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The labellar sugar receptor of *Drosophila*

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Summary. 1. The electrophysiology of the sugar receptor in labellar taste hairs of *Drosophila melanogaster* (Diptera) was investigated using 33 monosaccharides, oligosaccharides, glucosides, and amino acids which in calyptrate flies are known to bind to specific receptor sites or which may be assigned to specific receptor sites on the basis of structural criteria.

2. The pyranose site of *Drosophila* is very similar to the pyranose site of the calyptrate flies: regarding monosaccharides of the pyranose type three adjacent equatorial hydroxyl groups (C-2, C-3, C-4) seem to be important for stimulating effectiveness. On the other hand, it exhibits a more rigid stereospecificity with regard to the substituents at C-1 and C-5.

3. A furanose site as in calyptrate flies does not exist in *Drosophila*. First, D-galactose, phenylalanine and 2,5-anhydro-D-mannitol are not or nearly not stimulatory. Secondly, according to different sugar receptor responses after treatment of the taste hairs with papain, D-fucose binds to another receptor site than D-fructose. Thirdly, the effective conformation of D-fructose is not the furanose, but most probably the pyranose form as can be concluded from experiments with freshly prepared and equilibrium solutions of D-fructose.

4. The characteristic differences between the properties of the sugar receptors of *Drosophila* and of the calyptrate flies lead to the suggestion that the actual number of types of receptor sites in the various fly species is greater than assumed up till now. The broad specificity of the sugar receptors of flies may therefore result from a mosaic of different types of highly specific receptor sites.

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Introduction

One central aspect in chemoreception is the problem dealing with the molecular basis of specificity. In bacteria, the recognition of chemical compounds is enabled by many different types of receptor proteins. Each type shows a pronounced specificity and reacts to a very limited number of ligands (Lengeler 1980). Therefore the broad chemospecificity of the whole bacterial cell is based on a mosaic of diverse receptor proteins. In eukaryotes, the question for the broad specificity of certain chemosensitive receptor cells has not been answered up till now; one of the reasons is that, caused by the complex arrangement of many different types of chemosensitive receptor cells, it is difficult or impossible to record reactions from clearly identifiable populations of the same cell type. An exception is the sugar receptor of the calyptrate flies which has become, because of the easy electrophysiological access to single identifiable receptor cells, the best investigated eukaryotic chemosensitive receptor cell (Dethier 1976). Today we know that the broad specificity of the sugar receptor with regard to many carbohydrates, amino acids and fatty acids cannot be explained by a single type of receptor site (see Morita and Shiraishi 1985).

The first indirect indication in favour of more than one type of receptor site was the report of Dethier (1955) that in *Phormia regina* D-mannose inhibited the behavioural response to D-fructose, but not to D-glucose. This result led to the proposal of a 'glucose' and of a 'fructose' site (Evans 1963). Shimada and coauthors introduced a 'pharmacological' approach by blocking the electrophysiologically measured response of the sugar receptor selectively with group specific protein modi-

fying reagents. In this way they could directly demonstrate various types of receptor sites in *Boettcherisca* (Shimada et al. 1974; Shimada 1975; Shimada and Isono 1978; Shimada 1978): D-glucose and various other mono- and disaccharides could be assigned to the 'pyranose' site, D-fructose, D-fucose, D-galactose and several aromatic amino acids to the 'furanose' site, and several aliphatic amino acids and fatty acids to the 'carboxylate' site. The existence of three distinct types of receptor sites in a single chemosensitive receptor cell led to the explicit formulation of the concept of 'multiple receptor sites' (Shimada 1978). Additional types of receptor sites were discovered in other flies. In *Phormia*, Wieczorek and coauthors demonstrated that 4-nitrophenyl-α-glucoside as well as D-galactose bind to sites different from the pyranose and the furanose site (Wieczorek and Köppl 1982; Wieczorek et al. 1988). In the acalyptrate *Drosophila*, the pharmacological approach and the use of taste mutants revealed the existence of a pyranose, a furanose, and a trehalose site (Isono and Kikuchi 1974a; Kikuchi 1975; Rodrigues and Siddiqi 1981; Tanimura and Shimada 1981; Tanimura et al. 1982).

Drosophila is a preferred object of genetic research. The advantage of its use also for the investigation of the molecular basis of chemospecificity is evident, but more detailed knowledge about the specificity of its sugar receptor is needed. Therefore we examined the reaction spectrum of the sugar receptor of *Drosophila* in a more comprehensive way than it had been done before. Furthermore we believe that the comparison of the sugar receptor specificity of the acalyptrate *Drosophila* with that of the calyptrate flies will provide insights into aspects of the evolution of receptor sites. The results of this paper show a high degree of conformity between *Drosophila* and the calyptrate flies, but also characteristic differences. These differences may result from the existence of more types of receptor sites than assumed up till now.

Materials and methods

Two to four days old female adults of *Drosophila melanogaster* (Oregon R) from a laboratory culture were used for all investigations. Before the experiments they were fed for up to two days on 0.1 mol/l sucrose and water. All experiments were performed at an ambient temperature of 23 ± 1 °C, and at a relative humidity of 80–100%.

Tip-recordings (Hodgson and Roeder 1956) from the labellar L-hairs 1–3 (Fujishiro et al. 1984) were performed using recording capillaries with a tip diameter of 7–14 µm. Usually the electrolyte in which the stimuli were dissolved was 0.03 mol/l tri-choline citrate (made from choline dihydrogen citrate titrated to pH 7 with choline base). Contact with the input stage of the electrometer amplifier was achieved by a piece of silver wire. The indifferent electrode was an Ag/AgCl wire in a glass capillary (tip diameter 40 µm) which was filled with *Drosophila* Ringer solution (Ephrussi and Beadle 1936). This capillary was inserted through the intersegmental fold between thorax and abdomen and driven into the proboscis. The resistance of the preparation was 90–100 MΩ (30 Hz). Impulses were amplified and fed into a Z-80 microcomputer for processing (Piesch and Wieczorek 1982; Piesch 1983) either filtered (150 Hz/3.3 kHz, −3 dB) or unfiltered. Duration of stimulation was approximately 1 s with 3 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 receptor response was defined as the number of spikes obtained in the period of 50–350 ms after the onset of the stimulus (Wieczorek 1980). The control stimulus was 1 mol/l sucrose, applied at the beginning of an experiment and after every third up to fifth test stimulus. The receptor response to a test stimulus was related to the mean response of two enveloping control stimuli.

Treatment with papain was modified according to Tanimura and Shimada (1981) as follows. A capillary filled with 1 mg/ml freshly prepared papain in 0.02 mol/l sodium phosphate buffer pH 7 containing 0.1 mmol/l dithiothreitol was slipped over the tip of the taste hair for 3–7 min. After this procedure the hair tip was washed for 1 min with *Drosophila* Ringer solution. After at least 2 min after the end of the washing step the first test stimulus was applied. Analysis was made only of experiments in which the response of the sugar receptor to D-fructose was suppressed by papain treatment to a value of less than 40% compared to the response before treatment.

Chemicals used were usually of the highest purity commercially available and were supplied by the following firms: Fluka (L-arabinose, D-fructose, D-fucose, L-fucose, D-galactose, D-glucose, L-glucose, methyl-β-D-glucopyranoside, 4-nitrophenyl-α-D-glucopyranoside, trehalose, D-xylose), Merck ($CaCl_2 \times 2H_2O$, NaH_2PO_4, LiCl, NaCl, sucrose), Roth (maltose), Serva (2-deoxy-D-glucose, 6-deoxy-D-glucose, maltitol, maltotriose, maltotriitol, maltotetraitol, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, methyl-α-glucopyranoside, 4-nitrophenyl-α-D-maltoside, 4-nitrophenyl-α-D-maltotrioside, 4-nitrophenyl-α-D-maltopentaoside, 4-nitrophenyl-α-D-maltoheptaoside, L-sorbose, L-phenylalanine, L-valine), Sigma (2,5-anhydro-D-mannitol, choline base, choline dihydrogen citrate, DL-dithiothreitol, 3-O-methyl-D-glucose, papain).

Results

Use of tri-choline citrate as electrolyte

Recordings from labellar taste hairs of the calyptrate flies *Phormia* or *Boettcherisca* can easily be analyzed as the spikes elicited in the water, sugar and salt receptor have different and constant amplitudes. However, in *Drosophila*, the amplitudes of spikes recorded from these receptors are not constant but positively correlated with the spike frequency (Fujishiro et al. 1984). Therefore, the identification of the three receptors is very difficult especially for responses in which water and sugar receptor are involved, a usual situation when the stimulating effectiveness of sugars is tested. Using LiCl or NaCl as electrolytes we observed the same

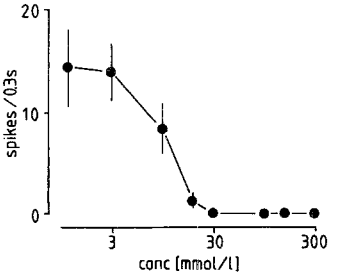

Fig. 1. Dose-response curve of the water receptor for tri-choline citrate ($n=4$–5). Vertical bars: SEM values

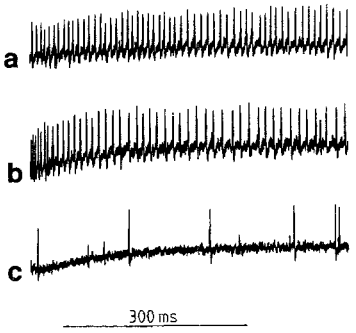

Fig. 2a–c. Responses of the sugar receptor to **a** 1 mol/l sucrose, **b** 0.1 mol/l sucrose and **c** 0.01 mol/l sucrose, all dissolved in 0.03 mol/l tri-choline citrate. The few small spikes are from the water receptor. All three (filtered) recordings are from the same receptor cell

Table 1. Dependence of sugar receptor responsiveness on the use of the electrolyte

	Electrolyte		*P*
	5 mmol/l NaCl	30 mmol/l CC	
1 mol/l sucrose	1.15±0.22	1.0	≤0.05
1 mol/l D-glucose	1.41±0.27	1.32±0.29	≤0.5
1 mol/l D-fructose	0.67±0.19	0.45±0.19	≤0.05

The data are from the same set of receptors ($n=10$ for sucrose and D-glucose; $n=8$ for D-fructose). Relative responses are shown as mean values ±SD. *CC* tri-choline citrate; *P* levels of significance according to two-tailed *t*-tests

phenomena as described by Fujishiro et al. (1984). But using tri-choline citrate at the concentration of 0.03 mol/l, the water receptor activity was inhibited (Fig. 1) and we no longer could observe the dependence between spike frequency and spike amplitude (Figs. 2 and 3a). With tri-choline citrate as electrolyte unfiltered spikes were purely monophasic at any spike frequency (Fig. 3b). Tri-choline citrate slightly depressed the sugar receptor response when compared with 0.005 mol/l NaCl as electrolyte, but there was no influence on the specificity when tested with three representative sugars

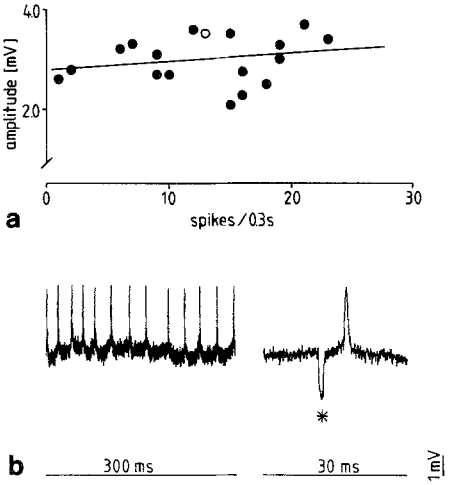

Fig. 3. a Relation between spike frequency and spike amplitude for the sugar receptor. Concentrations of sucrose dissolved in 0.03 mol/l tri-choline citrate range from 0.01 to 1 mol/l. Each point represents the mean amplitude and frequency of an unfiltered record. The calculated regression line has a slope of 0.015, meaning that an increase of 5 spikes/0.3 s leads to an increase in amplitude of maximally 0.08 mV. **b** Typical unfiltered response (empty circle in **a**). At the right the record is expanded to demonstrate a monophasic spike (upward deflection) and the recording quality (rectangular downward pulse marked by an asterisk, pulse duration 1 ms)

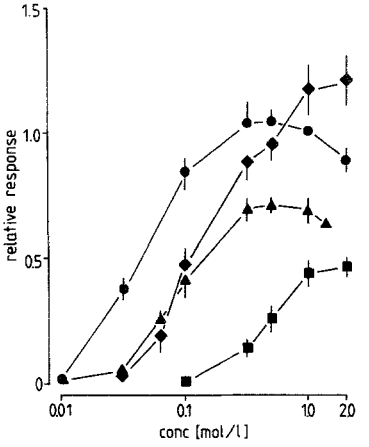

Fig. 4. Dose-response curves of the sugar receptor for sucrose (●, $n=7$), D-glucose (◆, $n=7$–10, 1 mol/l: $n=18$), D-fructose (■, $n=5$–8), and trehalose (▲, $n=6$–10). All substances dissolved in 0.03 mol/l tri-choline citrate. The values are normalized with regard to the response to 1 mol/l sucrose. Vertical bars: SEM values

(Table 1). Moreover, tri-choline citrate had no major influence on receptor sensitivity as the dose-response curve for sucrose (Fig. 4) showed an affinity comparable to that published by Fujishiro et al. (1984).

We did not further analyze the apparent discrepancy between the results of Fujishiro et al. (1984) and our observation of

monophasic spikes with constant amplitude, but there may still be a possible explanation. According to Fujishiro et al. (1984) the positive correlation between spike form (amplitude and shape) and spike frequency reflects the increase of inactivated dendritic sodium channels with increasing receptor potential. As Fujishiro et al. (1984) reported this dependence using 0.1 mol/l choline chloride as electrolyte, our use of citrate as anion may have been the cause for amplitude constancy and monophasic spike appearance. Citrate complexes calcium ions which usually occur in millimolar concentration in the extracellular solution. Calcium normally binds to negative membrane charges; a decrease in membrane bound calcium as a consequence of the established calcium citrate complexes would lead to a smaller transmembrane potential gradient without affecting the potential difference between the aqueous bulks of extracellular and intracellular medium (Hille 1976). A lower transmembrane potential gradient would tend to inactivate sodium channels also in the absence of a depolarizing receptor current. In this case dendritic spikes (Fujishiro et al. 1984), recorded as negative deflections, would be suppressed. A spike originating in the region of the cell body (see Morita and Shiraishi 1985) would then be recorded as a monophasic event, and, as a consequence, the spike form would no longer depend on the strength of the receptor current. We do not know how far reasonable amounts of citrate anions actually diffuse within the short time period at the beginning of a stimulus. However, the mean diffusion distance in e.g. 30 ms is 6 µm (the diffusion coefficient D being 6.61×10^{-6} cm^2 s^{-1}, Weast 1987) which is about 15–20% of the total length of a taste hair (Falk et al. 1976).

The reaction spectrum of the sugar receptor

Sucrose, D-glucose, D-fructose and trehalose. At first, we investigated the responses to sucrose, D-glucose, D-fructose and trehalose because receptor sites for these sugars had already been identified in *Drosophila* (Tanimura and Shimada 1981; Tanimura et al. 1982). The dose-response curves (Fig. 4) were different from the data of Tanimura and Shimada (1981) in two regards. First, D-glucose led to an approximately 15% higher maximal response than sucrose, whereas the maximal response for D-glucose as estimated from the data of Tanimura and Shimada (1981) was only 50% of that for sucrose. As this result was unexpected we tested in all further experiments the effectiveness not only of 1 mol/l sucrose (control stimulus), but also that of 1 mol/l D-glucose, and always found the same relation as for the dose-response curves in Fig. 4. Secondly, while we measured a maximal response for D-fructose only half of that for D-glucose and a threefold higher K_b value for D-fructose than for D-glucose, Tanimura and Shimada (1981) reported the same maximal responses and K_b values for D-glucose and D-fructose. The sensitivity of the sugar receptor for trehalose with a K_b value of about 0.09 mol/l seems to be comparable to the observations of Tanimura et al. (1982) who measured a behavioural threshold concentration of 0.06 mol/l.

The 'pyranose' site. To investigate the specificity of the pyranose site in *Drosophila* we tested the same monosaccharides as Shimada et al. (1974), and derivatives of D-glucose which differ only with regard to one C-atom or which may be good candidates for the pyranose site because of their structure (Table 2). Most of the sugars were first tested at a concentration of 1 mol/l to estimate maximal responses, and in case of stimulating effectiveness also at 0.1 mol/l. The sensitivity of the receptor could then be estimated roughly from the relation of responses at both concentrations. In this way a comparison not only with the electrophysiologically measured responses of the calyptrate flies was possible, but also with their behavioural thresholds (Table 2). The estimation of receptor sensitivity in addition to that of the maximal response appeared to be useful as the relation of both receptor parameters may be different for different sugars (for instance, compare maximal responses and threshold concentrations of the calyptrate flies for L-arabinose and L-sorbose in Table 2a).

Table 2a shows that receptor sensitivities were not different between *Drosophila* and the calyptrate flies. However, with regard to maximal responses the investigated substances could be arranged into three groups. (1) The same maximal responses in *Drosophila* and the calyptrate flies were caused by L-fucose and methyl-β-glucoside. (2) Higher maximal responses in *Drosophila* than in the calyptrate flies were caused by D-glucose, methyl-α-glucoside, maltose, 6-deoxy-D-glucose and maltotriose. (3) Lower maximal responses in *Drosophila* than in the calyptrate flies were caused by L-sorbose, L-glucose, L-arabinose, D-xylose, 2-deoxy-D-glucose and 3-O-methyl-D-glucose.

An unexpected observation was the stimulating effectiveness of 4-nitrophenyl-α-maltoside whereas 4-nitrophenyl-α-glucoside elicited no response (Table 2a). We therefore investigated how the responsiveness of the sugar receptor was influenced (1) by an increasing number of glucose units linked by α-1-4-glucosidic bonds, (2) by substances out of (1) which are linked by an α-glucosidic bond to 4-nitrophenol, and (3) by substances out of (1) whose reducing D-glucose is substituted by sorbitol. The respective dose-response curves (Fig. 5) were analyzed and the resulting receptor parameters are shown in Fig. 6 as functions of the chain length. The following results were obtained: (1) Sensitivity and maximal responses increased from maltose to maltopentaose, whereas longer glucose chains led to a decrease of both parameters. The exception was maltotriose with a clearly lowered maximal response, but the result seems to be valid as it is in line with that for maltotriitol. (2) The

Table 2. Responses of the sugar receptor of *Drosophila* to substances which in calyptrate flies probably bind (**a**) to the pyranose site or (**b**) to the furanose or carboxylate site

Stimulus	mol/l	*Drosophila*		*Phormia*	*Sarcophaga*	*Boettcherisca*	*Phormia*
		Rel. resp. (m ± SD)	*n*	Relative responses			Threshold conc. (mmol/l)
(**a**) Sucrose	1.0	1.0		1.0	1.0	1.0	10[h]
	0.1	0.83 ± 0.16					
D-glucose	1.0	1.15 ± 0.23	18	0.74–0.79	0.70	0.72[a]	130[a]
	0.1	0.46 ± 0.14	7				
L-fucose	1.0	0.73 ± 0.15	8	0.77–0.91			87[a]
	0.1	0.20 ± 0.09	8			0.50[d]	
L-arabinose	1.0	0.15 ± 0.10	8	0.45–0.52			536[a]
	0.3					0.26[d]	
	0.1	0	6				
L-sorbose	1.0	0.31 ± 0.09	8			0.54[a]	140[a]
	0.1	0	6				
D-xylose	1.0	0.08 ± 0.05	8				440[a]
	0.2					0.17[d]	
	0.1	0	6				
L-glucose	1.0	0.41 ± 0.14	8	0.64–0.69	0.49		230[h]
	0.2					0.35[d]	
	0.1	0	8				
6-deoxy-D-glucose	1.0	1.26 ± 0.24	8		0.35		
	0.1	1.01 ± 0.19	8				
2-deoxy-D-glucose	1.0	0	8		0.16		165[a]
3-O-methyl-D-glucose	1.0	0.03 ± 0.06	8		0.12		
Methyl-α-D-glucoside	1.0	1.28 ± 0.21	8	0.84–0.98	0.77	0.71[a]	69[a]
	0.1	0.74 ± 0.18	8				
Methyl-β-D-glucoside	1.0	0.20 ± 0.10	8		0.21	0.27[a]	150[h]
	0.1	0	8				
4-np-α-D-glucoside	0.01	0	9	0.50–0.95		0.21[f]	1[h]
4-np-α-maltoside	0.01	0.52 ± 0.19	10				
Maltose	1.0	1.21 ± 0.15	11	0.99–1.06			10[h]
	0.1	0.96 ± 0.25	19			0.66[d]	
Trehalose	1.0	0.68 ± 0.11	6	0.65–0.75			120[h]
	0.3					0.25[e]	
	0.1	0.41 ± 0.20	8				
Maltotriose	0.1	0.86 ± 0.17	24			0.30[e]	10[h]
(**b**) D-fructose	1.0	0.43 ± 0.13	8	0.39–0.61	0.54	0.65[a], 0.46[b]	6[a]
	0.1	0	5				
2,5-anhydro-D-mannitol	1.0	0.04 ± 0.06	8			0.27[g]	
D-fucose	1.0	0.32 ± 0.06	8	0.59–0.72			264[a]
	0.2					0.41[d]	
	0.1	0	8				
D-galactose	1.0	0.03 ± 0.03	8	0.40–0.45		0.53[a]	500[a]
L-phenylalanine	0.05	0	4			0.3[c]	1[h]
L-valine	0.05	0	4	0		0.3[c]	

Mean values ± SD are shown. 1.0 = response to 1 mol/l sucrose (mean response of all investigated sugar receptors was 25.1 ± 6.6 spikes). *np* = nitrophenyl. Values for *Phormia* from Wieczorek and Köppl 1982; maximal responses obtained with 0.8 or 1.0 mol/l, for 4-np-α-glucoside with 0.015 or 0.020 mol/l. Values for *Sarcophaga* from Jakinovich et al. (1971). Values for *Boettcherisca* from authors a–g.

[a] Hanamori et al. (1974); [b] Morita and Shiraishi (1968); [c] Shiraishi and Kuwabara (1970); [d] Shimada et al. (1974; estimation of relative responses: according to Fig. 1 of Morita and Shiraishi (1968), 0.1 mol/l sucrose elicits a response of 0.73 compared to 1 mol/l sucrose; therefore all values in Table 1 of Shimada et al. (1974) were related to the values for 0.1 mol/l sucrose in Table 2 and multiplied with 0.73); [e] Ninomiya and Shimada (1976; estimation of relative responses: according to Fig. 1 of Morita and Shiraishi (1968), 0.2 mol/l D-glucose elicits a response of 0.36 compared to 1 mol/l sucrose; therefore all values in Table 1 of Ninomiya and Shimada (1976) were related to the response to 0.2 mol/l D-glucose and multiplied with 0.36). [f] Hanamori et al. (1972); [g] Shimada et al. (1985); [h] Hansen (1978)

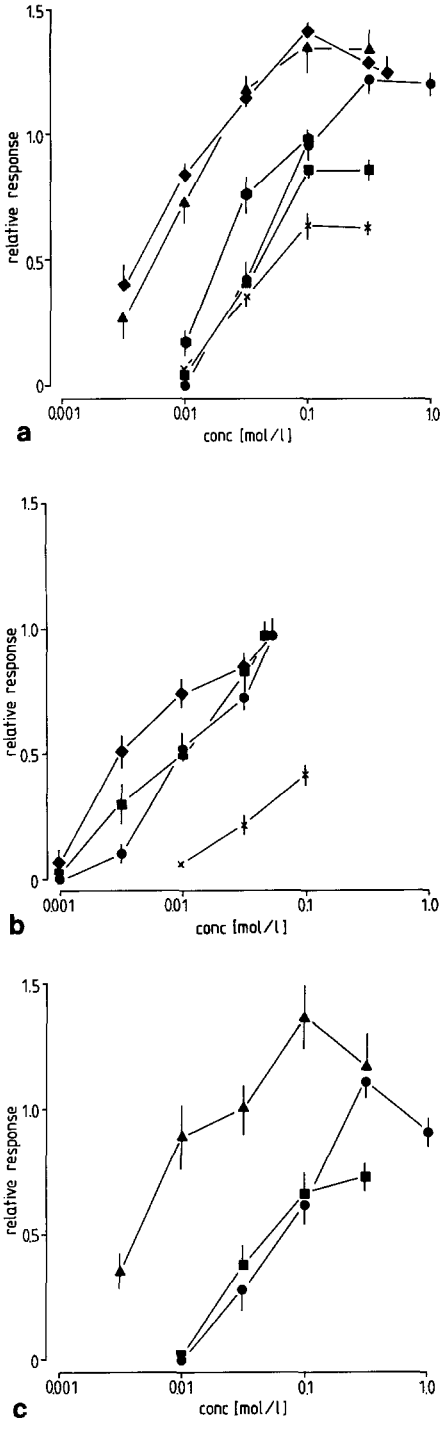

Fig. 5a–c. Dose-response curves of the sugar receptor for maltose and derivatives of maltose. All substances dissolved in 0.03 mol/l tri-choline citrate. The values are normalized with regard to the response to 1 mol/l sucrose. Vertical bars: SEM values. **a** Sugars: maltose (●, $n=11$–19), maltotriose (■, $n=10$–24), maltotetraose (▲, $n=6$–8), maltopentaose (◆, $n=14$–25), maltohexaose (●, $n=8$), maltoheptaose (×, $n=8$–10). **b** 4-Nitrophenyl-α-derivatives (NP-): NP-maltoside (●, $n=4$–10), NP-maltotrioside (■, $n=8$–9), NP-maltopentaoside (◆, $n=8$), NP-maltoheptaoside (×, $n=5$–8). **c** Sugar alcohols (reducing D-glucose substituted by sorbitol): maltitol (●, $n=8$), maltotriitol (■, $n=8$), maltotetraitol (▲, $n=6$–8)

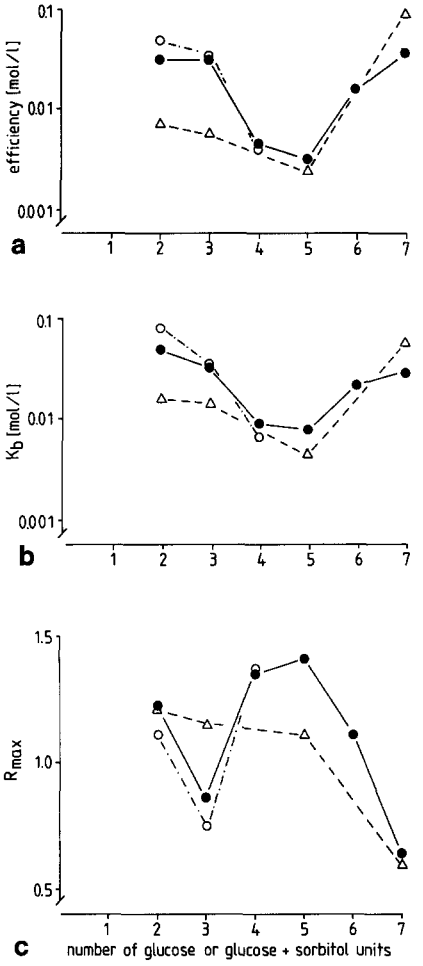

Fig. 6. Influence of chain length of the substances from Fig. 5 on the estimated **a** efficiency at the relative receptor response of 0.4 (1.0 = response to 1 mol/l sucrose), **b** concentration K_b leading to half maximal response, and **c** maximal response R_{max}. Chain length starts with maltose for the sugars (●), 4-nitrophenyl-α-maltoside for the glycosides (△), and maltitol for the sugar alcohols (○). Analysis of the dose-response curves from Fig. 5 was performed as follows. If R_{max} was not reached with the highest concentration used it was estimated by calculating the mean between (1) the response to the highest concentration and (2) the intercept on the y-axis of the double reciprocal Lineweaver-Burk plot obtained by connecting the values for the two highest concentrations. The K_b values were estimated from the dose-response curves taking into account the calculated R_{max} values. The 'iso-stimuli' curves in **a** show similar results like the curves of the K_b values in **b** demonstrating that both types of curves enable the estimation of sensitivity as function of the chain length. Because of the (partly) indirect determination of K_b values and maximal responses the strongest evidence may be derived from the 'iso-stimuli' curves in **a**; especially the K_b values and maximal responses for the nitrophenyl derivatives should be interpreted cautiously

sugar receptor was clearly more sensitive to the nitrophenyl derivatives than to the respective sugars, but with increasing chain length this effect diminished. The maximal responses for the nitrophenyl derivatives were comparable to that for

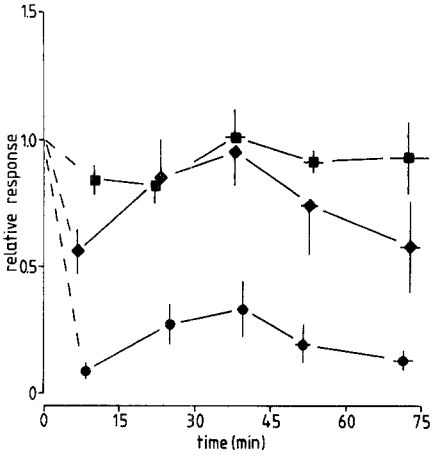

Fig. 7. Time course of relative responses of the sugar receptor to 0.5 mol/l D-glucose (■), 1 mol/l D-fucose (♦) and 1 mol/l D-fructose (●) after treatment with papain. All sugars dissolved in 0.03 mol/l tri-choline citrate. Dotted lines connect responses before treatment with the first responses after treatment. Vertical and horizontal bars: SEM values. Number of tested receptors from left to right for D-glucose: 6-4-5-6-4; for D-fucose: 13-9-10-6-6; for D-fructose: 15-11-8-7-6. Levels of significance (two-tailed *t*-tests) for differences between responses to D-fucose and those to D-fructose from left to right: ≤ 0.001-0.01-0.01-0.02-0.05

maltose and hardly changed up to 4-nitrophenyl-α-maltopentaoside. The low maximal response for 4-nitrophenyl-α-maltoheptaoside was in accordance with that for maltoheptaose. (3) Using sorbitol instead of the reducing glucose did not alter the responsiveness.

The 'furanose' site. Substances binding to the furanose site, the carboxylate site and a putative 'aryl' site of *Boettcherisca* (Shimada et al. 1974; Shimada and Isono 1978; Shimada et al. 1985) are listed in Table 2b. D-galactose, 2,5-anhydro-D-mannitol (furanose site) and L-phenylalanine ('aryl' site) were, as well as L-valine (carboxylate site), not or nearly not stimulatory for *Drosophila*. The effectiveness of D-fucose compared to D-fructose was considerably lower in *Drosophila* than in the calyptrate flies. As this result led us to the idea that D-fucose and D-fructose may bind to different receptor sites we treated the sugar receptor with the proteolytic enzyme papain and tested the receptor's responsiveness to both sugars and to D-glucose. Figure 7 shows that papain had only a small effect on the responsiveness of the sugar receptor to D-glucose, but abolished that to D-fructose to a large extent (compare Tanimura and Shimada 1981). The responsiveness to D-fucose was far less affected than that to D-fructose. The lack of stimulating effectiveness of the furanose sugar 2,5-anhydro-D-mannitol prompted us to investigate the stimulating conformation of D-fructose. We measured its effectiveness in equilibrium and in freshly prepared solutions (Hanamori et al. 1974; Wieczorek 1980). As shown in Table 3 the sugar receptor responded equally well to both types of stimuli at all sugar concentrations.

Table 3. Relative sugar receptor responses to D-fructose in equilibrium solutions or freshly prepared solutions ($t = 56 \pm 5$ s after dissolution

D-fructose	Equilibrium solution	Freshly prepared solution
1.0 mol/l (8)	1.0	1.04 ± 0.12
0.5 mol/l (8)	0.64 ± 0.14	0.68 ± 0.14
0.2 mol/l (7)	0.44 ± 0.19	0.44 ± 0.14

D-fructose was dissolved in 0.02 mol/l $CaCl_2$ which largely inhibited the activity of the water receptor. The data are from the same set of receptors (numbers in brackets). Mean values $\pm$SD are shown. For polarimetric measurement of the mutarotation of D-fructose (23 ± 0.1 °C) a Perkin-Elmer-241 polarimeter was used at a wavelength of 589 nm

Discussion

The 'pyranose' site: relation between molecular structure and stimulating effectiveness

Drosophila and the calyptrate flies. The results of this paper disclose many similarities between the reaction spectra of the sugar receptor of *Drosophila* and that of the calyptrate flies, especially considering substances which may bind to the pyranose site. These similarities are apparent particularly with regard to sensitivity, but, with regard to relative maximal responses there are major differences.

Five sugars contrast with the others since they are better stimulants in *Drosophila* than in the calyptrate flies: D-glucose, methyl-α-glucoside, maltose, maltotriose and 6-deoxy-D-glucose. All five C1-sugars are in accordance to the rule stated by Jakinovich et al. (1971) that for optimal stimulating effectiveness C-2, C-3 and C-4 must have equatorial hydroxyl groups. Evidently the differences at C-1 or C-5 do not cause any change of responsiveness in comparison with D-glucose.

Most other tested pyranose-like sugars are less stimulatory in *Drosophila* than in the calyptrate flies. They either do not obey the rule of three adjacent equatorial hydroxyl groups (Jakinovich et al. 1971; Hanamori et al. 1974) or show important differences from D-glucose with regard to substitution at the C-1 or C-5 position. The first explanation may apply to the non-stimulating 2-deoxy-D-

glucose, 3-O-methyl-D-glucose and D-galactose, and to the weakly stimulating L-arabinose. D-xylose, the epimer of L-arabinose at C-4, is structurally identical with D-glucose except for the substitution of the CH_2OH group at C-5 with a hydrogen, but the sugar receptor response is more than tenfold lower compared to D-glucose and more than threefold lower than in *Boettcherisca* (for a direct comparison the response of *Boettcherisca* at a concentration of 1 mol/l was estimated to be at least 0.3). The importance of the substitution at C-1 and C-5 is also apparent for the 1C-sugars L-sorbose and L-glucose. For a comparison with the C1-sugar D-glucose, both sugars have to be rotated 180° along the axis between C-4 and the ring oxygen (Hanamori et al. 1974). The new C-2, C-3 and C-4 then have equatorial hydroxyl groups. The new C-5 of L-sorbose bears axially a hydroxyl group instead of hydrogen, and the hydroxyl group at C-1 is substituted by hydrogen. In *Boettcherisca* the response to 1-deoxy-D-glucose is as high as that to D-glucose (Hanamori et al. 1972). Supposing the same relation for *Drosophila* too, the low stimulating effectiveness of L-sorbose (about half of that in the calyptrate flies) must be caused by the additional hydroxyl group at the new C-5. For L-glucose the rotation leads to a substitution of the CH_2OH group at the new C-5 by hydrogen or a hydroxyl group (depending on the previous α- or β-configuration); the new C-1 has an equatorial CH_2OH group. As the equatorial CH_3 group of methyl-β-glucoside at C-1 leads to extremely diminished stimulating effectiveness, substitution with an equatorial CH_2OH group in case of L-glucose could be one reason for the small stimulating effectiveness.

In summary it may be said that the existence of three adjacent equatorial hydroxyl groups is, like in the calyptrate flies, an absolute precondition for stimulating effectiveness. Even then substituents at C-1 and C-5 are very important. Although the tendency of change in responsiveness as a function of change of substituents is the same as in the calyptrate flies, stereospecificity in *Drosophila* seems to be more rigid because substitutions lead to more dramatic changes in responsiveness than in the calyptrate flies.

The role of substituents linked α-glycosidically to D-glucose. Like in calyptrate flies (see Hansen 1978), α-glycosidically linked aglykons lead to enhanced responsiveness of the sugar receptor. In the case of maltose and its derivatives the prolongation of the D-glucose chain up to maltopentaose leads to a continuously increasing affinity. A comparable dependence has been described for the substrates of amylase in *Bacillus subtilis* (Takagi et al. 1971). Binding of repetitive D-glucose sequences has been interpreted by a model assuming repetitive D-glucose subsites (Thoma et al. 1970). This model does not exclude the possibility that at certain chain lengths the hydrolysis rate may be diminished (Takagi et al. 1971); in our case this would correspond to the lowered maximal response for maltotriose.

The enhanced affinity for nitrophenyl derivatives may also be interpreted by repetitive binding regions for the nitrophenyl group. For this, the adjacent amino and imino groups of arginine residues would be good candidates because the distance of the hydrogens of both groups corresponds to the distance of the oxygens of the nitro group. However, the first NO_2 binding 'subsite' cannot be in close vicinity to the non-reducing D-glucose as 4-nitrophenyl-α-glucoside is not stimulatory.

The receptor site reacting to D-fructose

In the calyptrate flies *Boettcherisca* and *Phormia* the stimulating conformation of D-fructose is the furanose form (Shimada et al. 1974; Hanamori et al. 1974; Wieczorek 1980). Therefore the respective receptor site has been called 'furanose' site. Based on results from experiments using the pharmacological approach, Shimada et al. (1974) suggested that in *Boettcherisca* D-galactose and D-fucose bind to the furanose site, too. Our results with *Drosophila* show (1) that D-galactose is not stimulatory and (2) that D-fucose binds to a site other than that for D-fructose. Furthermore the effective conformation of D-fructose cannot be the furanose form for the following reasons. The sugar receptor responded equally well to freshly prepared and to equilibrium solutions of D-fructose, although the furanose content in equilibrium solutions was four times higher than in freshly prepared solutions. As the pyranose content in equilibrium and in freshly prepared solutions differed only by 25%, we conclude that the effective conformation of D-fructose is the pyranose and not the furanose form. Altogether our results clearly indicate that a furanose site as has been established in the calyptrate fly *Boettcherisca* does not occur in *Drosophila*.

Receptor sites: a mosaic on the dendritic membrane?

Wieczorek and coauthors (Wieczorek and Köppl 1978; Wieczorek 1980; Wieczorek et al. 1988) have shown that the furanose site of the sugar receptor

occurs in the water receptor of *Phormia*, too. This means that the expression of one type of receptor site may be independent of the expression of other types of receptor sites. The different reaction spectra of the sugar receptors in different types of taste hairs of *Phormia* may also be interpreted as indication for an independent expression of receptor sites (Wieczorek and Köppl 1982): if only those sugars are compared which, according to pharmacological criteria, bind to one type of receptor site, the reaction spectra are identical. The same is true if the reaction spectra of different calyptrate fly species like *Phormia, Boettcherisca* and *Sarcophaga* are compared. Thus different reaction spectra in different calyptrate fly species may result from different distributions of essentially the same set of receptor sites (Wieczorek and Köppl 1982). If this interpretation is expanded to the acalyptrate *Drosophila*, the comparison with the reaction spectra of calyptrate flies should enable conclusions on the existence and distribution of receptor sites. This suggestion forms the basis of the following considerations.

We have already discussed the comparatively rigid stereospecificity of the pyranose site. L-fucose, a sugar assigned pharmacologically to the pyranose site (Shimada et al. 1974), has only two adjacent equatorial hydroxyl groups, although it is a strong stimulus for the sugar receptor. In the experiments of Shimada et al. (1974) the sugar receptor response to L-fucose was not as strongly depressed by p-chloromercuribenzoate as that to D-glucose. Furthermore, pronase treatment of the labellar sugar receptor of *Phormia* led to results which gave no clear indication of the binding behaviour of L-fucose (Wieczorek et al. 1988). Last but not least a considerable fraction of L-fucose in aqueous solution consists of the furanose conformation (Hanamori et al. 1974). All these results may lead to the suggestion that L-fucose binds to a receptor site different from the pyranose site.

Another remarkable observation is the unusually high response of the sugar receptor to D-glucose which is more stimulatory than sucrose and as stimulatory as maltose. At the same time, various sugars which are structurally very similar to D-glucose are more stimulatory for *Drosophila* than for the calyptrate flies. For calyptrate flies, but also for the *Drosophila* strains investigated by other authors (Isono and Kikuchi 1974a; Isono and Kikuchi 1974b; Kikuchi 1975; Tanimura and Shimada 1981) sucrose and maltose are stronger stimuli than D-glucose. These differences are perhaps an indication for the existence of a second 'pyranose' site in our *Drosophila* strain which reacts very specifically to D-glucose or derivatives which are only slightly different from D-glucose.

According to our results, following sites established in the calyptrate flies do not occur in *Drosophila*: the carboxylate and the 'aryl' site (*Boettcherisca*), the furanose site (*Boettcherisca, Phormia*) and the site reacting to 4-nitrophenyl-α-glucoside (*Phormia*). On the other hand there is a strong indication for a receptor site in *Drosophila* reacting to fructopyranose.

The comparison of reaction spectra of the calyptrate flies and *Drosophila* has led us to the idea that the actual number of diverse types of receptor sites in flies may be substantially higher than assumed up till now. A similar situation is observed in bacteria: more than 40 different chemoreceptor proteins, many of them reacting to sugars, are known for the enterobacterium *Escherichia coli* (Lengeler 1980). For ten sugars tested in *Drosophila* (sucrose, D-glucose, L-fucose, L-arabinose, L-sorbose, D-xylose, trehalose, maltose, D-fructose and D-galactose) ten different receptor proteins with rigid specificity occur in *E. coli*. In contrast to the situation in *E. coli*, only four types of receptor sites for these ten sugars have been identified in flies. Provided that our idea mentioned above is valid, the concept of multiple receptor sites (Evans 1963; Shimada 1978) would have to be understood in such a way that the broad specificity of the sugar receptors of flies results from a mosaic of different types of highly specific receptor sites.

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