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

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Abstract

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 magnitude of responses to sugars. Other similar metabolic impairments might also be related to the enhanced gustatory neural responses 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.

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Enhanced Responses of the Chorda Tympani Nerve to Sugars

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, Yoko-
hama, Japan) and water. The rats were maintained in the laboratory for 2–20 wk after arrival to permit them to acclimate to their surroundings before experiments began (8–9 wk old, 250–300 g at the start of experiments).

The VMH was destroyed bilaterally by an electrolytic lesion according to the procedure described by Saito et al. (1985) with slight modifications. Briefly, rats were anesthetized with pentobarbital sodium (40 mg/kg ip) and were placed in the stereotaxic instrument and positioned with the nose bar set 5 mm above the interaural line. The electrode, consisting of an insulated stainless steel insect pin with exposed tip, was positioned 0.6 mm lateral to the Bregma and 9.0 mm below the skull surface. A direct anodal current of 1.2 mA for 4 s was passed through the electrode. The current pass was repeated three times with 30-s interval. For sham controls, the same surgical procedures were performed except that no current was passed. Only animals that developed hyperphagia and rapid gain in body weight compared with sham-lesioned rats were used. Sham-lesioned animals were used as controls for possible damage to nerve fibers by the procedure. We designated the early progressive phase of obesity as the dynamic phase (2 wk after creating VMH lesions) and the late phase of obesity as the static phase (15–18 wk after VMH lesions). At the end of the experiments, localization of the lesions was verified microscopically in serial frontal sections of the brain stained with cresyl violet.

Dietary obese rats and insulin-deficient diabetic rats have been also prepared to provide a comparison with VMH-lesioned rats. To induce dietary obesity, some rats were maintained on a high-fat diet for 18–20 wk. The high-fat diet contained the following (g/kg): 330 lard (Oriental Yeast), 50 corn oil (Oriental Yeast), 200 casein (Oriental Yeast), 225 corn starch (Oriental Yeast), 100 sucrose (Oriental Yeast), 50 cellulose (Oriental Yeast), 35 minerals (AIN76 mineral mixture; Oriental Yeast), and 10 vitamins (AIN76 vitamin mixture; Oriental Yeast). Control animals were given a standard laboratory chow (MR stock; Japan SLC, Shizuoka, Japan). Diabetic rats were made with a single-bolus intraperitoneal injection of STZ (60 mg/kg; Sigma, St. Louis, MO) dissolved in 0.1 M citric buffer (pH 4.2). The diabetic animals with fasting blood glucose concentrations of >300 mg/dl were remained untreated throughout the duration of the study.

Neural recording procedure

The CT nerve responses to various taste stimuli were recorded as previously described (Shimizu and Tonosaki 1999). The electrical activity of the whole CT nerve was fed to an AC amplifier and previously described (Shimizu and Tonosaki 1999). The electrical activity of the whole CT nerve was fed to an AC amplifier and previously described (Shimizu and Tonosaki 1999). The electrical activity of the whole CT nerve was fed to an AC amplifier and previously described (Shimizu and Tonosaki 1999). The electrical activity of the whole CT nerve was fed to an AC amplifier and previously described (Shimizu and Tonosaki 1999). The electrical activity of the whole CT nerve was fed to an AC amplifier and previously described (Shimizu and Tonosaki 1999).

**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).

**FIG. 1.** Chorda tympani nerve responses to various taste stimuli in ventromedial area of hypothalamus (VMH)-lesioned rats. Representative recordings of the integrated responses from the chorda tympani nerve to various taste stimuli in dynamic phase (2 wk after creating VMH lesion; A) and static phase (15–18 wk after creating VMH lesions; B) are shown. Similar results were obtained in 5 independent experiments. Suc, sucrose; QHCl, quinine-HCl; MSG, monosodium glutamate.
TABLE 1. Plasma glucose, insulin, and leptin concentrations in dynamic and static phase of VMH-lesioned rats

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<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>
</tr>
<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 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.
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.

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 suppressive effect of leptin. In accordance with this, intraperitoneal injection of leptin to db/db mice does not affect the CT nerve responses to sweeteners, whereas leptin substantially reduces those in lean control animals (Kawai et al. 2000). In the present study, we observed that the CT nerve responses to sugars in VMH-lesioned obese rats were greater than those in control rats. Similarly, high-fat diet-induced obese rats have greater responses to sugars as compared with rats maintained with a standard laboratory chow. These results conflict with the suppressive effect of leptin on taste reactivity to sugar because plasma leptin concentration of these rats is elevated as in db/db mice, but leptin receptors in taste cells are intact in contrast to db/db mice. This apparent discrepancy could be reconciled by supposing that leptin resistance is induced in taste cells. In fact, the early progressive phase of obesity was not accompanied by changes in CT nerve responses, suggesting that the enhancement of CT nerve responses to sugars is established on a time scale of several months. It is generally considered that a major mechanism for leptin resistance is a defect in the blood-brain-barrier transport system that delivers leptin to the sites of action in the hypothalamus (Caro et al. 1996; Schwartz et al. 1996; Van Heek et al. 1997). However, in addition to the defect in leptin access, it has been demonstrated that a signaling defect also exists in leptin-responsive 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.
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; Mathaeti et al. 1986; Partridge 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 evidences 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; Chandrashekhar 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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**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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<tr>
<td></td>
<td>Control</td>
<td>High-Fat</td>
</tr>
<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>3.2 ± 0.5</td>
<td>17.9 ± 3.9**</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1**</td>
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</table>

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.

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REFERENCES


