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Trehalose Sensitivity of the Gustatory Receptor Neurons Expressing Wild-type, Mutant and Ectopic *Gr5a* in *Drosophila*

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Introduction

Among ~70 candidate gustatory receptor genes identified from the *Drosophila* genome, *Gr5a* is the only gene that is allelic to a known gene, *Tre*, which controls taste sensitivity. *Tre* was discovered as an X-linked genetic polymorphism (Tre^{+} and Tre^{01}) among wild populations and laboratory strains (Tanimura *et al.*, 1982). Recent molecular studies on *Gr5a* and other gustatory receptor genes supported the finding that *Tre* encodes a functional gustatory sugar receptor and have provided novel information on the sugar sensitivity in the gustatory receptor neurons. *Gr5a* was found to be one of the candidate gustatory receptor genes discovered from the *Drosophila* genome database (Clyne *et al.*, 2000), with the locus in close proximity to *Tre* (Berkeley Drosophila Genome Project; http://flybase.net).

Mutations were obtained using a P-element insertion near the *Tre* locus (Isono *et al.*, 1998). Subsequent molecular analysis of the mutations provided key information to prove that *Tre* is identical to *Gr5a* (Dahanukar *et al.*, 2001; Ueno *et al.*, 2001). The induced mutations and the spontaneous mutation Tre^{01} provide a clue to the understanding of how a specific chemosensory receptor protein contributes to the sensitivity of the receptor neurons and the feeding response. In this paper we present physiological and behavioral data for the gustatory receptor TRE encoded by *Gr5a* in wild-type, mutant and transformant flies and discuss the sugar sensitivity of the gustatory receptor neurons.

Results

The dosage of the receptor TRE and the gustatory sugar sensitivity

A feeding preference test (Ueno *et al.*, 2001) to various concentrations of trehalose against control 2 mM sucrose was investigated in a trehalose-sensitive strain *Canton-S* (Tre^{+}), an insensitive strain *Oregon-R* (Tre^{01}) and the F1 females (Tre^{+}/Tre^{01}) from the cross of the two strains (Figure 1A). Trehalose sensitivity was defined as the concentration of trehalose that gives a preference index of 0.5. The mean sensitivity was estimated to be 9.8, 54 and 21 mM for *Canton-S*, *Oregon-R* and the F1 females, respectively. Thus the F1 females showed an intermediate value of the two parents, as was reported previously (Tanimura *et al.*, 1982). We then carried out a simpler preference test with a fixed concentration of 20 mM trehalose versus 2 mM sucrose in transformant flies where a 4.6 kb *Eco*RI–*Not*I genomic fragment containing the *Gr5a* gene or a 7.2 kb *Hin*dIII–*Eco*RI fragment containing the *CG3171* gene was ectopically introduced. A total of 10 independent transformants for each gene was obtained with a host strain $w^{1118}Tre^{01}$ and was mapped for each insertion. The mean preference index, based on nine *CG3171* transformants and nine *Gr5a* transformants, is shown in Figure 1B. *CG3171* transformant flies did not significantly modify the preference for both hemizygotes and homozygotes for the insertions, supporting the previous report by Dahanukar *et al.* (2001) but not supporting the result of Ishimoto *et al.* (2000), where ectopic *CG3171* rescued induced *Tre* mutation. In *Gr5a* transformants, however, the preference index was noticeably increased as was previously reported in an experiment where *Gr5a* was shown to rescue $Tre\Delta^{EP5}$ and $Tre\Delta^{EP19}$ mutations (Dahanukar *et al.*, 2001). Note that ectopic *Gr5a* homozygotes almost fully rescued Tre^{01} on the host X-chromosome. The *Gr5a* hemizygotes showed an intermediate sensitivity. Therefore we conclude that the *Gr5a* or Tre^{+} allele positively and gene-dose-dependently contributes to the trehalose sensitivity regardless of its ectopic or intrinsic origin. Hemizygous *Gr5a* transformants carrying homozygous intrinsic Tre^{01} showed a similar intermediate trehalose sensitivity as in Tre^{+}/Tre^{01} heterozygous F1 females. Therefore it is not supported that Tre^{01} negatively contributes to decrease trehalose sensitivity.

Effect of *Tre* mutations on the sugar sensitivity of the receptor neurons

Extracellular recordings from the tips of the labellar taste hairs of *Drosophila* provide information on neural activity of the unit

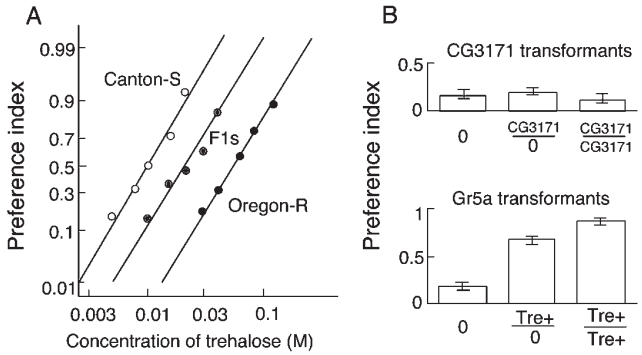

Figure 1 Preference to trehalose as measured by a two-choice feeding test in flies homozygous or heterozygous for Tre+/Tre01 alleles. **(A)** Dependency of preference index on the concentrations of trehalose solutions in *Canton-S* (open circles), *Oregon-R* (filled circles) and their reciprocal F1 females from the cross of *Canton-S* and *Oregon-R* male (gray circles). **(B)** Preference index of 20 mM trehalose versus 2 mM sucrose in a total of nine independent transformants carrying an ectopically introduced *CG3171* fragment (upper) or nine independent transformants carrying a *Gr5a* fragment (lower). Flies with hemizygous (middle) and homozygous (right) insertions are compared with the control host $w^{1118}Tre^{01}$ flies (left). Means and SDs were based on 100–200 female flies in (A) and 300–700 females for each construct in (B).

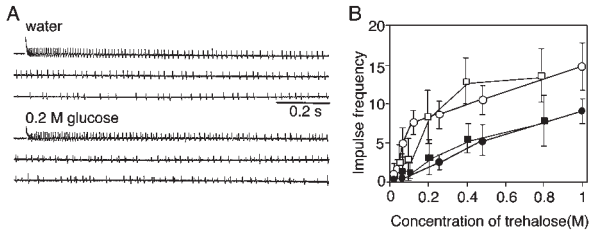

Figure 2 Electrophysiological activity of gustatory neurons recorded from the tip of a labellar taste sensillum. **(A)** Typical recordings of nerve impulses in *Canton-S* when the sensilla is stimulated by control water (upper trace) and 0.2 M glucose solution (lower trace). **(B)** Mean numbers of impulses from 0.2 to 0.4 s after onset of stimulation with various concentrations of trehalose are compared for *Canton-S* (open circles), *EP(X)496* (open squares) and the two mutant strains *Oregon-R* (filled circles) and Δ*EP3* (filled squares). Means and SDs were obtained from 5–18 recordings of the labellar long-type sensilla from 6–10 male and female flies.

gustatory receptor neurons. Stimulation with a sugar solution usually evokes a train of impulses different from the impulses evoked by water stimulation (Figure 2A; Fujishiro *et al.*, 1984). The number of impulses arising from sugar-sensitive neurons from 0.2 to 0.4 s after onset of the stimulation was compared in two Tre^+ and two *Tre* mutant strains (Figure 2B): *Canton-S* (Tre^+), *EP(X)496* (Tre^+), which is a parent strain used to induce P-element excision mutations by Isono *et al.* (1998), *Oregon-R* (Tre^{01}) and a P element excision mutant Δ*EP3* ($Tre\Delta^{EP3}$).

All four strains responded normally to sucrose stimulation (data not shown). Stimulation with trehalose solutions gave different responses depending on *Tre* alleles: very good responses were obtained to various concentrations of trehalose in the two Tre^+ strains (*Canton-S* and *EP(X)496*), while the response was noticeably reduced in Δ*EP3*, as was reported by Dahanukar *et al.* (2001) for two Δ*EP* mutations, $Tre\Delta^{EP5}$ and $Tre\Delta^{EP19}$. The responses of Δ*EP3* and *Oregon-R*, however, were not totally extinguished. Both strains showed similar trehalose sensitivities at higher trehalose concentrations. By comparing the concentration–response relationships of the four strains, it was suggested that 5–10 times higher trehalose concentrations are necessary to attain a similar level of response in *Oregon-R* and Δ*EP3* as compared with the two Tre^+ strains, suggesting a corresponding decrease in the trehalose sensitivity by the *Tre* mutations.

Discussion

In the feeding preference test we observed a gene-dose-dependent, positive contribution of Tre^+ allele to the gustatory trehalose sensitivity (Figure 1A,B). In the electrophysiological experiment it was also shown that normal gene-dose of intrinsic or ectopic Tre^+ ensures trehalose sensitivity of the receptor neurons (Figure 2B; Dahanukar *et al.*, 2001). Therefore the amount of the functional gustatory receptor TRE encoded by Tre^+ may be produced gene-dose dependently.

On the other hand, Tre^{01} contribution was not apparently observed in the feeding preference tests. In the receptor neurons Tre^{01} did not contribute to the electrophysiological trehalose sensitivity since Tre^{01} flies (*Oregon-R*) showed a trehalose sensitivity not significantly higher than in Δ*EP3*, where the *Gr5a* gene is severely disrupted and considered to produce no functional mRNAs and the receptor proteins (Ueno *et al.*, 2001). Tre^{01} is a polymorphic amino residue substitution Thr218Ala in the second intracellular loop domain of the seven-transmembrane protein TRE (Ueno *et al.*, 2001). It was also recently shown that, among the polymorphic sites in *Gr5a*, Thr218Ala is exclusively involved in controlling the trehalose sensitivity (Inomata *et al.*, 2004). Taken together, the present results suggest that Tre^{01} is a null mutation that inactivates the receptor function in an all-or-none fashion. The mutation may abolish binding interaction of the receptor with a sugar ligand or interaction with G protein and/or its activation. Future structure–function studies of Tre^{01} may provide clues to understanding the molecular mechanism of the receptor function and the gustatory transduction mechanism.

Where does the residual sensitivity to trehalose observed in *Oregon-R* and Δ*EP3* come from? The behavioral and physiological analysis in the present study suggests that the residual trehalose sensitivity is one-fifth to one-tenth of the Tre^+ trehalose sensitivity. Since mutations in TRE does not severely affect the sensitivity to sucrose and other sugars, other sugar receptor(s) must also be co-expressed in the neurons. The co-expressed receptor(s) may be mainly tuned to different subset of sugars but also weakly tuned to trehalose.

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