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A Specific Receptor Site for Glycerol, a New Sweet Tastant for *Drosophila*: Structure–Taste Relationship of Glycerol in the Labellar Sugar Receptor Cell

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Abstract

Glycerol, a linear triol, is a sweet tastant for mammals but it has not previously been recognized to stimulate the sense of taste in insects. Here we show by electrophysiological experimentation that it effectively stimulates the labellar sugar receptor cell of *Drosophila*. We also show that in accord with the electrophysiological observations, the behavioral feeding response to glycerol is dose dependent. 3-Amino-1,2-propanediol inhibited the response of the sugar receptor cell to glycerol, specifically and competitively, while it had almost no effect on responses to sucrose, D-glucose, D-fructose and trehalose. In the null *Drosophila* mutant for the trehalose receptor (Δ*EP19*), the response to glycerol showed no change, in sharp contrast with a characteristic drastic decrease in the response to trehalose. The glycerol concentration–response curves for I-type and L-type labellar hairs were statistically indistinguishable, while those for sucrose, D-glucose, D-fructose and trehalose were clearly different. These all indicate the presence of a specific receptor site for glycerol. 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.

Key words: chemoreception, electrophysiology, fly, inhibitor, mutant, receptor model

Introduction

Animals select and ingest nutrients among various substances in the environment according to their own requirements and food habits. They must detect various chemicals and discriminate them strictly, which may explain the evolution of multiple stereospecific receptor sites in insect taste cells. For example, the labellar sugar receptor cell of the fleshfly, *Boettcherisca peregrina*, responds to certain sugars, amino acids and nucleotides. Combining both pharmacological methods and analysis of the structure–taste relationships of stimulants, at least five different sites (pyranose, furanose, aryl, alkyl and nucleotide sites) have been revealed in a single sugar receptor cell (Shimada *et al.*, 1974; Shimada, 1987; Furuyama *et al.*, 1999). Their stereospecificity for each ligand is rigid. For example, sucrose and D-glucose react with the pyranose site and D-fructose, L-phenylalanine, L-valine and adenosine 5′-diphosphate (ADP) bind to the furanose, aryl, alkyl and nucleotide sites, respectively. Most of these receptor sites have been suggested to be G-protein-coupled receptors (GPCRs; Koganezawa and Shimada, 1997). In the fly genus *Phormia*, 4-nitrophenyl-α-glucoside and D-galactose were proven to bind to sites different from the pyranose and furanose sites (Wieczorek and Köppl, 1982; Wieczorek *et al.*, 1988). The housefly, *Musca domestica*, was reported to respond to lactose, which is instimulative to other flies and the specific site for lactose was suggested to be present in the sugar receptor cell (Schnuch and Seebauer, 1998). Lactose, abundant in milk, is of high nutritional value only for *M. domestica* (Galun and Fraenkel, 1957). The differences in receptor sites among different species of flies may therefore be related to their specific food requirements.

In *Drosophila*, the use of taste mutants and the pharmacological approach have revealed the existence of a pyranose, a 'fructose', a trehalose and other sites in a single sugar receptor cell (Isono and Kikuchi, 1974; Rodrigues and Siddiqi, 1981; Tanimura and Shimada, 1981; Tanimura *et al.*, 1982; Wieczorek and Wolff, 1989; Ueno *et al.*, 2001). Recent genetic studies have suggested that a family of GPCR genes, the *Gr* genes, comprising at least 56 members, encode *Drosophila* taste receptors. One of these genes, *Gr5a*,

was the first to be proven functionally to be a specific trehalose receptor (Clyne *et al.*, 2000; Dahanuker *et al.*, 2001; Dunipace *et al.*, 2001; Scott *et al.*, 2001; Ueno *et al.*, 2001; Chyb *et al.*, 2003). Thus, various sweet taste receptors with rigid stereospecificity for their ligands may have evolved in the flies.

In mammals, however, receptors for sweet and umami taste are apparently far fewer in number. T1Rs are a small family of only three GPCRs and candidate receptors, among which T1R2 and T1R3 associate 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).

Glycerol tastes sweet to humans (Moskowitz, 1971) and the ability of glycerol to stimulate taste receptors has been studied electrophysiologically in the gerbil (Jakinovich and Oakley, 1976). In behavioral tests on *Phormia*, however, glycerol showed no attractiveness when applied to the tarsi or single labellar hairs (Dethier, 1955). In *Boettcherisca,* preliminary electrophysiological studies (unpublished data) revealed that glycerol did not stimulate the labellar sugar receptor cell at all.

In the present study, we report electrophysiological evidence that glycerol stimulates the labellar sugar receptor cell of *Drosophila*, together with corresponding behavioral data. The effects of glycerol are compared to those of sucrose (and D-glucose), D-fructose and trehalose, which are presumed to be typical ligands specific for each of the three receptor sites, that is, the pyranose, 'fructose' and trehalose sites, respectively. Several approaches are used to present substantial evidence for the presence of a specific receptor site for glycerol in *Drosophila*.

Materials and methods

Flies

Strains of *Drosophila melanogaster* were maintained on a standard cornmeal agar medium at 25°C. *Canton*-S, *w cx* and *EP(X)496* were used as wild types. Δ*EP19* was isolated by imprecise excision of a P-element inserted near the taste receptor gene *Tre* (*Gr5a*) that controls gustatory sensitivity to a disaccharide trehalose. The mutant has a small genomic deletion that uncovers the whole promotor, 5′-untranslated region and the N-terminal domain of the coding sequence of the gene and shows noticeably reduced feeding preference to trehalose (Ueno *et al.*, 2001).

Female adult flies (4–6 days old) were fed on cornmeal agar medium for 3 days and then on 100 mM sucrose before the electrophysiological experiments. All the experiments were performed at an ambient temperature of 22 ± 1°C and at a relative humidity of 80–100%.

For behavioral tests, flies were used 1–2 days after eclosion. Prior to the tests, flies were allowed to feed on 100 mM sucrose solution for 2 h and then starved for 24 h, but supplied with distilled water.

Tip-recording method

A glass capillary (tip diameter 50–60 μm) filled with *Drosophila* Ringer solution (Ephrussi and Beadle, 1936) was inserted from the abdomen through the head and into the proboscis and served as an indifferent electrode. Tip-recordings (Hodgson *et al.*, 1955) from the labellar I and L hairs (Falk *et al.*, 1976; Nayak and Singh, 1983; Shanbhag *et al.*, 2001; Hiroi *et al.*, 2002) were performed using a recording capillary with a tip diameter of 30–40 μm. Usually, the electrolyte in which the stimulants were dissolved was 5 mM choline chloride (Wako Pure Chemical Industries Ltd, Tokyo, Japan). The recording electrode was connected to a preamplifier (MEZ-7101; Nihon Koden Ltd, Tokyo, Japan) and electric signals were further amplified, digitized (sampling rate = 20 kHz), stored on computer and analyzed using MacLab/4s (ADInstrument Ltd). Duration of stimulation was ~1 s with 3–5 min intervals to exclude adaptation effects. The solution at the tip of the recording capillary was renewed before stimulation by pressing a drop of fluid out of the capillary before touching a taste hair. The magnitude of the response was defined as the number of spikes obtained in the period from 0.15 to 0.35 s after the onset of the stimulus. Most electrophysiological data were obtained from I-type labellar hairs except those in Figure 7.

Behavioral tests

Determination of amount of intake

Tests were carried out by using micro test plates with 60 small wells (10 μl each; Nunc, Denmark) (Tanimura *et al.*, 1982) filled with glycerol solutions (3–1000 mM) prepared with 1% agar (Difco, noble) and 0.125 mg/ml brilliant blue FCF (a blue food dye). About 20 female flies were introduced into the plate. After being left to feed for 30 min at 25°C in the dark, flies were killed by freezing at –20°C. The amount of intake was determined colorimetrically after homogenization of the flies which had ingested the test solutions with the dye. Twenty female flies were homogenized in a centrifugation tube with a Teflon pestle in 200 μl of modified concentration of phosphate buffer, pH 7.4, including 75% ethanol. After centrifugation at 15 000 *g* for 60 min, the absorbance of the supernatant was measured at 630 nm.

Proboscis extension reflex (PER) for tarsal and labellar stimulation

Flies were fixed by attaching the dorsal thorax to the tip of a glass capillary (Narishige G-1) with nail vamish after 24 h starvation. For labellar stimulation, their legs were also immobilized. They were then placed in a humidified chamber for 6 h. Before the tests they were satiated with water. Flies were selected for tests only if they showed a clear PER upon touching the whole labellum or legs with a drop of 1 M sucrose solution. The results were expressed as the number of flies exhibiting a PER to test solutions.

Chemicals

Meso–erythritol, L-threitol, 1,2,3-butanetriol, 1,2-butanediol, (*R*)-1,2-propanediol, (*S*)-1,2-propanediol, 1,3-propanediol, 2-methyl-1,3-propanediol, 1-propanol, 2-propanol, ethylene glycol, 2-amino-1,3-propanediol and 3-amino-1,2-propanediol were purchased from Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan). Xylitol, D-threitol, D-(+)-glyceraldehyde, DL-glyceraldehyde, (±)-3-methoxy-1,2-propanediol and D-(+)-trehalose were from Sigma-Aldrich Corp. (St Louis, MO). Sucrose and D-(+)-glucose were from Wako Pure Chemical Industries (Tokyo, Japan). D-(+)-fructose, L-serine and ethanol were from Nacalai Tesque Inc. (Kyoto, Japan). In the inhibitor experiments, all the stimulants and inhibitors were dissolved in 1.8 M L-serine buffer (pH 8.4–8.6).

Results

Glycerol stimulates the sugar receptor cell (S cell)

Figure 1 shows the records from an I-type sensillum, which is known to have only two taste cells (Falk *et al.*, 1976; Nayak and Singh, 1983; Shanbhag *et al.*, 2001; Hiroi *et al.*, 2002). An L-type sensillum has typically four taste cells, each of which responds to sugar (S cell), water (W cell) or salts (L1 and L2 cells) (Hiroi *et al.*, 2002). Since the I-type sensilla lack W cells, W (water) spikes were never observed in Figure 1a when 5 mM choline chloride was used as the stimulus (which usually elicits only W spikes). In Figure 1b, the I-type sensillum responded to 250 mM sucrose and elicited only one type of regular spikes (S spikes) with equal intervals. Stimulation of the sensillum with 1M glycerol also elicited only one type of regular spikes with equal intervals (Figure 1c). Figure 1d shows the response to a mixture of 250 mM sucrose and 1 M glycerol, which resulted in one type of regular spikes with a higher frequency than each tastant alone. Since stimulation with 250 mM sucrose elicits S spikes, one of the spike types elicited in the response to the mixture including 250 mM sucrose must be an S-type spike. It is, therefore, concluded that glycerol stimulates S cells.

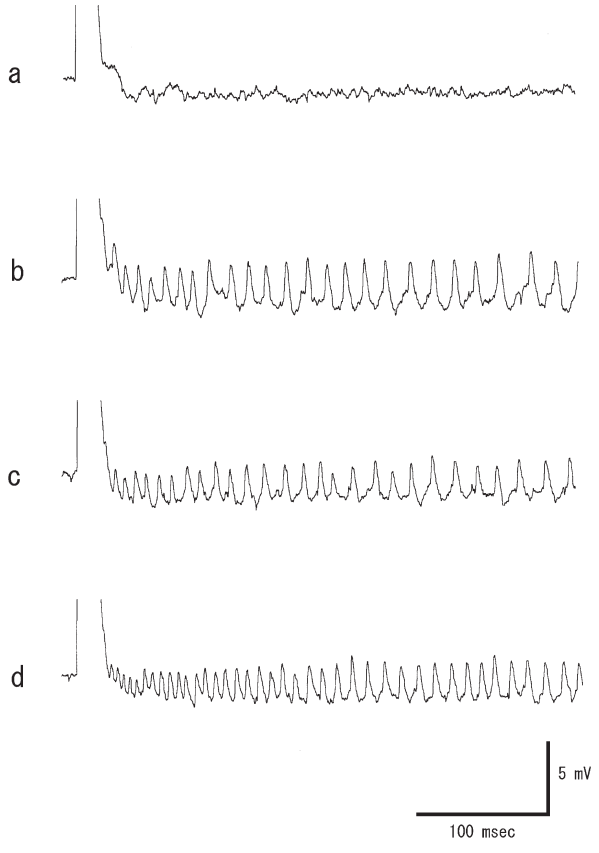

Figure 1 Records of typical responses of an I-type labellar hair to stimulation with **(a)** 5 mM choline chloride electrolyte alone, **(b)** 250 mM sucrose, **(c)** 1 M glycerol and **(d)** 250 mM sucrose + 1 M glycerol. Note that there are no W (water) spikes in **(a)**. Sucrose and glycerol were dissolved in 5 mM choline chloride electrolyte.

Figure 2 shows the concentration–response (C–R) curve for glycerol from the S cell. The maximum response (R_m), the stimulus concentration at one-half of R_m (K) and the Hill coefficient were determined using the least-squares method based on the Hill equation. They were 24.0 ± 1.7 impulses/0.2 s, 324 ± 84 mM 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.

Feeding response to glycerol

Behavioral tests are one criterion to know whether glycerol stimulates S cells, since most sugars that stimulate S cells should elicit a PER, where the flies were obsereved to spread their labellar lobes and begin sucking. Figure 3a shows the dependence of the amount of intake (µl/fly) on the concentration of glycerol. The intake volume of glycerol at 15 mM is significantly larger than water alone, while the ingested volume at 1 M was 0.31 µl, which is comparable with that of glucose (data not shown). In Figure 3b, PER was also

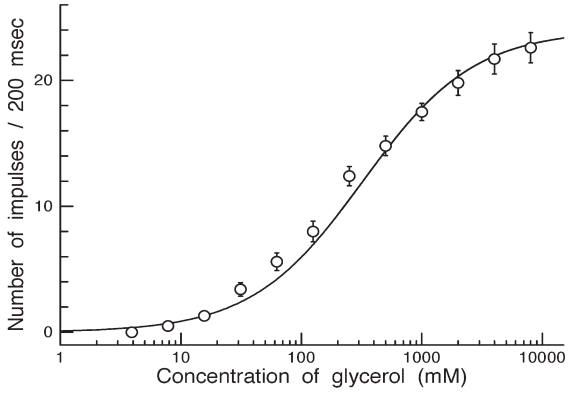

Figure 2 Concentration–response curve (mean value ± SEM, $n = 10$) for glycerol obtained from I-type labellar hairs. The ordinate represents the magnitude of the response measured as the number of impulses per 200 ms. The continuous curve is a least-squares fit of the data based on the Hill equation.

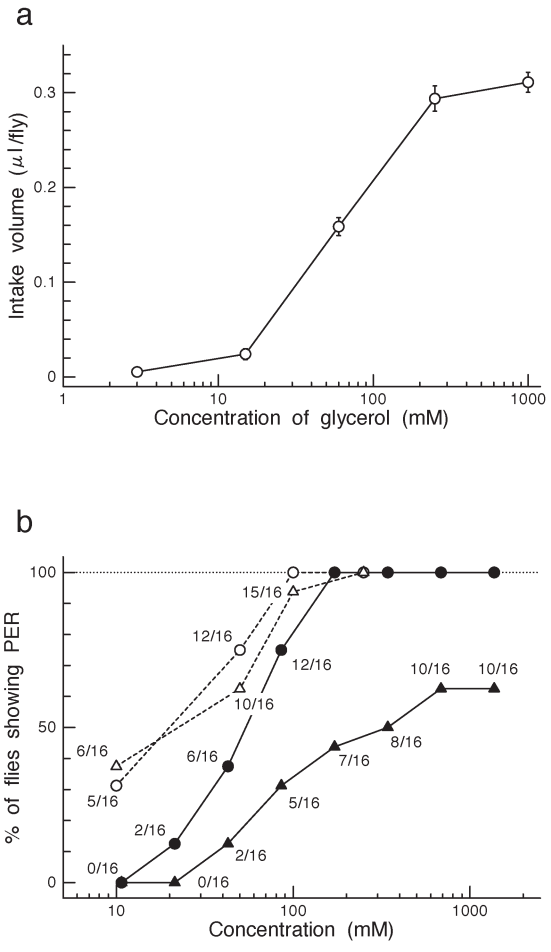

Figure 3 Feeding response to glycerol. **(a)** The ordinate indicates the ingested volume of glycerol solution per fly in μl. The vertical bars associated with each circle are the range of standard error (n = 10–12). **(b)** The percentage of flies showing PER is plotted against concentration of glycerol for labellar stimulation (filled circles) and tarsal (filled triangles) stimulation. For comparison, PER is also plotted against concentration of sucrose for labellar (open circles) and tarsal (open triangles) stimulation. The numerators and denominators of fractions attached to each symbol are the number of flies showing PER and the total numbers tested, respectively. Fractions corresponding to 100% were omitted.

clearly dependent on the concentration of glycerol. On stimulating the labellum with glycerol, PER approaches 100%, but only rises to 63% at maximum upon stimulating tarsi. This suggests a difference in the information processing between labellar and tarsal stimulation. On the other hand, PERs approaced 100% for both labellar and tarsal stimulation with sucrose, owing to its strong stimulus strength. These behavioral results may support the electrophysiological conclusion that glycerol stimulates S cells.

3-Amino-1,2-propanediol and 2-amino-1,3-propanediol, specific inhibitors of the response to glycerol

3-Amino-1,2-propanediol and 2-amino-1,3-propanediol themselves were instimulative for the sugar receptor cell in 1.8 M L-serine buffer (pH 8.4–8.6, itself also instimulative

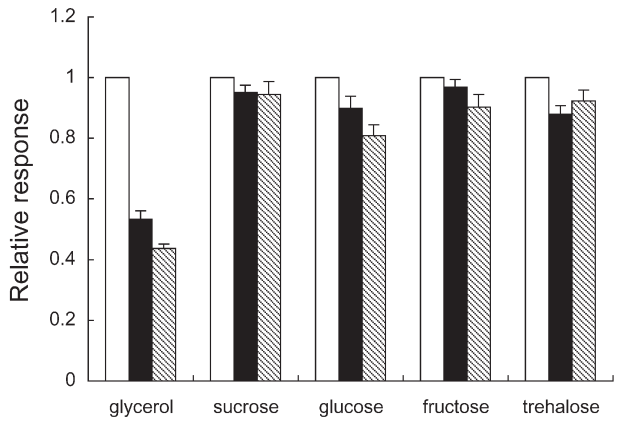

Figure 4 Relative responses of I-type labellar hairs to typical stimulants alone and to those mixed with inhibitors (mean values ± SEM, n = 10). The stimulants are 250 mM glycerol, 250 mM sucrose, 500 mM D-glucose, 500 mM fructose and 500 mM trehalose. Relative response on the ordinate is the ratio of the magnitude of the response to each stimulant alone (open bars) versus that mixed with 100 mM 3-amino-1,2-propanediol (filled bars) and that with 200 mM 2-amino-1,3-propanediol (hatched bars). All test solutions were made up in 1.8 M L-serine buffer.

for the sugar receptor cell). However, they clearly inhibited the response to glycerol. Figure 4 compares the control responses to glycerol and four sugar tastants alone with those mixed with 100 mM 3-amino-1,2-propanediol or 200 mM 2-amino-1,3-propanediol. The four sugar tastants are presumed to be typical ligands specific for each of the three known receptor sites, namely pyranose (for sucrose and glucose), 'fructose' and trehalose sites, respectively. Both 100 mM 3-amino-1,2-propanediol and 200 mM 2-amino-1,3-propanediol specifically inhibited the response to glycerol, while their effects on the responses to other sugars were negligible (Figure 4).

Effects of 3-amino-1,2-propanediol on the C–R relationships of glycerol

Figure 5 shows the C–R curves of glycerol alone and when mixed with 100 mM 3-amino-1, 2-propanediol in 1.8 M serine buffer. Both curves are sigmoid. From the curves fitted by the Hill equation, R_m, K and the Hill coefficient were determined using the least-squares method (Table 1). The difference in the values for glycerol alone between Table 1 and Figure 2 may be due to the different solvents; 1.8 M L-serine buffer versus 5 mM choline chloride. The two curves in Figure 5 clearly differ in K-value, but not in the maximum response, R_m. The K-value for the mixture of glycerol with 100 mM 3-amino-1,2-propanediol was three times that for glycerol alone, while the R_m values were indistinguishable. The inhibition therefore seems to be competitive and the inhibitor may bind with the specific site for glycerol. Both Hill coefficients are close to unity, but the slope of the C–R curve for glycerol mixed with 3-amino-1,2-propanediol was

slightly steeper than that with glycerol alone, reflecting a slightly larger Hill coefficient.

Response to glycerol in null mutant for trehalose receptor

As shown in Figure 6a, the C–R curves for glycerol were indistinguishable between the null mutant (Δ*EP19*) deficient in trehalose receptor and the wild type (*EP(X)496*), whereas the response to trehalose was typically much less sensitive, but still remained distinguishable in the mutant (Figure 6b).

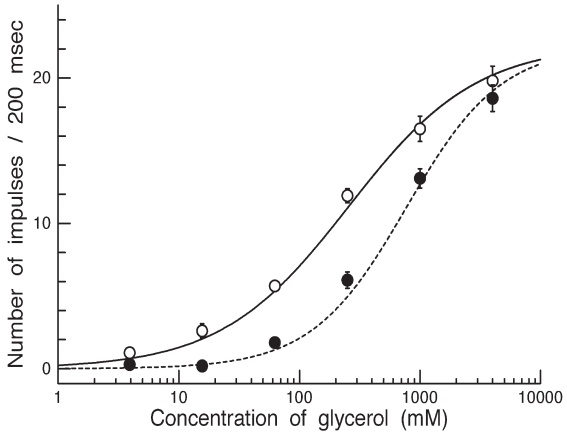

Figure 5 Concentration–response curves obtained from I-type labellar hairs (mean values ± SEM, $n = 10$) for glycerol alone (open circles) and for glycerol mixed with 100 mM 3-amino-1,2-propanediol (filled circles). Continuous lines are least-squares fits of the data based on the Hill equation. All test solutions were made up in 1.8 M L-serine buffer.

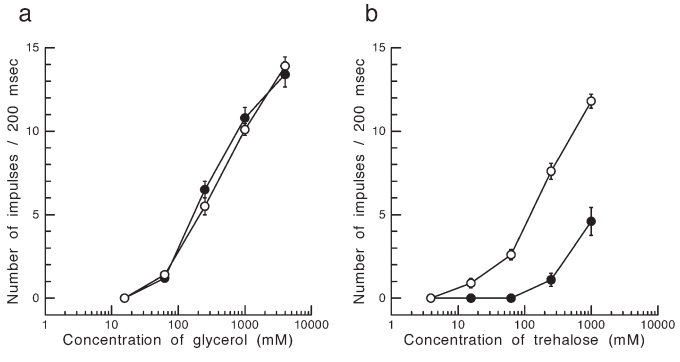

Figure 6 Comparison of concentration–response curves (mean values ± SEM, $n = 10$) obtained from I-type labellar hairs for **(a)** glycerol and **(b)** trehalose between the wild type, *EP(X)496* (open circles) and the null mutant, Δ*EP19* (filled circles).

Therefore, the glycerol site appears to be different from the trehalose site (receptor).

Comparison of C–R curves for glycerol and various sugars between I- and L-type sensilla

The C–R curves for glycerol were found to be indistinguishable between the I-type and L-type sensilla (Figure 7). The curves for the four sugars were, however, significantly different: the magnitude of each response from the L-type sensilla being statistically larger than that from the I-type. This difference in the C–R curves between glycerol and the four sugars is compatible with there being a specific receptor site for glycerol.

Comparison of the stimulating activities of glycerol derivatives and related compounds

The stimulatory effectiveness of various derivatives of glycerol and related compounds were examined systematically. The concentration for each compound was 1 M. The structure of each is shown in Figure 8 and the results obtained for their stimulating effectiveness are summarized in Table 2.

Comparison among linear polyols

The order of stimulating effectiveness of polyols is as follows, using the letter codes in Table 2 and Figure 8: triols (a, f) > diols (g, h, i, j, q) > tetrols (c, e, with the exception of d) > pentols (b) > monools (o, p). The triols are the most stimulative; none is as effective as glycerol.

Comparison of diol derivatives with glycerol

Since the diols (h, i, j) are stimulative, but less effective than glycerol, one of the three hydroxyl groups of glycerol, regardless of the position, is not essential for stimulation, but necessary to enhance the efficacy of stimulation. When the third hydroxyl group of glycerol was replaced by a methoxyl group (–OCH_3; n) or a double-bonded oxygen (=O; l, m), the derivatives became much less stimulative. This may indicate the role of the hydroxyl group as a hydrogen donor in hydrogen bond formation. When derivatives (h, i) had one extra (g) or one less (q) methyl group at the third carbon atom, the response was much reduced. Derivatives with three carbon atoms, therefore, appear to have the optimal stimulating effectiveness. Substituents larger than hydrogen, such as a methyl (f) or a hydroxyl methyl group (c, d, e), at the third carbon atom of glycerol decreased stimulating effectiveness. This may indicate the presence of a

Table 1 The K, R_m and Hill coefficients for glycerol alone and glycerol in the presence of 3-amino-1,2-propanediol

Stimulus	K (mM)	R_m (impulses/200 ms)	Hill coefficient	No. of test
Glycerol	256 ± 47	22.3 ± 1.7	0.82 ± 0.06	10
Glycerol + 3-amino-1,2-propanediol	772 ± 179	22.2 ± 2.1	1.11 ± 0.08	10

Mean values ± SEM are shown.
All chemicals were dissolved in 1.8 M L-serine buffer and data were obtained from I-type labellar hairs.

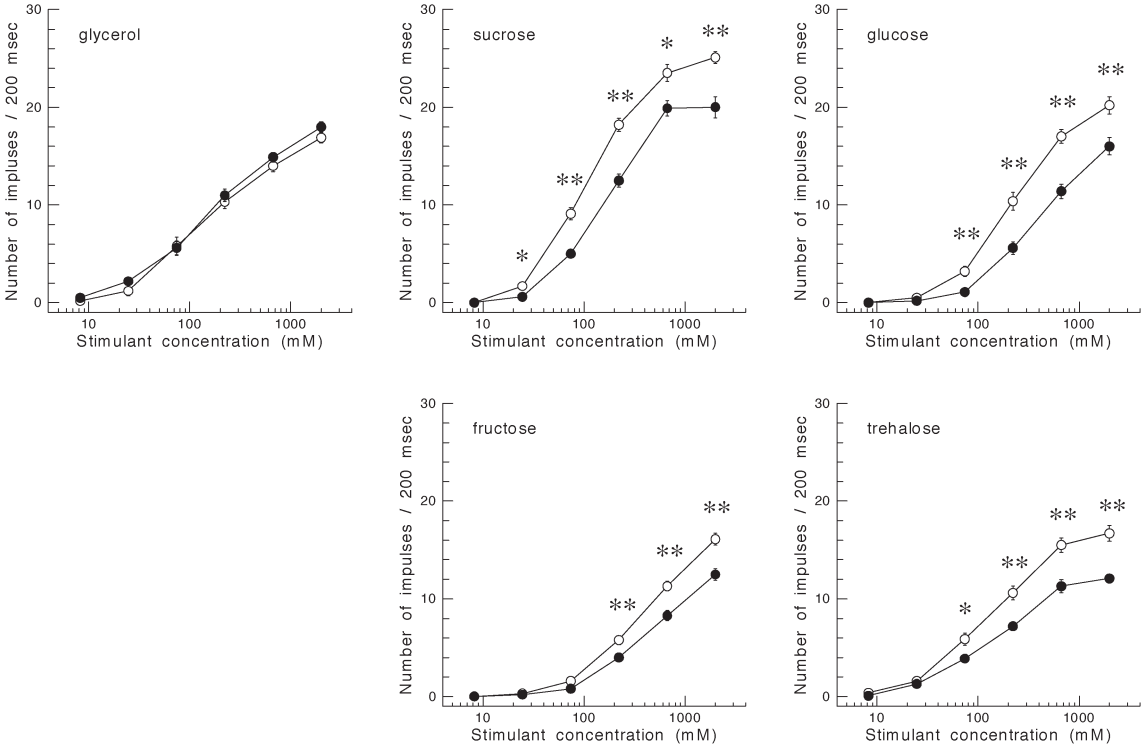

Figure 7 Comparison of concentration–response curves (mean values ± SEM, $n = 10$) for glycerol, sucrose, D-glucose, D-fructose and trehalose between L-type (open circles) and I-type (filled circles) labellar hairs. Asterisks indicate significant differences between the two hairs (*$P < 0.05$; **$P < 0.01$, Mann–Whitney U-test).

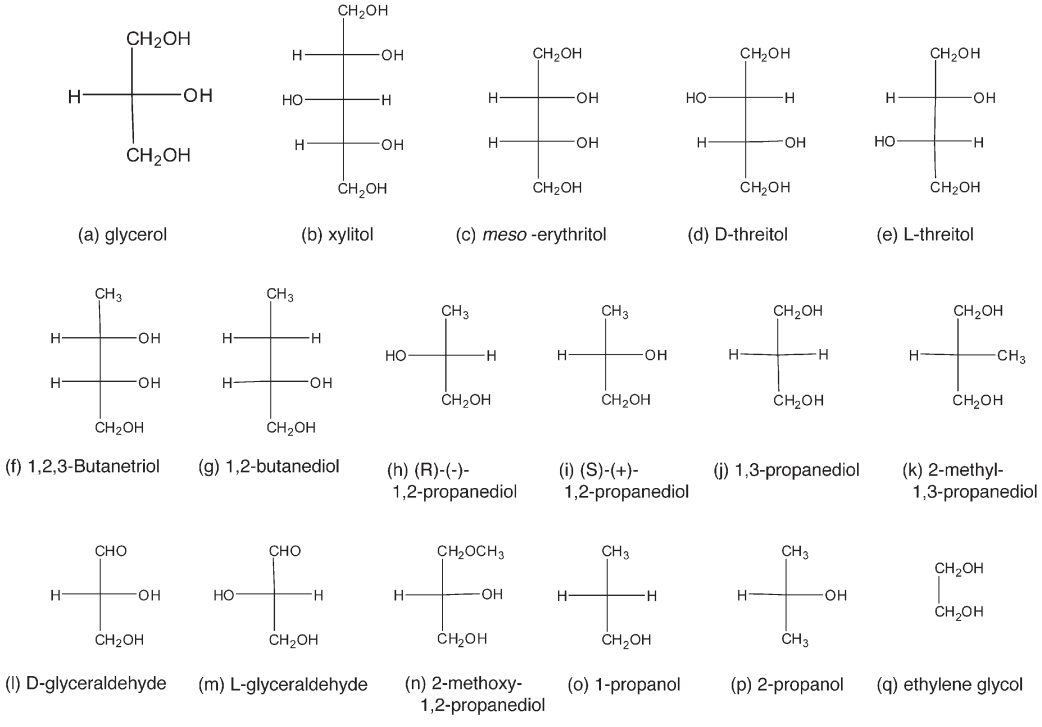

Figure 8 Structure of glycerol derivatives and related compounds.

Table 2 Stimulatory effectiveness of glycerol derivatives and related compounds

Stimulus	Relative response (mean ± SEM)	No. of test
(a) Glycerol	1.0	29
(b) Xylitol	0.02 ± 0.007	15
(c) Meso-erythritol	0.06 ± 0.012	10
(d) D-Threitol	0.23 ± 0.021	10
(e) L-Threitol	0.03 ± 0.014	10
(f) 1,2,3-Butanetriol	0.78 ± 0.023	13
(g) 1,2-Butanediol	0.06 ± 0.011	10
(h) (*R*)-(–)-1,2-Propanediol	0.47 ± 0.02	11
(i) (*S*)-(+)-1,2-Propanediol	0.57 ± 0.017	11
(j) 1,3-Propanediol	0.39 ± 0.026	11
(k) 2-Methyl-1,3-propanediol	0.41 ± 0.021	11
(l) D-Glyceraldehyde	0.21 ± 0.035	11
(m) L-Glyceraldehyde	0.08 ± 0.015	11
(n) 2-Methoxy-1,2-propanediol	0.01 ± 0.005	11
(o) 1-Propanol	0 ± 0	10
(p) 2-Propanol	0 ± 0	10
(q) Ethylene glycol	0.12 ± 0.021	11

steric barrier of the glycerol site near the third carbon atom of the stimulant, glycerol. Inferences about the third carbon atom of the derivatives can be applied equally to the first carbon atom owing to the symmetry of glycerol. Replacement of a hydrogen at the second carbon atom by a methyl group (k) did not affect the stimulating effectiveness of 1,3-propanediol (j).

Discussion

Glycerol is a sweet tastant for mammals, but was previously thought to be instimulative for the taste of insects (Dethier, 1955; Moskowitz, 1971; Jakinovich and Oakley, 1976). The present study, however, has provided the evidence that glycerol effectively stimulates the labellar sugar receptor cell of *Drosophila* (Figure 1).

A new receptor site specific for glycerol

3-Amino-1,2-propanediol inhibited the response to glycerol, specifically and competitively, compared with almost no effects on those to sucrose, D-glucose, D-fructose and trehalose (Figures 4 and 5). In the null *Drosophila* mutant for the trehalose receptor (Δ*EP19*), the response to glycerol did not show any change, in sharp contrast with a characteristic drastic decrease in the response to trehalose (Figure 6). Note that the results of the present study indicate that in the *Drosophila* trehalose mutant the response to trehalose is greatly reduced but not abolished, in contrast to the report of Dahanuker *et al.* (2001). The C–R curves for glycerol from both I- and L-type labellar hairs were statistically indistinguishable, while those for sucrose, D-glucose, D-fructose and trehalose were clearly different (Figure 7). These all indicate the presence of the specific receptor site for glycerol (glycerol site) different from the pyranose, 'fructose' and trehalose sites in *Drosophila*.

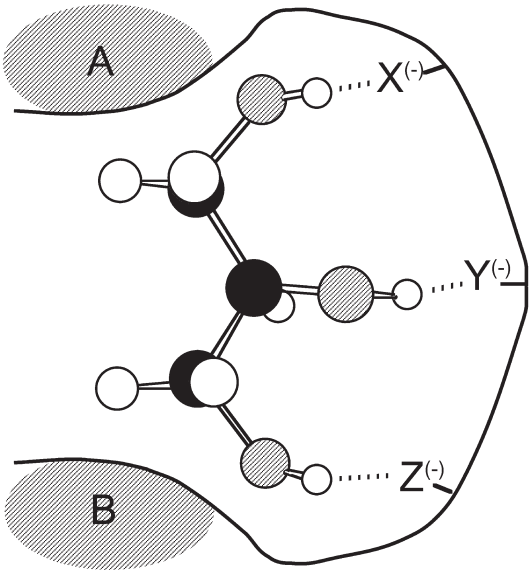

Figure 9 Model of the glycerol site. The site has three subsites, X, Y, Z and two steric barriers, A and B. open circles, hydrogen atoms; filled circles, carbon atoms; hatched circles, oxygen atoms.

Glycerol site model

By comparing the effectiveness of various derivatives of glycerol, we propose a model of the specific glycerol site composed of three subsites (X, Y and Z) and two steric barriers A and B (Figure 9). The three subsites form hydrogen bonds with the three hydroxyl groups of glycerol, which are necessary to evoke an effective response in the sugar receptor cell. X, Y and Z are proton acceptors. The two steric barriers take part in the structural specificity of glycerol for stimulation. They fit the chain of three carbon atoms of glycerol but cannot accommodate a sugar with a carbon ring, such as glucose. The absolute configuration of glycerol in the glycerol site in Figure 9 is shown only tentatively since it has not yet been determined (Uzawa *et al.*, 1990).

The relative response of 0.78 of 1,2,3-butanetriol (which has four carbon atoms) can be explained by the steric hindrance to CH_3 added to glycerol at the third carbon atom. Threitols (c, d, e) and xylitol (b), with more than three carbon atoms, are expected to undergo similar steric hindrance. Propanediols (h, i, j, k) are moderately stimulative but less so than glycerol since they have only two hydroxyl groups. Due to strong steric hindrance to the

methoxy group, 3-methoxy-1, 2-propanediol (n) is almost instimulative. Ethylene glycol, with two carbon atoms and two hydroxyl groups shows low stimulation. With their aldehyde group, glyceraldehydes (l, m) cannot bind to X or Z due to the property of the proton acceptor and so they, too, show a low level of stimulation. Propanols (o, p) are instimulative because they have only a single hydroxyl group.

The specific inhibition of the glycerol response by 3-amino-1,2-propanediol and 2-amino-1,3-propanediol may be caused by the presence of a protonated amino group ($-NH_3^+$), which forms an ionic bond with X, Y, or Z. The interaction may be stronger than a hydrogen bond and may freeze any conformation changes at the glycerol site required to evoke the response.

This glycerol site model contrasts with that for pyranose (cf. Shimada *et al.*, 1974; Shimada, 1987). Specificity is much more rigid in the former than the latter, although for both sites three hydrogen bond formations are necessary to elicit the full response of the sugar receptor cell. Among the chemicals examined so far, glycerol is the most stimulative and the only natural substance that reacts with the glycerol site. On the other hand, D-glucose, sucrose, maltose and various other oligosaccharides with a pyranose ring residue react effectively with the pyranose site. The difference in specificity may reflect the difference in function. The glycerol site is specialized to detect glycerol, which is a marker indicating the presence of yeast (see below). In contrast, the pyranose site is relatively generalized for detecting substances that are a source of energy (rather than other kinds of nutrient).

Biological implications of the glycerol site

The presence of various receptor sites of taste cells revealed so far in different fly species appears to be related to their particular food habits. Yeast is a main component of the food of the fruitfly *Drosophila*. It releases ethanol as the product of fermentation, which attracts the fruitfly. It 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. The fruitfly *Drosophila* can sense concentrations of 15 m M glycerol (Figure 2) and ingest it (Figure 3a). It is therefore deduced that the fruitfly 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 may have evolved specific receptor sites in the labellar sugar receptor cells.

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