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Stereospecificity of the Receptor Site for Glycerol, a New Sweetener, in a Labellar Sugar Receptor Cell of *Drosophila*

Takaya Koseki[1], Masayuki Koganezawa[1], Akira Furuyama[1], Kunio Isono[2] and Ichiro Shimada[1]

[1]Department of Developmental Biology and Neuroscience, Graduate School of Lifesciences, Tohoku University, Sendai 980-8578, Japan and [2]Laboratory of Information Biology, Graduate of School of Information Science, Tohoku University, Sendai 980-8578, Japan

Correspondence to be sent to: Ichiro Shimada, e-mail: ishimada@mail.tains.tohoku.ac.jp

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Introduction

In 2001 there were remarkable developments in the research of sweet taste receptors of mammals and insects. In mammals, T1R2 and T1R3 are G protein-coupled receptors (GPCRs) with very long N-terminal extracellular domains, as shown in Figure 1. They have been shown to associate into a heterodimer and to function as a broadly tuned sweet receptor for various sugars, artificial sweeteners and D-amino acids (Nelson *et al.*, 2001; Li *et al.*, 2002). In *Drosophila*, on the other hand, TRE (trehalose sensitivity/Gr5a) is a GPCR with a short N-terminal extracellular domain. It was functionally identified and proved to respond specifically to the disaccharide trehalose (Clyne *et al.*, 2000; Dahanuker *et al.*, 2001; Dunipace *et al.*, 2001; Scott *et al.*, 2001; Ueno *et al.*, 2001; Chyb *et al.*, 2003). Is such a rigid specificity as that of TRE, which contrasts with mammalian T1R2 and T1R3, a general feature of the sweet taste receptors of insects? This question may be answered through the study of a specific receptor for glycerol, a new sweet tastant for *Drosophila* (Koseki *et al.*, 2004).

Glycerol stimulates the sugar receptor cell

Glycerol, a linear triol, is a sweet tastant for mammals but it was thought to be nonstimulative for the taste of insects (Dethier, 1955). Here we show electrophysiologically that it effectively stimulates the labellar sugar receptor cell of *Drosophila*. From the concentration–response curve for glycerol, the maximum response (R_m), the stimulus concentration at one-half of R_m (K), and the Hill coefficient were calculated from the Hill equation using the least-squares method: they are 24.0 ± 1.7 impulses/0.2 s, 0.324 ± 0.084 M and 0.94 ± 0.06, respectively (mean ± SEM). The Hill coefficient is close to one, indicating no cooperativity in the response to glycerol and suggesting a 1:1 ligand–receptor interaction.

A glycerol site model

The stimulatory effectiveness of various derivatives of glycerol and related compounds was examined systematically. Figure 2 summarizes the results obtained for their stimulating effectiveness in bar graph. The concentration for each compound was 1.0 M, which is close to the maximum response for most chemicals. Note that none are more stimulative than glycerol itself. The glycerol site was characterized by comparing the effectiveness of various derivatives of glycerol. Based on this structure–taste relationship of glycerol, a model is proposed for the glycerol site including three subsites and two steric barriers, which cannot accommodate carbon-ring-containing sugars such as D-glucose.

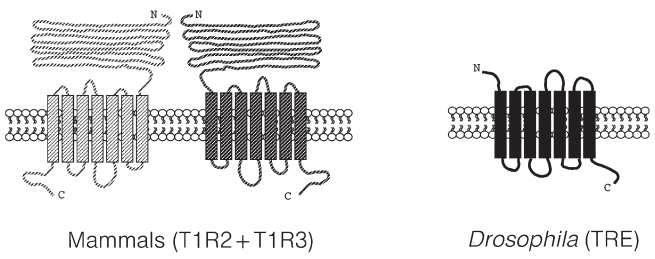

Figure 1 Mammalian and *Drosophila* sweet taste receptors.

Specificity of the glycerol site

Our model suggests rigid specificity for the glycerol site. This specificity can be confirmed by approaches other than structure-taste relationship: through inhibitors, for example. In the course of examining the effectiveness of glycerol derivatives, it was found that 2-amino-1,3-propanediol and 3-amino-1,2-propanediol are nonstimulative, but have a clear inhibitory effect on the response to glycerol. They inhibited the response to glycerol, specifically and competitively as compared with the almost total lack of effects of the reagent on the responses to the four sugars sucrose, D-glucose, D-fructose and trehalose.

The concentration-response curves for glycerol were found to be indistinguishable for I-type and L-type hairs. The curves of the responses to these four sugars, however, were significantly different. The magnitude of each response from an L-type hair is statistically larger than that from an I-type hair. This difference in the curves for glycerol and the four sugars is compatible with the presence of a specific receptor site for glycerol.

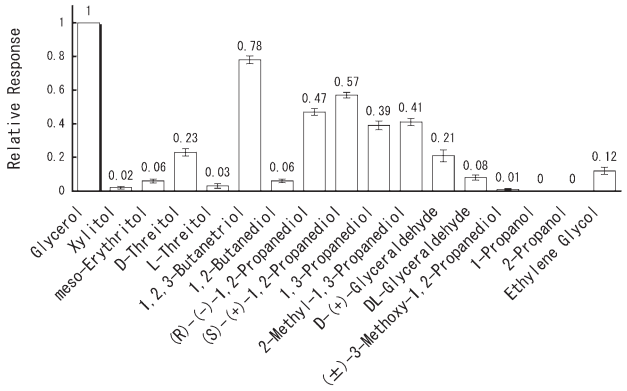

Figure 2 Stimulatory effectiveness of glycerol derivatives and related compounds. Relative response means the ratio of each response to the control response to glycerol. Figures on each bar are the mean values of the relative responses. The error bars indicate standard errors (n = 10–15).

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Gr5a was shown to be the gene *Tre* of the receptor for trehalose (Ueno *et al.*, 2001). *EP(X)496* is the wild-type strain with a normal *Gr5a* trehalose receptor and Δ*EP19* is a deletion mutant deficient in *Gr5a*. The concentration–response curves for glycerol were indistinguishable between Δ*EP19* and *EP(X)496*, whereas the response to trehalose was typically much less sensitive in the mutant. Therefore, the glycerol site appears to be different from the trehalose site.

Biological meanings of the glycerol site

With all the results presented so far, it can be concluded that the glycerol site is a rather specific, unique sweet taste receptor in *Drosophila*. What, then, are the biological implications of the glycerol site? A staple food of the fruit fly *Drosophila* is yeast, which releases ethanol as a product of fermentation. Ethanol attracts the fruit fly. Yeast also synthesizes glycerol (Gancedo *et al.*, 1968), the intracellular concentration of which approaches 0.9 M (André *et al.*, 1991). This glycerol is rapidly released from yeast cells upon hypo-osmotic shock (Kayingo *et al.*, 2001). Glycerol is therefore abundant around yeast. It is deduced that the fruit fly is first attracted by the scent of ethanol vapor released from yeast, and subsequently locates and feeds on the yeast by detecting the presence of the less labile released glycerol, for which the fly has evolved specific receptor sites in the labellar sugar receptor cells.

Finally, it is interesting to compare the diversity and specificity of insect sweet taste receptors with those of mammals. Insects have many more types of sweet taste receptor than mammals. There are presumed to be eight sweet receptors of GR family 2 in *Drosophila* and in the corresponding GR family 2 of *Anopheles* (malaria mosquito), while there are only two mammalian sweet taste receptors, T1R2 and T1R3 (Nelson *et al.*, 2001; Hill *et al.*, 2002; Li *et al.*, 2002). Regarding specificity, the insect TRE receptor responds to trehalose alone, but T1R2+3 (a heterodimer) responds to almost all mammalian sweet tastants. Together with the glycerol site and other receptor sites of the flies (Shimada *et al.*, 1974; Shimada, 1987; Furuyama *et al.*, 1999), rigid specificity is a general feature of sweet taste receptor of insects. This is also seen in the amino acid identity between sweet taste receptors of different species in the two groups: 70% of mice and humans, which is much higher than the mere 26% between *Drosophila* and *Anopheles*.

Therefore, it appears that different evolutionary strategies may be at work in insects and mammals regarding the evolution of sweet taste receptors: divergence and specialization in the former, and limited mutation and modification in the latter.

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