SHORT COMMUNICATION

Neural and behavioral taste responses to D-amino acids in mice

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Introduction

It is generally known that most D-amino acids taste sweet to human, although most L-amino acids taste bitter. Psychophysical measurements of perceived taste intensity suggests that sweetness for D-amino acids tends to be greater with increasing molecular weight¹⁻³⁾. For example, sweetness is greater for D-tryptophan, D-phenylalanine and D-histidine (molecular weight > 150) than D-alanine, D-serine and D-valine (molecular weight < 120). In animal studies, only a few papers on taste responses to D-amino acids have been published yet. Kasahara et al.⁴⁾ used two bottle preference test in mice and found a similar molecular weight dependency in the order of the preference magnitude for several D-amino acids, although the two bottle preference test by itself was not suitable for qualitative measurement of taste substances. Ninomiya et al.⁵⁻⁷⁾ measured qualitative similarities among taste substances by using a conditioned taste aversion technique and reported that the ability to perceive "sweetness" of D-phenylalanine (D-phe) varies among mouse strains. A taste aversion conditioned to D-phe generalized to sweet substances in C57BL/6 CrSlc mice (D-phe sweet taster), whereas it gener-

alized to the isomer L-phenylalanine but not to the sweet substances in BALB/c CrSlc and C3H/HeSlc mice (D-phe nonsweet taster). However, in a series of their studies, they used only three D-amino acids and did not thoroughly investigate possible difference in taste responses among D-amino acids.

The present study, therefore, expanded the stimulus array of D-amino acids (eight in total) and examined characteristics of D-amino acids as sweet taste stimuli by comparing both behavioral similarities between D-amino acids vs. sucrose and effects of a specific inhibitor for sweet taste responses, Pro-nase E⁸⁾, on the chorda tympani nerve responses to D-amino acids in D-phe sweet and nonsweet taster mouse strains.

Materials and Methods

Subjects were adult male and female mice of C57BL/6CrSlc (D-phe sweet taster) and BALB/cCrSlc (D-phe nonsweet taster) strains, ranging in weight from 20 to 35 g. In behavioral experiments, we used a conditioned taste aversion (CAT) paradigm. Each animal was conditioned to avoid one of 8 D-amino acids and distilled water, forming 8 experimental groups and one control group of 5-12 each in each strain. The conditioning stimulus

(CS) for each of the 8 experimental groups was either; 0.1 M D-alanine, 0.1 M D-serine, 0.1 M D-valine, 0.1 M D-leucine, 0.1 M D-methionine, 0.1 M D-histidine, 0.1 M D-phe and 0.03 M D-tryptophan dissolved in distilled water. The CS for the control group was distilled water. These solutions were also used as test stimuli (TSs). The other TS employed was 0.5 M sucrose dissolved in distilled water.

Procedures for the CTA technique were the same as those described in previous studies^{5,6}. Briefly, from the first to the fifth day was a training period, during which the animal was trained to drink distilled water from a single drinking tube via a circular window (5 mm diameter) in the test cage. The tip of a drinking tube was located 2 mm outside the window. This arrangement prevented contact of the tip of the tube with the animal's lip. The licks were detected by a lickometer with a photo lick sensor and recorded on a stripchart recorder. On the sixth day, each animal was given access to one of the 8 CSs more than 20 trials of 10-sec periods of presentation of the CS, and then given an intraperitoneal injection of LiCl (230 mg/kg), the unconditioned stimulus, to induce gastrointestinal malaise. Control mice drank distilled water before the LiCl injection. The seventh day was a recovery period. On the eighth and ninth day, the number of licks for each of TSs including the CS and distilled water given by each animal was counted during the first 10 sec after the animal's first lick.

For data analysis, the strength of the CTA to each stimulus was expressed as a percent suppression according to the formula: percent suppression = $[1 - (\text{numbers of licks for 10 sec of experimental animal} / \text{number of licks for 10 sec of control animal})] \times 100$.

For electrophysiological experiments, animals were the same as those used for behavioral experiments. Under sodium pentobarbital anesthesia (40-50 mg/kg), integrated responses of the chorda tympani nerve of each animal to various taste stimuli were recorded before and after the treatment of the tongue with 2 % Pronase E for 10 min. This lingual treatment with Pronase for 10 min. suppressed chorda tympani responses to 0.5 M sucrose

by about 10% of the control level in both strains⁹. Procedures of dissection of the nerve, recording of the nerve responses and taste stimulation were described elsewhere¹⁰. Taste stimuli were 8 D-amino acids mentioned above, and 0.5 M sucrose and 0.1 M NH_4Cl . For data analysis, the magnitude of the integrated response at 10 sec 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.

Results and Discussion

Figure 1 shows both licking suppression rate of sucrose after the aversion conditioning for each of 8 D-amino acids (filled circles: BALB/c strain; filled triangles: C57BL/6 strain) and percent reduction (control=100) of the chorda tympani responses to each of 8 D-amino acids after the lingual treatment with Pronase (open circles: BALB/c strain; open triangles: C57BL/6 strain). Each value indicates the mean of the results obtained from each 5-12 mice. This figure was illustrated to make a comparison easier between behavioral and neural responses for each D-amino acid in each strain [filled vs. open symbols in left (BALB/c strain) and right (C57BL/6 strain) side of the figure]. It is clear that in both strains the magnitude of percent reduction of the chorda tympani response by Pronase was higher for D-amino acid, to which the conditioning aversion produced higher licking suppression rates of sucrose. Correlation coefficients between values for behavioral and neural response measurements were significantly high in both strains (BALB/c: $r=0.933$, $P<0.001$; C57BL/6: $r=0.834$, $P<0.01$), suggesting correspondence between behavioral and neural responses in both strains. In this figure, D-amino acids were arranged in the order of molecular weight from the bottom lower to the top higher. Apparently, both neural and behavioral response values tend to be higher for D-amino acids with higher molecular weight in two strains and are plotted in a symmetrical fashion in the figure, except values for

Fig. 1 Licking suppression rate of sucrose after the aversion conditioning for each of 8 D-amino acids (filled circles: BALB/c strain; filled triangles: C57BL/6 strain) and percent reduction (control=100) of the chorda tympani responses to each of 8 D-amino acids after the lingual treatment with Pronase (open circles: BALB/c strain; open triangles: C57BL/6 strain). Each value indicates the mean of the results obtained from each 5~12 mice. D-amino acids are arranged in the order of molecular weight (Mol. wt.) from the bottom lower to the top higher. Abbreviations: Try: D-tryptophan; Phe: D-phenylalanine; His: D-histidine; Met: D-methionine; Leu: D-leucine; Val: D-valine; Ser: D-serine; Ala: D-alanine; r : a correlation coefficient between behavioral and neural response measures for 8 D-amino acids in each strain [filled vs. open symbols in left (BALB/c strain) and right (C57BL/6 strain) side of the figure]. **: $P < 0.01$; ***: $P < 0.001$.

D-phe in BALB mice. As shown in Table 1, correlation coefficients between molecular weight and licking suppression rate of sucrose and between molecular weight and percent reduction of the chorda tympani response by Pronase are significantly high ($P < 0.01$ or 0.05) in both strains, although in BALB/c mice data for D-phe were excluded. These results indicate that except D-phe mice possess a similar molecular weight dependency in the order of the magnitude of sweet taste responses to D-amino acids to that shown in

human¹⁻³⁾, and in other aspect neural and behavioral response measures for D-amino acids employed in the present study are to some extent comparable with human psychophysical measures for perceived sweetness for D-amino acids. Previous genetic studies^{6,7)} have demonstrated that sweet taste response to D-phe is controlled by a single gene, *dpa*, on chromosome 4. The strain difference specifically observed in response to D-phe suggests that BALB mice presumably lack the ability to perceive "sweetness" for only D-phe among

Table 1 Correlation coefficients between molecular weight and licking suppression rate of sucrose (behavioral response value) and between molecular weight and percent reduction of the chorda tympani response by pronase (neural response value) for D-amino acids in BALB/c and C57BL/6 strains

	BALB/c ^a	C57BL/6 ^b
Behavioral response value	0.840*	0.802*
Neural response value	0.878**	0.766*

^a: Calculation was based on the data for 7 D-amino acids. Data for D-phenylalanine were excluded.

^b: Calculation was based on the data for 8 D-amino acids. Statistical significance, *: $P < 0.05$; **: $P < 0.01$.

8 D-amino acids tested, and that the postulated *dpa* gene^{6,7)} selectively controls sweet taste sensitivity to D-phe in mice.

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