin levels were significantly increased during this period.

Chorda tympani nerve responses to various taste stimuli in diet-induced obese and STZ-diabetic rats

The representative recordings of the CT nerve responses to various taste stimuli from rats maintained by high-fat diet and STZ-diabetic rats are shown in Figs. 3 and 4, respectively. As expected, STZ-diabetic rats had fourfold higher plasma glucose concentration with a marked reduction in insulin levels (~15% of mean control values) when compared with control animals (Table 2, right). Plasma leptin was <0.5 ng/ml in all STZ-diabetic rats (Table 2, right).

Table 1. Plasma glucose, insulin, and leptin levels in VMH-lesioned rats

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<tr>
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<th>Dynamic Phase</th>
<th>Static Phase</th>
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<tr>
<td></td>
<td>Sham</td>
<td>VMH</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>133.9 ± 8.6</td>
<td>142.5 ± 13.1</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.3 ± 0.3</td>
<td>4.0 ± 0.6**</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>3.5 ± 0.3</td>
<td>15.4 ± 2.8**</td>
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Data are means ± SE (n = 5). Dynamic phase, 2 wk after creating ventromedial area of hypothalamus (VMH) lesions; static phase, 15–18 wk after VMH lesions; sham, sham-operated control rats. *P < 0.05 vs. sham control (2 wk); **P < 0.01 vs. sham control (2 wk); †P < 0.01 vs. sham control (15–18 wk); ‡P < 0.01 vs. VMH-lesioned rats of dynamic phase.
the leptin receptor and that leptin directly increases K
leptin. nerve responses to sugars in addition to the direct effects of metabolic impairments might be related to the enhanced CT suppressive effect of leptin on the taste receptor cells (Ninomiya et al. 1996; Lee et al. 1996), it is reasonable to suppose that the enhanced sugar responsiveness in leptin-resistant hypothalamic neurons in the mice fed high fat diet (El-Haschimi et al. 2000). In addition, it has been shown recently that skeletal muscle becomes resistant to the direct effects of leptin, such as increase in lipid oxidation and triglycerol breakdown, during the development of obesity (Steinberg and Dyck 2000; Steinberg et al. 2002). It is thus possible that the leptin signaling pathway in the taste cells is down-regulated by prolonged hyperleptinemia in high-fat diet-induced obese rats as well as in VMH-lesioned obese rats.

We found that STZ-induced diabetic rats have higher CT nerve responses to sugars as in VMH-lesioned obese rats. This result seems to conflict with that reported by Ninomiya et al. (1995), who have demonstrated that STZ-induced diabetic mice show no significant difference in CT nerve responses to sugars compared with the control mice. This difference is probably derived from the difference in duration of diabetic state. In fact, we failed to observe significant changes in CT nerve responses to sugars 2 wk after STZ injection, whereas the enhancement of sugar responses of CT became evident after 4 wk of STZ injection. It should be noted, however, that the plasma leptin was reduced much by 2 wk after STZ injection (Table 2) (Havel et al. 1998). If leptin exerts a tonic inhibitory effect on sweet taste-sensing mechanism in taste cells under the normal conditions, the reduction of circulating leptin may result in an increased sensitivity of taste cells immediately. The evidence that the time course of increase in CT responses to sugars does not coincide with a reduction of serum leptin level suggests that some chronic factors, including lowered leptin levels, alters the taste-sensing mechanism under the diabetic and obese conditions.

Recently, it has been demonstrated that taste cells express the leptin receptor and that leptin directly increases K+ conductance, resulting in hyperpolarization and reduction of cell excitability (Kawai et al. 2000). Considering that db/db mice express a defective leptin receptor (Chen et al. 1996; Chu et al. 1996; Lee et al. 1996), it is reasonable to suppose that the enhancement of CT response to sugars is due to the lack of the direct suppressive effect of leptin on the taste receptor cells (Ninomiya et al. 1995, 2002), our results suggest some common metabolic impairments might be related to the enhanced CT nerve responses to sugars in addition to the direct effects of leptin.

FIG. 4. The chorda tympani nerve responses to various taste stimuli in streptozotocin (STZ)-induced diabetic rats. The representative recordings of the integrated responses of chorda tympani nerve to various taste stimuli in STZ-induced diabetic rats are shown. Similar results were obtained in 5 independent experiments.

except for fructose, but not to NaCl, HCl, QHCl, and MSG, are substantially increased in the VMH-lesioned obese rats; these changes were evident in the static phase of the VMH syndrome 15–18 wk after operation but not during the early progressive phase of obesity (the dynamic phase; 2 wk after creating VMH lesions), and the enhanced responsiveness to sugars of the CT nerves are common among the other obese and diabetic models (i.e., high-fat diet-induced obese and STZ-induced diabetic rats). In addition, measurements of plasma leptin concentrations in these obese and diabetic animals have revealed that changes in the responses of the CT nerve to sugars are not necessarily correlated with plasma leptin levels. Although it has been postulated that the enhanced sugar responsiveness in db/db mice is largely attributable to the lack of the direct suppressive effect of leptin on the taste receptor cells (Ninomiya et al. 1995, 2002), our results suggest some common metabolic impairments might be related to the enhanced CT nerve responses to sugars in addition to the direct effects of leptin.

FIG. 5. Relative responses to sugars in the chorda tympani of high-fat diet-induced obese rats and STZ-induced diabetic rats. Steady-state responses of the integrated chorda tympani nerve activity to sugars in high-fat diet-induced obese rats (A) and STZ-induced diabetic rats (B) were normalized to that to 0.1 M NH₄Cl, which was taken as unity (1.0). The values were presented as means ± SE (n = 5). Suc, 0.5 M sucrose; Mal, 0.5 M maltose; Glc, 0.5 M glucose; Fru, 0.5 M fructose.

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Because of insulin deficiency of CT responses to sugars. Under the condition and reduction of insulin action are likely to underlie the speculation, however, that a chronic increase in plasma glucose-sensing system in taste cells are not known. We

TABLE 2. Plasma glucose, insulin, and leptin levels in diet-induced obese and STZ-diabetic rats

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<th>Diet-Induced Obese Rats</th>
<th>STZ-Diabetic Rats</th>
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<td></td>
<td>Control</td>
<td>High-Fat</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>134.5 ± 7.8</td>
<td>188.6 ± 21*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.3 ± 0.3</td>
<td>4.4 ± 0.7**</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>3.2 ± 0.5</td>
<td>17.9 ± 3.9**</td>
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Data are means ± SE (n = 5). High-fat, high-fat diet-induced obese rats; STZ, streptozotocin (STZ)-induced diabetic rats. *P < 0.05 vs. respective control; **P < 0.01 vs. respective control.

At present, the putative chronic factors that affect the sugar-sensing system in taste cells are not known. We speculate, however, that a chronic increase in plasma glucose and reduction of insulin action are likely to alter the altered sensitivity of CT responses to sugars. Under the diabetic condition, tissue glucose utilization may be limited because of insulin deficiency or insulin resistance. Furthermore, persistent hyperglycemia has been known to reduce the brain blood flow and the glucose transport across the blood-brain barrier (Gjedde and Crone 1981; Harik and LaManna 1988; Matthaei et al. 1986; Pardridge et al. 1990), both of which would result in a lowered supply of glucose to the brain. Thus these conditions can be recognized as an insufficiency of intracellular glucose, in spite of the presence of excess extracellular glucose. Considering that enhancement of sugar responsiveness of CT is correlated with an increase in sugar intake behavior (Ninomiya et al. 1995, 2002), the enhancement of CT nerve responses to sugars in diabetic rats is inferred to be an adaptive change to satisfy the demand for glucose of brain and peripheral tissues. In accordance with this, we found that VMH-lesioned obese rats showed a strong preference for starch, which is a source of glucose but possesses different taste quality than sugars (Y. Shimizu, T. Takewaki, and K. Tonosaki, unpublished observation).

One of the characteristics observed commonly among VMH-lesioned obese rats, high-fat diet-induced obese rats and STZ-diabetic rats is that CT nerve responses to fructose were unchanged in contrast to those to glucose, sucrose, and maltose. Several lines of evidence have suggested that there are multiple receptor sites for sugars in taste cells (Beidler and Tonosaki 1985; Jakinovich 1976; Jakinovich and Goldstein 1976; Tonosaki and Funakoshi 1984, 1989). Recently, receptors for sweet taste stimuli including sugars were identified as T1Rs (Li et al. 2002; Margolskee 2002; Nelson et al. 2001). However, in contrast to bitter taste receptors T2Rs that constitute a large multigene family (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000), T1Rs family is small and the only one form of functional sweet receptor is known to be a heterodimer of T1R2 and T1R3 (Li et al. 2002; Nelson et al. 2001). Available data have suggested that the heterodimer of T1R2 and T1R3 can be activated by most of sweet-tasting substances including fructose, glucose, sucrose, and maltose (Li et al. 2002). Thus the molecular basis for the multiple receptor sites for sugars is not so far established. Since several G protein α-subunits have been identified in taste receptor cells (Kusakabe et al. 1998; McLaughlin et al. 1992; Ruiz-Avila et al. 1995), it is possible that coupling patterns of the T1Rs and G proteins is related to the sweet taste specificity.

In fact, co-expression experiments of T1R2/T1R3 complex with various Go15 chimeras in which the five-residue C-terminal tail was replaced by those of other G proteins showed that sucrose induced an increase in intracellular calcium when some specific chimeras were present (Li et al. 2002). Although the precise mechanism for the fructose-sensing system is not clear, it may be separate from other sugar receptors, and the factors that promote enhancement of responsiveness for glucose, sucrose, and maltose are ineffective for this putative fructose-sensing mechanism. In support of this notion, it has been reported that preabsorptive recognition of fructose and glucose in small intestine is made in a different manners (Mei 1978, 1985).

Physiological significance of the differences of the responses between fructose and other sugars are not known. It has been demonstrated that fructose ingestion induces insulin resistance and hyperlipidemia in rats (Elliott et al. 2002). This disadvantage of fructose as an energy source may be related to the lack of adaptive changes in taste sensing system for fructose. In accordance with this, our preliminary experiments with two-bottle preference tests (glucose vs. fructose) show that the relative preference of glucose solution was increased day by day, and the rats become to drink glucose solution exclusively within a week (Y. Shimizu, T. Takewaki, and K. Tonosaki, unpublished observation).

One of the striking characteristics of VMH-lesioned rats is their hyperreactivity to QHCl adulterated in foods (Corbit and Stellar 1964; Graff and Stellar 1962; Weingarten 1982). We failed to detect changes in CT nerve responses to QHCl in VMH-lesioned rats. It should be mentioned, however, that changes in ingestive behavior are not necessarily accompanied by the corresponding changes in taste nerve reactivity. Accordingly, it seems likely that hyperreactive behavior to QHCl in VMH-lesioned rats depends on the differential responsiveness of higher centers that receive taste information. Further study is needed to establish the neurophysiological bases for finickiness of VMH-lesioned obese rats.

In summary, the present study shows that the responsiveness of the CT nerve to sugars is enhanced during the development of diabetes and obesity. Although leptin is established as an important regulator of the sweet-taste-sensing system, our results have suggested that some chronic factors, including high blood glucose, inefficiency of insulin action, or leptin resistance may be related to the enhancement of CT nerve responses to sugars.

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