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 Tre01) 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 Tre01 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 (Tre01) and the F1 females (Tre+/Tre01) 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 EcoRI–NotI genomic fragment containing the Gr5a gene or a 7.2 kb HindIII–EcoRI fragment containing the CG3171 gene was ectopically introduced. A total of 10 independent transformants for each gene was obtained with a host strain w1118Tre01 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 TreΔEP5 and TreΔEP19 mutations (Dahanukar et al., 2001). Note that ectopic Gr5a homozygous almost fully rescued Tre01 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 Tre01 showed a similar intermediate trehalose sensitivity as in Tre+/Tre01 heterozygous F1 females. Therefore it is not supported that Tre01 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...
gustatory receptor neurons. Stimulation with a sugar solution (filled squares) and the two mutant strains trehalose are compared for from 0.2 to 0.4 s after onset of stimulation with various concentrations of solutions. Means and SDs were obtained from 5–18 recordings of the labelar long-type sensilla from 6–10 male and female flies.

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), EP04/496 (open squares) and the two mutant strains Oregon-R (filled circles) and ∆EP3 (filled squares). Means and SDs were obtained from 5–18 recordings of the labelar long-type sensilla from 6–10 male and female flies. All four strains responded normally to sucrose stimulation (data not shown). Stimulation with trehalose solutions gave different responses depending on Tre allelic mutant. 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 ∆EP3, as was reported by Dahanukar et al. (2001) for two ∆EP mutations, Tre∆EP3 and Tre∆EP10. The responses of ∆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 ∆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, Tre01 contribution was not apparently observed in the feeding preference tests. In the receptor neurons Tre01 did not contribute to the electrophysiological trehalose sensitivity since Tre01 flies (Oregon-R) showed a trehalose sensitivity not significantly higher than in ∆EP3, where the GrSa gene is severely disrupted and considered to produce no functional mRNAs and the receptor proteins (Ueno et al., 2001). Tre01 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 GrSa, Thr218Ala is exclusively involved in controlling the trehalose sensitivity (Inomata et al., 2004). Taken together, the present results suggest that Tre01 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 Tre01 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 ∆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.

References


