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The Role of the *dpa* Locus in Mice

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Introduction

Genetic approaches to sweetener receptor mechanism in mice have provided evidence for existence of at least two loci influencing sweet sensitivity on the mouse chromosome 4: one is *dpa* [D-phenylalanine (D-Phe) sensitivity] (Ninomiya *et al.*, 1987), the other is *Sac* (saccharin preference)(Lush, 1989). The *Sac* locus has recently been found to encode a G-protein coupled receptor candidate, T1R3. Subsequent studies suggested that its heterodimer with T1R1 or T1R2 may function as an amino acid or a sweet taste receptor (Nelson *et al.*, 2001). With regard to the *dpa* locus, analyses on taste responses in sweet taster (C57BL) or non-sweet taster strains (BALB) demonstrated that the site of action of the locus is at the peripheral taste system and the locus influences responses not only to D-Phe but also to other sweet substances (Ninomiya and Funakoshi, 1993). Yet details of the role of the *dpa* locus remain unknown.

The scope of the present proceeding is to introduce the possible role of the *dpa* locus. We first touch upon strain differences of mice in sweet responses to D-Phe. We then mention mouse genetic studies of sweet receptor mechanisms for D-Phe. In the last part, we report the present knowledge on the *dpa* locus obtained from various analyses by using the *dpa* congenic strain (CG) having BALB background except for a DNA segment that includes the locus derived from the D-Phe responsive C57 strain.

Strain differences of mice in sweet taste response to D-phenylalanine

By using an electrophysiological approach with mice, we found prominent strain differences in whole nerve and single fiber responses of the chorda tympani nerve to two sweet tasting amino acids, D-Phe and L-proline (L-Pro) (Ninomiya *et al.*, 1984a). The whole chorda tympani responses to D-Phe were greater in C57BL/6-CrSlc (C57) mice than in BALB/cCrSlc (BALB) and C3H/HeSlc (C3H) mice. Response patterns across single fibers for D-Phe and L-Pro correlated with those for sucrose only in C57 mice. Subsequent behavioral studies that employed a conditioned taste aversion paradigm demonstrated consistent strain differences in behavioral responses to these amino acids (Ninomiya *et al.*, 1984b). A taste aversion conditioned to D-Phe generalized to sugars, saccharin and D-tryptophan in C57 mice, whereas in BALB and C3H mice it generalized to the isomer L-Phe (a bitter taste to humans), but not to the sweet substances.

Genetic approaches to sweet taste receptor mechanisms for D-phenylalanine

We further investigated the genetic basis for strain differences in behavioral and neural responses to D-Phe. We tested whether conditioned aversion of 0.1 M D-Phe would generalize to 0.1–0.5 M sucrose, phenotypes in F1 and F2 from C57 (sweet taster) and BALB (non-sweet taster) strains were clearly segregated into two groups with phenotypic ratios consistent with the expected Mendelian single locus model. We designed the gene, *dpa*, which has a major effect on sweet taste sensitivity to D-Phe (Ninomiya *et al.*, 1987). Concomitant measurements of the neural responses obtained from single chorda tympani fibers of each generation suggested clear correspondence with behavioral responses. That is, response patterns of sweet taster mice were comparable between D-Phe and sucrose, whereas non-sweet tasters show similar response patterns to D-Phe and quinine or HCl.

Various analyses of *dpa* locus by using the *dpa* congenic strain

To further evaluate genes controlling taste responses to D-Phe, CG was established using standard techniques (Ninomiya, 1991). The segment of the chromosome of the sweet taster C57 (donor), carrying the gene responsible for D-Phe, was transferred onto non-sweet taster BALB (partner) background by continuous backcrossing. Selection at each generation was made by using the conditioned taste aversion paradigm for behavioral measurement of the taste similarities between D-Phe and 0.1 M sucrose as described above. Behavioral responses of this CG strain to D-Phe as well as other sweeteners were not different from those of the donor C57 strain.

Neurophysiological studies were then undertaken to further characterize these three strains (Ninomiya, 1991). In both CG and C57 mice, chorda tympani responses to 0.1 M D-Phe was inhibited by the lingual treatment with the proteolytic enzyme mixture pronase E, and D-Phe responses were enhanced with the addition of saccharin, whereas these response characteristics were not observed in BALB mice. Relative response magnitudes to 0.5 M sucrose and 0.02 M saccharin were about twice as great for these strains than for BALB mice. The greater response to sucrose was evident at the concentration range between 0.03 and 1.0 M. Responses to other sweet tasting amino acids L-alanine and D-tryptophan, and to HCl and quinine were not different among three strains.

Gurmarin (a peptide isolated from the plant, *Gymnema sylvestre*) is known in rat to specifically inhibit responses to sweeteners but not to other basic taste stimuli, such as NaCl, HCl and quinine (Imoto *et al.*, 1991). In mice, the gurmarin inhibition of sweetener responses is strain- and nerve-specific (Ninomiya and Imoto, 1995; Ninomiya *et al.*, 1997). The relative response to sucrose (0.01–1.0 M) significantly decreased to ~50% of control after lingual exposure to gurmarin in ANOVA. The PLSD *post hoc* test indicates significant differences in responses at 0.1–1.0 M in C57 and at 0.3–1.0 M in CG. In BALB mice, no such gurmarin inhibition was observed (Figure 1). The sweet responses to D-Phe observed in C57 and CG almost

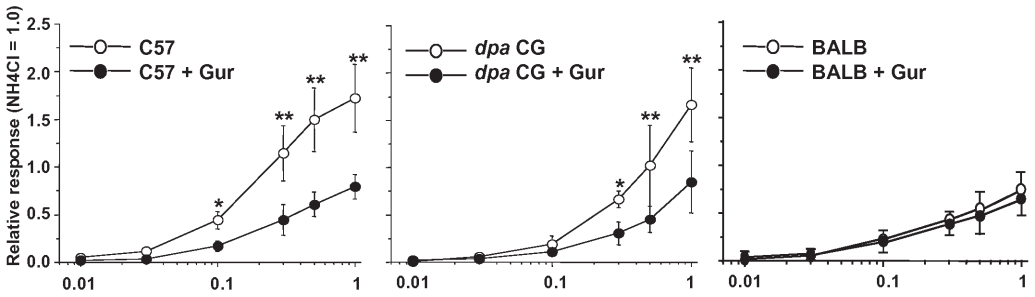

Figure 1 Concentration–relative response (NH_4Cl = 1.0) relationships for sucrose before (open circles) and after (filed circles) 4.8 μM gurmarin (Gur) lingual treatment in chorda tympani nerves in C57, CG and BALB mice. Vertical bars indicate SDs. Data were obtained from four mice for each strains. PLSD *post hoc* test, $*P < 0.05$; $**P < 0.01$ versus before Gur.

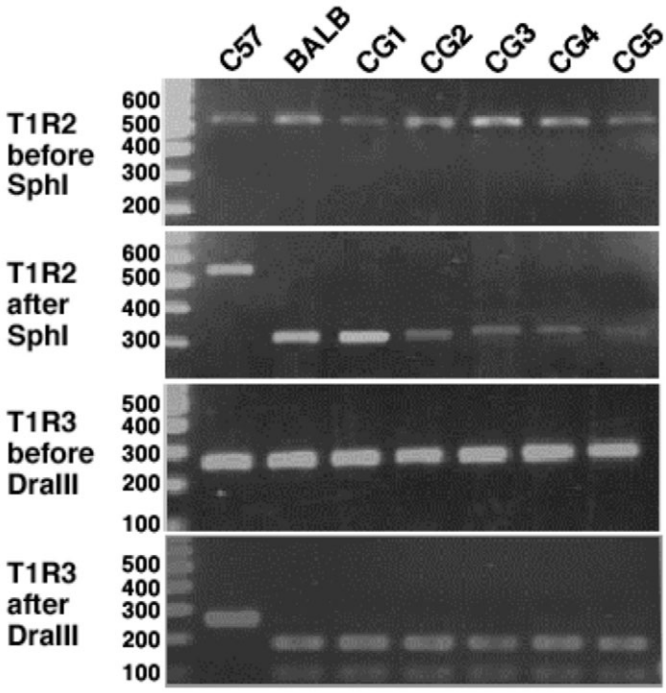

Figure 2 Gene typing of *T1R2* and *T1R3* in CG mice by using PCR with allele-specific primers and restriction enzymes.

disappeared after gurmarin lingual treatment; this suggests that the *dpa* locus may control gurmarin sensitive component in fungiform papillae in mice.

To investigate whether the sweet sensitivity to D-Phe in CG mice could be caused by *T1R2* or *T1R3* derived from the donor C57 strain, gene typing of these genes in CG, C57 and BALB strains was performed as reported previously (Montmayeur *et al.*, 2001). We amplified the *T1R2* or *T1R3* DNA segment containing the SNP region from each strain DNA by using specific PCR primers, and then digested the segment with SphI for *T1R2*, DraIII for *T1R3*, respectively. The amplified *T1R2* and *T1R3* PCR products from both CG and BALB, but not C57 mice, were completely digested with these restriction enzymes (Figure 2).

These results with CG mice suggest that the gene segment derived from C57 may control gurmarin-sensitivity of receptor and transduction system for sweet stimuli in fungiform papillae independently of the action of the T1R locus.

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