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Analysis of Concentration-response Relationship for Enhanced Sugar Responses of the Chorda Tympani Nerve in the Diabetic *db/db* Mouse

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Abstract

Chorda tympani responses to sugars were greater in diabetic (*db/db*) than in non-diabetic control mice. A kinetic analysis suggested that the greater sugar responses in *db/db* mice were unlikely due to the increased number of sugar receptors. **Chem. Senses 21: 59–63, 1996.**

Introduction

The *db/db* mouse is known as a genetic model of non-insulin-dependent diabetes (NIDD), in which a single gene defect (*db* gene), leads to the expression of diabetes with preceding hyper-insulinemia, hyperglycemia and extreme obesity (Colman and Hummel, 1967, 1974). Our recent study (Ninomiya *et al.*, 1995) demonstrated that responses of the chorda tympani nerve to sugars, but not to salts, acid and quinine HCl, were greater in *db/db* mice than in non-diabetic control mice, and behavioral preference for sugars was also higher in *db/db* than in control mice. The results of electrophysiological experiments using infant *db/db* and streptozotosin-induced diabetic mice suggested that the greater neural responses to sugars in *db/db* mice are genetically determined. However, underlying mechanisms for genetic induction of the high sugar sensitivity have not yet been investigated.

In the present study, therefore, as the first step to clarify the mechanisms, we compared characteristics of the stimulus-receptor interaction for sugars in *db/db* and control mice by using a kinetic analysis of concentration–response relationships in responses of the chorda tympani nerve to sugars. Since the integrated responses of the chorda tympani nerve to sugars might include response components which are evoked through receptor mechanisms other than 'sweet receptor proteins' (Pfaffmann, 1974; Iwasaki and Sato, 1986), to exclude this possible component, we recorded the chorda tympani responses to sugars before and after the lingual treatment with a proteolytic enzyme, pronase (Hiji, 1975) and used the pronase-inhibited response component to sugars for the kinetic analysis.

Methods, results and discussion

Subjects

Diabetic and non-diabetic male littermates were obtained from mating pairs of the C57Bl/KsJ-db mouse strain originally supplied from the Jackson Laboratories (Bar Harbor, ME). In this strain, the closely-linked mutant fur color gene, misty (m), has been incorporated into stocks for maintenance of *db*, in repulsion (*db*+/+*m*) to facilitate identification of

heterozygotes for breeding. Therefore, adult diabetic mice with a black fur color (*db*+/*db*+: 8–16 weeks of age, 50–60 g body weight) and non-diabetic mice with a grey (misty) fur color (+*m*/+*m*: 8–16 weeks of age, 20–35 g body weight) were selectively obtained from the stock. Diabetic mice with *db/db* genotype were referred to as *db/db* mice, whereas nondiabetic control mice with +/+ genotype are referred to as control mice.

Measurement of blood glucose levels of diabetic and non-diabetic mice

Before the start of experiments, blood glucose level of each mouse was measured by using a blood glucose autoanalyser (Reflorax; Manheim-Toho Co.). Blood of the animal was taken from the tail vein. The obtained blood glucose levels were 10.7 ± 1.1 mmol/l (n = 10) in control mice, and 35.3 ± 6.2 mmol/l (n = 8) in *db/db* mice.

Recording of the mouse chorda tympani responses

Mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (40–50 mg/kg body wt) and maintained at a surgical level of anesthesia with supplemental injections of pentobarbital sodium. The trachea was cannulated and the mouse was then fixed in the supine position with a headholder to allow disection of the chorda tympani nerve. The nerve was exposed at its exit from the lingual nerve and cut near its entrance to the bulla. For whole nerve recording, the entire nerve was placed on a silver wire electrode. An indifferent electrode was positioned nearby in the wound. Neural responses resulting from chemical stimulation of the tongue were amplified (Iyodenshikogaku K-1) and displayed on an oscilloscope screen (Nihon Kohden VC-10). Whole nerve responses were integrated and displayed on a recorder (Nihon Kohden WS-641G). The time constant of the integrator was 0.3 s.

Chemical stimulation

Solutions used for chemical stimuli were: 0.1 M NH_4 Cl, 0.1 M NaCl, 0.01 M HCl, 0.02 M quinine HCl, 0.1 mM–1.0 M sucrose, 0.1 mM–1.0 M fructose, 0.1 mM–1.0 M glucose and 0.1 mM–1.0 M maltose. These chemicals were dissolved in distilled water at about 25°C. During chemical stimulation of the tongue, test solution flowed for about 30 s at the same flow rate as the distilled water used for rinsing the tongue (0.5 ml/s). The tongue was rinsed during the interval of more than 1 min between successive stimulations. To obtain the pronase-inhibited response component for each stimulus, chorda tympani responses of animals were measured before and after the lingual treatment with pronase E (2%) for 15 min, which was disolved in 50 mM phosphate buffer (pH = 7.0, 37°C) immediately before the treatment.

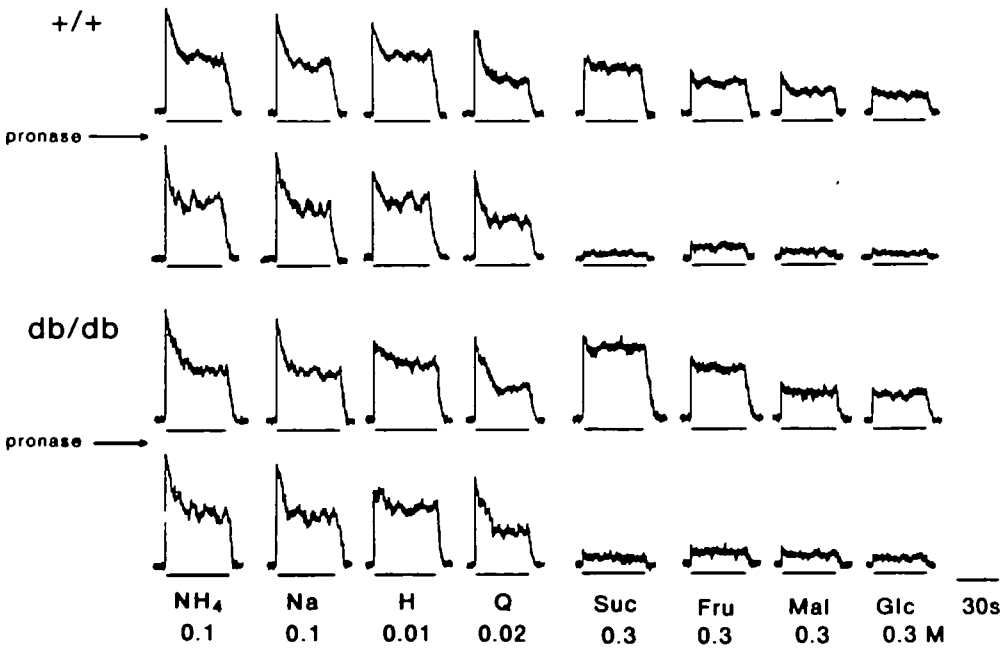
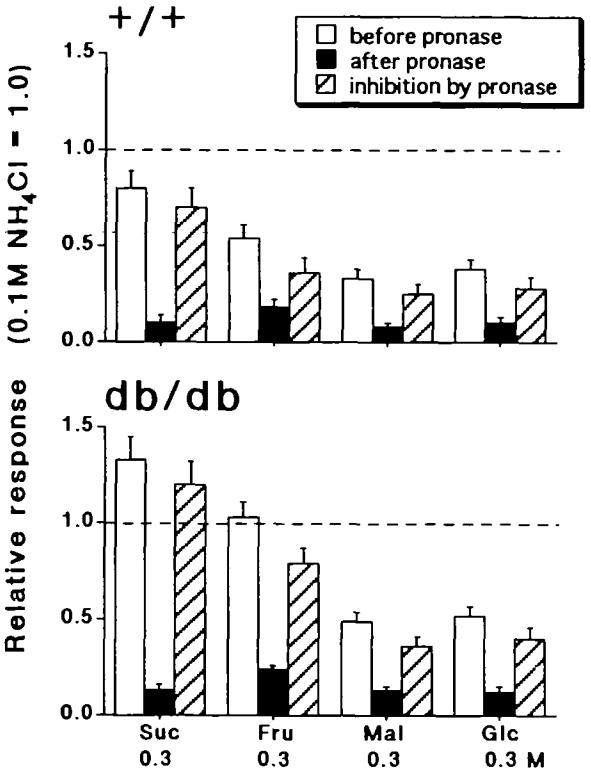


Figure 1 Sample recordings of integrated responses of the chorda tympani nerve of diabetic (*db/db*) and control (+/+) mice to eight taste stimuli before and after the lingual treatment with pronase. Abbreviations, NH_4 0.1: 0.1 M NH_4Cl; Na 0.1: 0.1 M NaCl; H 0 01: 0.01 M HCl; Q 0.02: 0.02 M quinine HCl; Suc 0.3: 0.3 M sucrose; Fru 0.3: 0.3 M fructose; Mal 0.3: 0.3 M maltose; Glc 0.3: 0.3 M glucose.



Adaptation of the tongue to the buffer without pronase had no effect on the responses to taste stimuli. To maintain the pronase inhibition, the tongue was repeatedly treated with pronase for 5 min with a 5-min inter-treatment interval during recording taste responses.

Data analysis

In the analysis of whole nerve responses, the magnitude of the integrated response at 20 s after stimulus onset was measured. Relative response magnitude for each stimulus was calculated when the response magnitude to 0.1 M NH_4Cl was taken as a unity (1.0) and this was used for statistical analysis.

Figure 1 shows integrated responses of the chorda tympani

Figure 2 Relative magnitudes of chorda tympani responses to 0.3 M sugars before (open column) and after (filled column) pronase treatment, and magnitudes of pronase-inhibited response component (striped column) in diabetic (*db/db*) and control (+/+) mice. Mean relative responses (±SD) are plotted. Data were obtained from eight diabetic and 10 control mice. Abbreviations are the same as those described in Figure 1.

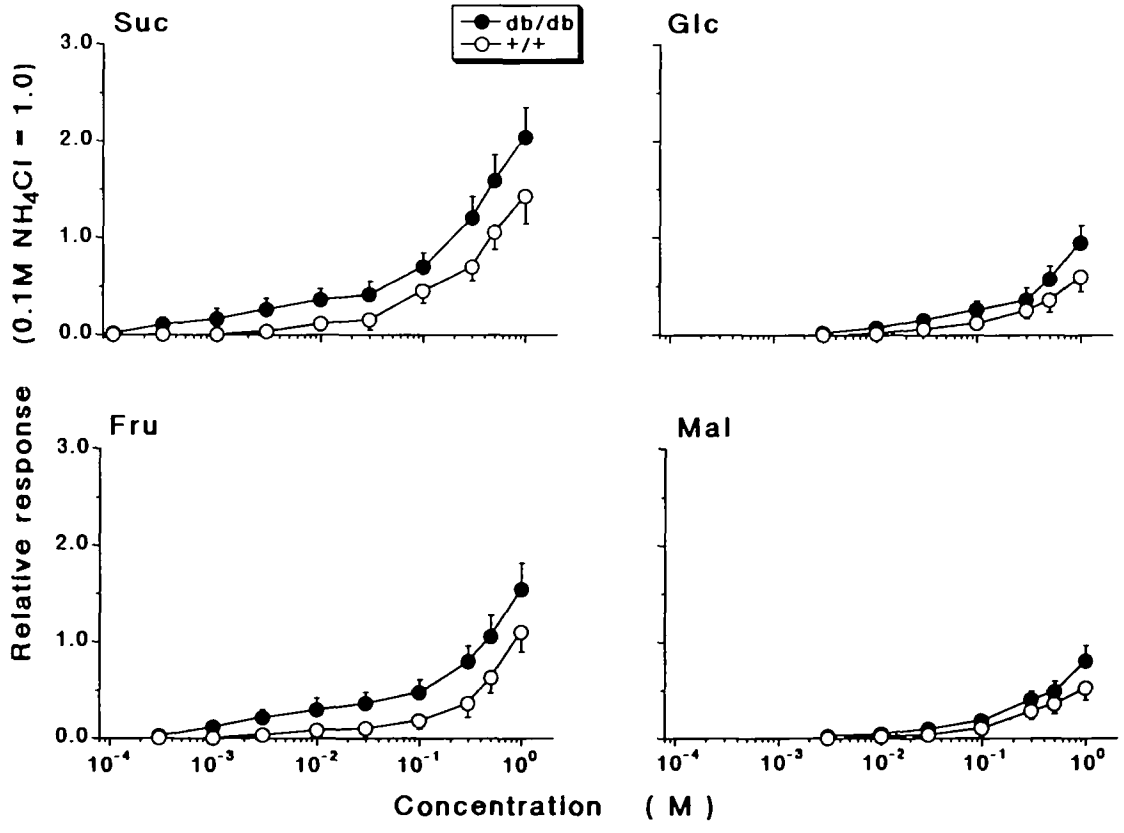


Figure 3 Concentration-response relationships of pronase-inhibited response components of the chorda tympani nerve for four sugars in diabetic (*db/db*: filled circles) and control (+/+: open circles) mice. Mean relative responses (±SD) are plotted. Data were obtained from eight diabetic and 10 control mice. At all concentrations tested, except 1.0 M maltose, relative magnitudes of responses to four sugars were significantly greater in *db/db* mice than in control mice (Student *t*-test, $P<0.05$). Abbreviations are the same as those described in Figure 1.

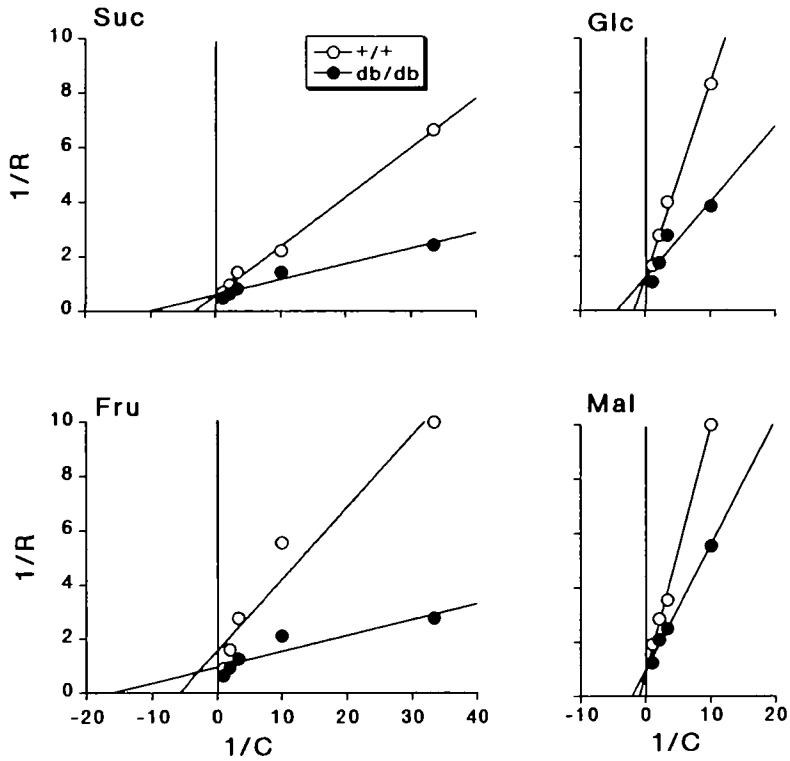


Figure 4 Double reciprocal plots of concentration-response relationships of pronase-inhibited response components of the chorda tympani nerve for four sugars in diabetic (*db/db*) and control (+/+) mice. Mean values obtained from eight diabetic and 10 control mice are used. Abbreviations are the same as those described in Figure 1.

nerve to eight taste stimuli before and after the lingual treatment with pronase in the *db/db* and control mice. As reported previously (Ninomiya *et al.*, 1995), magnitudes of responses to sugars before pronase were larger in the *db/db* mouse than the control mouse, whereas no such difference was observed in response to NH_4Cl, NaCl, HCl and quinine HCl (see also Figure 2). This suggests a specific increase in responses to sweet substances in the *db/db* mouse. Responses to sugars were selectively suppressed by pronase in both mice. It is noted that the suppression of sugar responses was not complete. Small residual responses to each sugars were observed. As shown in Figure 2, relative magnitudes of responses to 0.3 M sugars before pronase in *db/db* mice were 1.4 (maltose), 1.5 (glucose), 1.7 (sucrose) and 1.9 (fructose) times greater than those in control mice (Student *t*-test, $P<0.05$). In both groups, responses to 0.3 M sugars before pronase were greater in the order of sucrose>fructose>maltose = glucose, whereas residual responses after pronase were in the order of fructose>maltose = sucrose = glucose. Response to sucrose was most greatly suppressed by pronase among four sugars. The magnitude of the response component for sugars that was suppressed by pronase (pronase-inhibited response component) was also greater in *db/db* than in control mice [1.4 (maltose), 1.5 (glucose), 1.7 (sucrose) and 2.2 (fructose) times; Student *t*-test, $P<0.05$], suggesting that the enhanced sugar responses in *db/db* mice were evoked mainly through acitivation of pronase-inhibited receptor components.

Concentration-response relationships for pronase-inhibited response components for four sugars in *db/db* and control mice are shown in Figure 3. At all concentrations tested, the relative magnitudes of responses to four sugars were significantly greater in *db/db* than in control mice (Student *t*-test, $P<0.05$). Also, thresholds for sugars were lower in *db/db* than in control mice. Based on the data for sugars at limited concentration ranges which produced more reliable responses (0.03–1.0 M of sucrose and fructose, and 0.1–1.0 M of glucose and maltose) in *db/db* and control mice, double reciprocal plots (Lineweaver and Burk, 1964) were made and shown in Figure 4. The dissociation constant (K_d value) for each sugar in *db/db* mice obtained from each plot was ~0.10 M for sucrose, ~0.06 M for fructose, ~0.48 M for maltose or ~0.23 M for glucose, which was smaller than that in control mice (~0.31 M for sucrose, ~0.18 M for

fructose, ~1.0 M for maltose or ~0.57 M for glucose). In contrast, the magnitude of the maximum response (V_{max}) for each sugar in *db/db* mice (1.69 for sucrose, 1.03 for fructose, 1.04 for maltose or 0.83 for glucose) was not largely different from that in control mice except that for fructose (1.69 for sucrose, 0.65 for fructose, 1.11 for maltose or 0.79 for glucose). If the V_{max} values would be comparable with the number of functional receptors, it is conceivable that the enhanced sugar responses in *db/db* mice were unlikely due to increase of functional sugar receptors at least for sucrose, glucose and maltose.

Our previous study (Ninomiya *et al.*, 1995) showed that the *db/db* mice at 7–9 days of age already have their high sugar sensitivity comparable with that in adult *db/db* mice. This mouse age is in accordance with the age when the first typical symptom of *db/db* mice, hyperinsulinemia, manifests itself (Basabe *et al.*, 1986). Therefore, we suggested that in *db/db* mice the *db* gene acts on both pancreatic B cells and taste cells at this early stage, and produces the high sugar sensitivity. Several investigations have suggested similar responsiveness of pancreatic B and taste cells to anomers of glucose (Vlahopoulos and Jakinovich, 1986; Niki *et al.*, 1989), alloxan (Cooperstein and Lazarow, 1969; Zawalich, 1973), some proteolytic enzymes (Krause *et al.*, 1973; Hiji, 1975), or p-nitrophenyl-D-glucopyranoside (a inhibitor of sugar taste response) (Vlahopoulos and Jakinovich, 1986; Niki *et al.*, 1989). However, the proposed mechanism of glucose recognition is different between the two types of cells (Niki *et al.*, 1989). The taste cell is thought to recognize glucose at its receptor site on the cell membrane, whereas the pancreatic B cell is believed to metabolize glucose for signal production for insulin release. Taking these facts together with the present results that showed no prominent difference in V_{max} values at least for sucrose, glucose and maltose but difference in K_d values for these sugars between *db/db* and control mice, it is conceivable that the *db* gene may act on (a) common factor(s) involved in the intracellur transduction mechanisms in the two types of cells. Future extensive studies are, however, needed to answer to these possibilities.

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