

## STIMULATION OF THE GERBIL'S GUSTATORY RECEPTORS BY MONOSACCHARIDES

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### SUMMARY

The gustatory responses of the Mongolian gerbil were tested with a large number of monosaccharides. Electrophysiological methods were used to record from the chorda tympani nerve. Methyl glycosides which have structural features in common with sucrose are the most effective monosaccharides for eliciting a neural response. Among the monosaccharides tested, efficacy appears to be highest in D-pyranosides having equatorial substituents at the C-2 and C-4 positions and axial substituents at the C-1 position. A C-5 hydroxymethyl group is not required. Similarities in the structural requirements for taste response in the fly and gerbil are discussed.

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### INTRODUCTION

A putative protein sugar-receptor in the tongue of the rat has been reported<sup>20</sup>. Presumably, this protein complexes with sugar to bring about an electrical change within the gustatory cell. A correlation of the electrophysiological effectiveness of different sugars as gustatory stimulants with the protein's interaction, as measured by ultraviolet difference spectroscopy, with the same sugars, was reported<sup>19</sup>. Biochemical isolation of a sugar receptor would be facilitated by additional physiological characterization. In the previous paper<sup>25</sup> it was shown that in gerbils, sucrose is the most stimulatory disaccharide. To account for this finding a 'sucrose receptor site' was postulated. Since sucrose can be considered as a fructofuranoside ( $\alpha$ -D-glucopyranosyl- $\beta$ -D-fructofuranoside) or a glucopyranoside ( $\beta$ -D-fructofuranosyl- $\alpha$ -D-glucopyranoside) it could be more stimulatory than other disaccharides if there were two monosaccharide receptor sites, one site accommodating a glucose molecule and a second

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site a fructose molecule. Comparison of molecular configuration with gustatory stimulating ability of disaccharides and their component monosaccharides has led others to the conclusion<sup>1,2</sup> that the stimulatory effectiveness of a disaccharide depends upon the effectiveness of its constituent monosaccharides. However, in the gerbil, both turanose and palatinose were electrophysiologically less effective gustatory stimuli than sucrose even though these 3 molecules have identical monosaccharide constituents<sup>25</sup>. Hence, the nature of the glycosidic linkage joining the two monosaccharide residues must play an important role in determining gustatory effectiveness. Furthermore, since the fructose moiety of turanose and palatinose occupies a reducing position which may exist in solution as a mixture of furanose and pyranose isomers the reduced effectiveness observed for these two disaccharides could result from a paucity of one type of isomer or competitive interaction of the less complementary isomers for either a single sucrose receptor site or a monosaccharide site. The difficulty of interpreting gustatory reception with an isomeric mixture has been recognized and overcome by using cyclitols, sugar glycosides of well defined structures and freshly prepared sugar solutions which have not had time to mutarotate<sup>6,11,13,26</sup>.

The possibility that the putative sucrose receptor site is composed of two subunits was investigated using methyl glycosides of glucose and fructose and other monosaccharide sugars of known configuration.

#### MATERIALS AND METHODS

##### *Animals*

Male and female Mongolian gerbils, *Meriones unguiculatus*, were used<sup>27</sup>.

##### *Sugars*

Sugars were obtained from Pfanstiehl Laboratories, Waukegan, Ill. and Sigma Chemical Co., St. Louis, Mo.

The following sugars were prepared by the methods of the authors cited: 1,5-anhydro-D-glucitol<sup>12</sup>, m.p. 143–144 °C, 1,5-anhydro-D-galactitol<sup>12</sup>, m.p. 112–113 °C, 1,5-anhydro-D-mannitol<sup>13</sup>, m.p. 153–155 °C,  $\alpha$ -D-glucopyranosyl fluoride<sup>18</sup>, m.p. 118–125 °C with decomp., methyl 2-deoxy- $\alpha$ -D-arabino-hexopyranoside<sup>24</sup>, m.p. 91–93 °C, methyl  $\alpha$ -D-fructofuranoside<sup>5</sup>, m.p. 80–82 °C, methyl  $\beta$ -D-fructofuranoside<sup>5</sup>, chromatographically pure syrup, methyl  $\beta$ -D-fructopyranoside<sup>5</sup>, m.p. 121–123 °C, methyl  $\alpha$ -D-fructopyranoside prepared from the tetraacetate<sup>23</sup> which was deacetylated by treatment with sodium in methanol, was obtained as a chromatographically pure and salt-free syrup after passage through a mixed bed ion exchange resin (Amberlite MB-3).

Melting points were determined on a Fisher–Johns melting-point apparatus and are uncorrected. All evaporations were conducted *in vacuo* at 35–40 °C on a rotary evaporator. Purity of the glycosides was evaluated by descending paper chromatography on Whatman No. 1 paper with ethyl acetate–pyridine–water (2.5:1:3.5) as the solvent. Sugars were visualized with silver nitrate–NaOH (see ref. 40) or *p*-anisidine–HCl<sup>22</sup>. Thin-layer chromatography was carried out on activated Silica Gel G glass

plates using isopropanol–ethyl acetate–water (20 75 5) for glycosides and benzene–methanol (95 5) for acetylated sugars. These sugars were visualized by spraying with  $\text{H}_2\text{SO}_4$  (50% v/v, in ethanol) and heating 15 min at 120 °C.

#### *Taste solutions*

All compounds were dissolved in distilled water and stored at 2 °C and used immediately or up to 7 days later. Reducing sugar solutions were prepared approximately 24 h before use and were allowed to reach mutarotational equilibrium at room temperature. Both  $\alpha$ - and  $\beta$ -D-glucose were used within 3 min after dissolving the sugar. Because of instability, fresh  $\alpha$ -D-glucopyranosyl fluoride solution was prepared immediately before each experiment.

#### *Exposing the nerve, stimulus presentation and electrophysiology*

Exposure of the chorda tympani nerve and electrophysiological recording were done through the middle ear. Stimulation procedures have been previously described<sup>25</sup>.

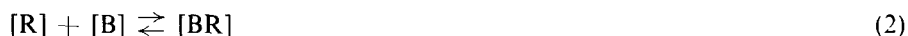
#### *Concentration–response curves*

The  $\text{CR}_{50}$  and maximum response were measured directly from semilogarithmic plots<sup>25</sup>. Determinations of dissociation constants ( $K_d$ ) for the monosaccharides were done by means of a Beidler plot<sup>4</sup>.

Theoretical curves for the interaction of two substances (A and B) with one receptor site (R) were drawn from the following equations:



and



In the above equilibrium reactions [A] and [B] are the concentrations of the respective sugars. [AR] and [BR] are the concentrations of the sugar-receptor complexes. Therefore,

$$K_A = \frac{[\text{A}][\text{R}]}{[\text{AR}]} \quad (3)$$

and

$$K_B = \frac{[\text{B}][\text{R}]}{[\text{BR}]} \quad (4)$$

are the dissociation constants of [AR] and [BR], respectively. Assuming the response (Resp) is proportional to the combined effects of [AR] and [BR] then

$$\frac{\text{Resp}}{\text{Resp}_{\max}} = \frac{\text{sites filled}}{\text{total sites}} = \frac{[\text{AR}] + [\text{BR}]}{[\text{AR}] + [\text{BR}] + [\text{R}]} \quad (5)$$

By substituting equations 3 and 4 into equation 5 we get

$$\frac{\text{Resp}}{\text{Resp}_{\text{max}}} = \frac{K_B [A] - K_A [B]}{K_B [A] + K_A [B] + K_A K_B} \quad (6)$$

## RESULTS

### *General observations*

The taste nerve of the gerbil responds with increased activity to increasing concentrations of monosaccharides applied to the tongue as previously described for disaccharides.<sup>25</sup> 'Off' discharges of the type seen with divalent cation stimulation<sup>27</sup> were never observed with monosaccharides. The concentration-response curve was sigmoidal on semilog coordinates. Every sugar tested over the applied concentration range reached a maximum response. The slight depression of the response previously seen with the strongest solutions of disaccharides<sup>25</sup> was observed infrequently with monosaccharides.

### *Effect of mutarotation*

A demonstration of the effect of mutarotation on stimulation by a reducing sugar can be seen in Fig. 1. This figure presents for comparison  $\alpha$ -D-glucopyranose,  $\beta$ -D-glucopyranose and the mutarotated mixture. In the 5 animals tested,  $\alpha$ -D-glucopyranose was more stimulatory than  $\beta$ -D-glucopyranose in the middle part of

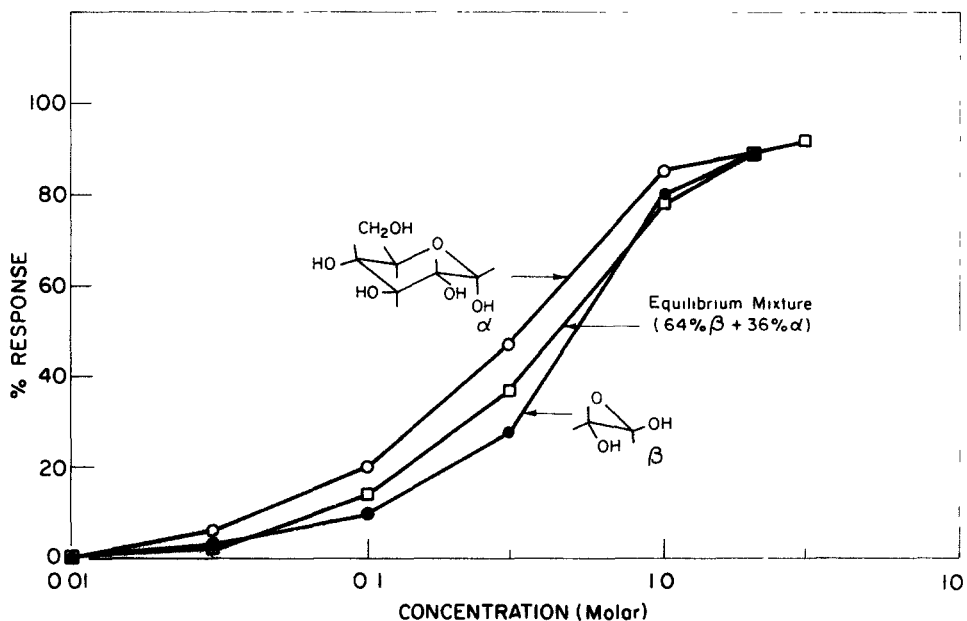


Fig. 1 Semilogarithmic plot of the mean integrated responses of the gerbil's (N = 5) chorda tympani nerve to  $\alpha$ -D-glucopyranose (○),  $\beta$ -D-glucopyranose (●) and the equilibrated mixture of D-glucose mutarotational isomers (□) applied to the tongue. Each response is represented as a percentage of the maximum response of sucrose. Measurements were made within 3 min of dissolving the dry anomeric species.

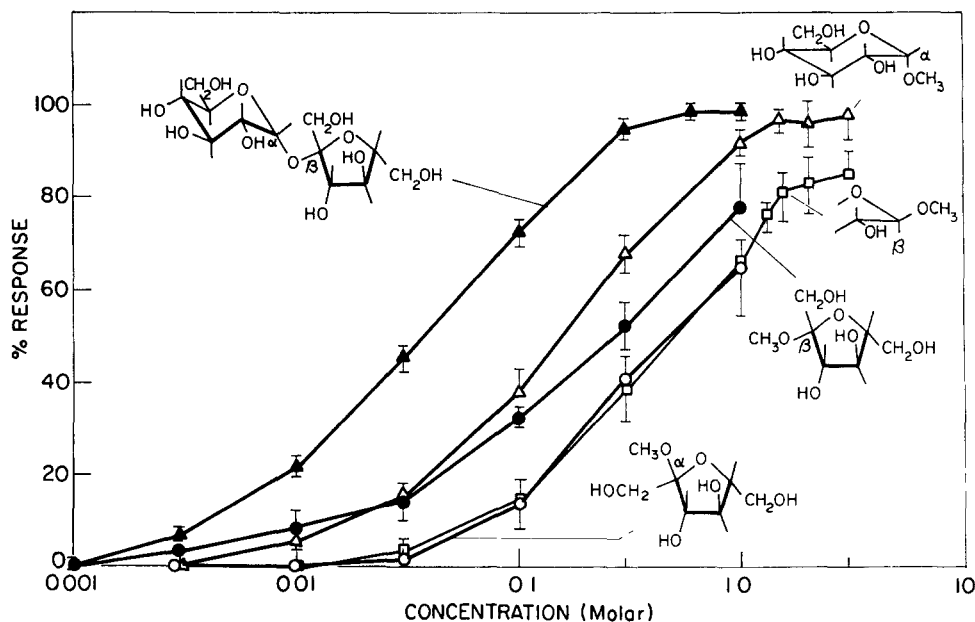


Fig 2 Mean integrated response of the chorda tympani nerve discharge in the gerbil to sucrose (▲)  $N = 49$ , methyl  $\alpha$ -D-glucopyranoside (▬)  $N = 15$ , methyl  $\beta$ -D-glucopyranoside (□)  $N = 5$ , methyl  $\beta$ -D-fructofuranoside (●)  $N = 5$  and methyl  $\alpha$ -D-fructofuranoside (⊖)  $N = 5$ . Responses are relative to the maximum sucrose response. Bars represent 95% confidence intervals.

the response range. The equilibrium mixture was intermediate in its stimulatory effectiveness.  $\alpha$ -D-Glucopyranose was a slightly better stimulant at low concentrations (5/5 responded at 0.05 M) than the  $\beta$ -anomer (3/5 responded), but at high concentrations (2 M) they were equal. The maximum responses of each of these two sugars did not exceed the sucrose maximum.

#### Comparison of methyl $\alpha$ - and $\beta$ -D-glucopyranosides, methyl $\alpha$ - and $\beta$ -D-fructofuranosides, methyl $\alpha$ - and $\beta$ -D-fructofuranosides

Neither fructose nor any of the 6 methyl glycosides investigated were better stimuli than sucrose (Figs 2 and 3). The two methyl glycosides that most closely resemble the two carbohydrate moieties of sucrose, methyl  $\alpha$ -D-glucopyranoside and methyl  $\beta$ -D-fructofuranoside, were the most stimulatory of the 6 methyl glycosides. Methyl  $\beta$ -D-fructofuranoside had a lower threshold (0.003 M) than methyl  $\alpha$ -D-glucopyranoside (0.01 M). The latter, however, evoked the greater response at the higher concentrations. Furthermore, the shape of the concentration-response curve for methyl  $\beta$ -D-fructofuranoside (Fig 2) had a somewhat different shape than the curve of the other methyl glycosides but was similar to the concentration-response curve of equilibrated D-fructose (25 °C) (see Fig 7). With the exception of methyl  $\beta$ -D-fructofuranoside and equilibrated D-fructose (25 °C) Beidler plots<sup>4</sup> for the sugars tested yielded a straight line (Fig 3A and B). The straight line of a Hill plot<sup>21</sup> with a slope of one for the sugars (Fig 4A and B), with the exception of D-fructose and methyl  $\beta$ -D-fructofuranoside, suggested simple monomolecular interactions. The

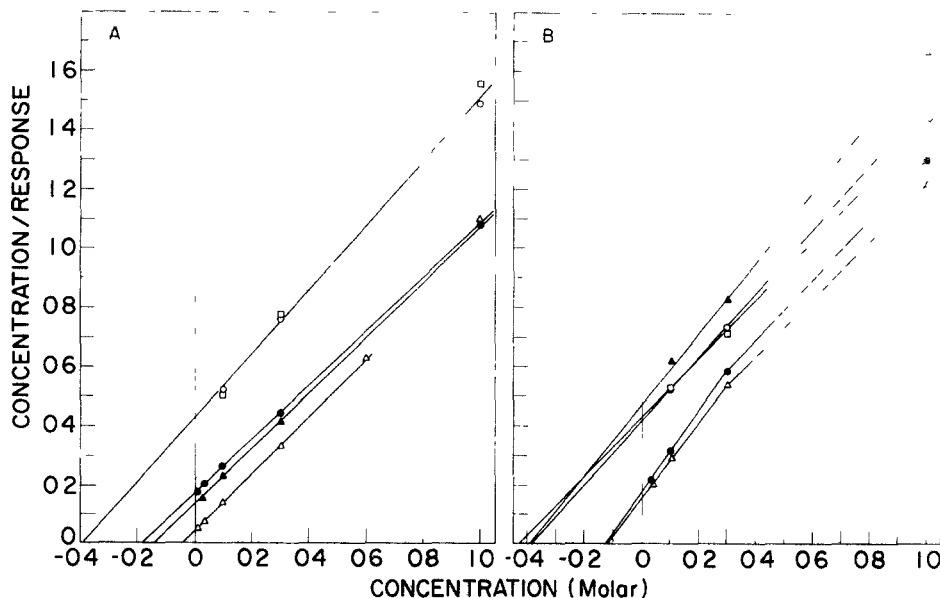


Fig 3 A Beidler plot of gerbil's integrated taste response to various glycosides: Sucrose (○), methyl  $\alpha$ -D-xylopyranoside (●), methyl  $\alpha$ -D-glucopyranoside (▲), methyl  $\beta$ -D-glucopyranoside (◐) and methyl  $\beta$ -D-xylopyranoside (◻) B Beidler plot of gerbil's integrated taste response to D-fructose (△) (equilibrated at 25 °C) and the 4 methyl fructosides: methyl  $\alpha$ -D-fructofuranoside (◑), methyl  $\beta$ -D-fructofuranoside (●), methyl  $\alpha$ -D-fructopyranoside (◻), methyl  $\beta$ -D-fructopyranoside (▲)

downward deflection of the curve in the Beidler plot for these two sugars (Fig 3B) was not an artifact caused by a single deviant response because the same trend was observed in each of the animals tested. As seen in the plot (Fig 3B) equilibrated D-fructose (25 °C) was a slightly better stimulus than methyl  $\beta$ -D-fructofuranoside and was vastly superior to the two fructopyranosides and the  $\alpha$ -fructofuranoside.

#### *Anomeric substitution*

Replacement on the anomeric carbon (C-1) of  $\alpha$ -D-glucopyranose by a number of substituent groups led to molecules of different stimulatory effectiveness (Fig 5). An equatorial hydroxyl or methoxyl group at the anomeric carbon, as seen in methyl  $\beta$ -D-glucopyranoside or  $\beta$ -D-glucopyranose, dramatically reduced the effectiveness of the sugar as a stimulus (Figs 1 and 2). A similar effect was observed between methyl  $\alpha$ -D-xylopyranoside and methyl  $\beta$ -D-xylopyranoside (Fig 3A).

#### *Epimeric substitution*

Replacement or reorientation of the various hydroxyl groups of methyl  $\alpha$ -D-glucopyranoside at positions C-2, C-4, or C-5 of the D-pyranoside ring had variable effects on the ability of the resulting molecule to stimulate. Methyl  $\alpha$ -D-mannopyranoside, the C-2 epimer, and methyl  $\alpha$ -D-galactopyranoside, the C-4 epimer of the corresponding glucoside, were considerably poorer stimuli compared to the glucoside (Fig 6B). Two other pyranosides, methyl  $\alpha$ -D-fructopyranoside and methyl

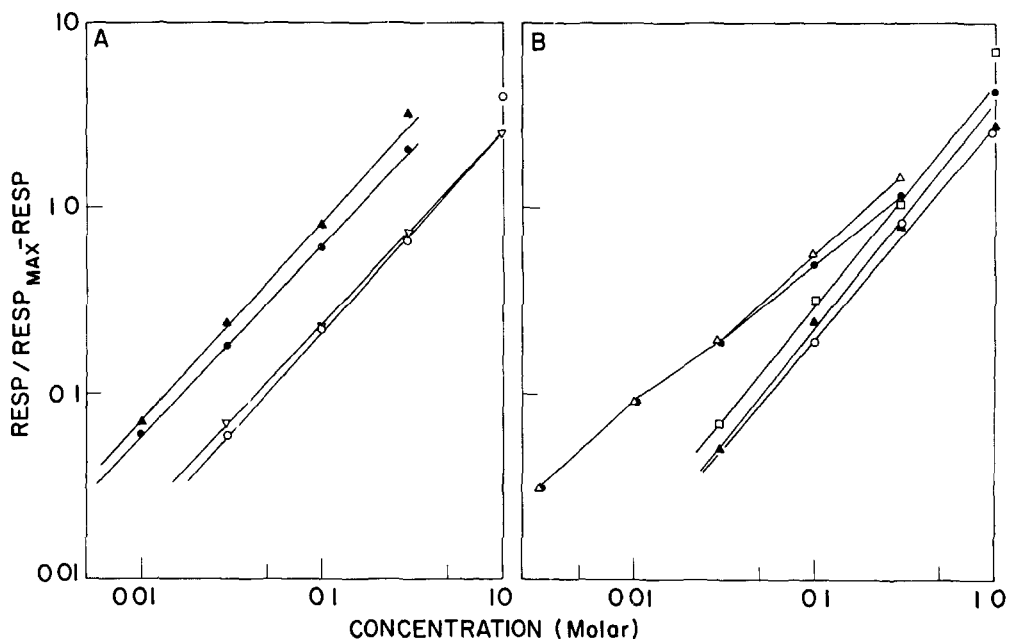


Fig 4 A Hill plot of data from Fig 3A Methyl  $\alpha$ -D-glucopyranoside ( $\bullet$ )  $n = 1.06$ , methyl  $\alpha$ -D-xylopyranoside ( $\blacktriangle$ )  $n = 1.10$ , methyl  $\beta$ -D-glucopyranoside ( $\square$ )  $n = 1.04$ , methyl  $\beta$ -D-xylopyranoside ( $\circ$ )  $n = 1.07$  For the meaning of  $\text{Resp}/\text{Resp}_{\text{max}} - \text{Resp}$  and  $n$  see text B Hill plot of data from Fig 3B Methyl  $\beta$ -D-fructopyranoside ( $\bullet$ ), methyl  $\alpha$ -D-fructofuranoside ( $\circ$ )  $n = 1.14$ , methyl  $\beta$ -D-fructopyranoside ( $\blacktriangle$ )  $n = 1.18$ , methyl  $\alpha$ -D-fructopyranoside ( $\square$ )  $n = 1.12$ , fructose (equilibrated, 25°C) ( $\circ$ )

$\beta$ -D-fructopyranoside, which have axial C-5 hydroxyl groups, were poor stimuli (Fig 3B) The response to the 2-deoxy derivative, methyl-2-deoxy- $\alpha$ -arabino-hexopyranoside, was inferior to the response of methyl  $\alpha$ -D-glucopyranoside Stimulation with methyl  $\alpha$ -D-xylopyranoside which lacks a C-6 hydroxy methyl group was equaled to the response of methyl  $\alpha$ -D-glucopyranoside (Fig 6A) 1,5-Anhydro-D-mannitol, which is similar to  $\alpha$ - and  $\beta$ -D-mannopyranoside in all respects but which lacks an anomeric hydroxyl group, also was a poor stimulus (Fig 7)

#### Reducing sugars

Comparison of the thresholds and  $\text{CR}_{50}$ s of the reducing sugars equilibrated at 25°C showed that the order of effectiveness as gustatory stimulants was D-fructose > D-mannose > D-xylose > D-galactose > D-glucose (Fig 7) The most striking feature of the family of curves in Fig 7 is the greater effectiveness of sucrose compared to the other sugars

#### Competitive interaction

The theoretical curve for competitive interaction was determined from the dissociation constant measured from a Beidler plot (Fig 8, inset) The responses evoked by a mixture of 0.3 M methyl  $\alpha$ -D-glucopyranoside and increasing concentrations of sucrose fit the theoretical curve closely (Fig 8) A mixture of 1.0 M methyl  $\alpha$ -D-

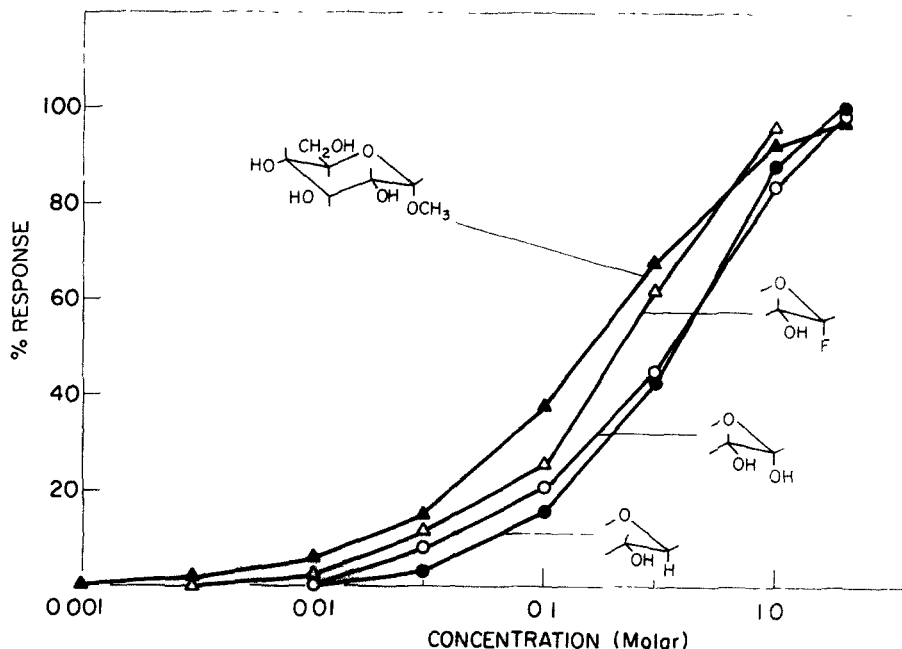


Fig 5 Effect of the substituent group at position C-1 on the stimulatory ability of D-glucopyranose Responses are relative to the maximum sucrose response Methyl  $\alpha$ -D-glucopyranoside ( $\blacktriangle$ ),  $\alpha$ -D-glucopyranosyl fluoride ( $\triangle$ ),  $\alpha$ -D-glucopyranose ( $\circ$ ), 1,5-anhydro-D-glucitol ( $\bullet$ )

glucopyranoside and sucrose evoked responses slightly greater than predicted by the curve and the mixture of 0.1 M methyl  $\alpha$ -D-glucopyranoside and sucrose evoked slightly lower responses than expected. In no case did the response to the mixture ever exceed the maximum response evoked by sucrose alone.

#### DISCUSSION

In the present study the gustatory effectiveness of reducing and non-reducing monosaccharides was determined by recording taste responses in the chorda tympani nerve of the Mongolian gerbil. The order of effectiveness of the equilibrated reducing sugars at a single concentration (0.3 M) was found to be D-fructose > D-mannose > D-xylose > D-glucose > D-galactose. To date the order fructose > mannose > glucose > galactose has been found in all mammals studied comparing these sugars at a single concentration. These include the human<sup>8</sup>, gerbils including *Meriones libycus*, *Meriones shawi*, *Psammomys obesus*<sup>27</sup>, rat<sup>31</sup>, hamster<sup>32</sup> and dog<sup>2</sup>. The electrophysiological threshold for fructose and galactose in the rat and hamster is 0.05 M but the threshold is 0.1 M for glucose<sup>17</sup>. This is much higher than the gerbil's threshold for 3 other sugars: fructose (0.003 M), glucose (0.03 M) and galactose (0.03 M).

Most results obtained with the chorda tympani nerve of the gerbil may be fitted by Beidler's taste equation<sup>4</sup> first proposed for the NaCl response in the rat. Except for fructose and methyl  $\beta$ -D-fructofuranoside, the responses of most of the



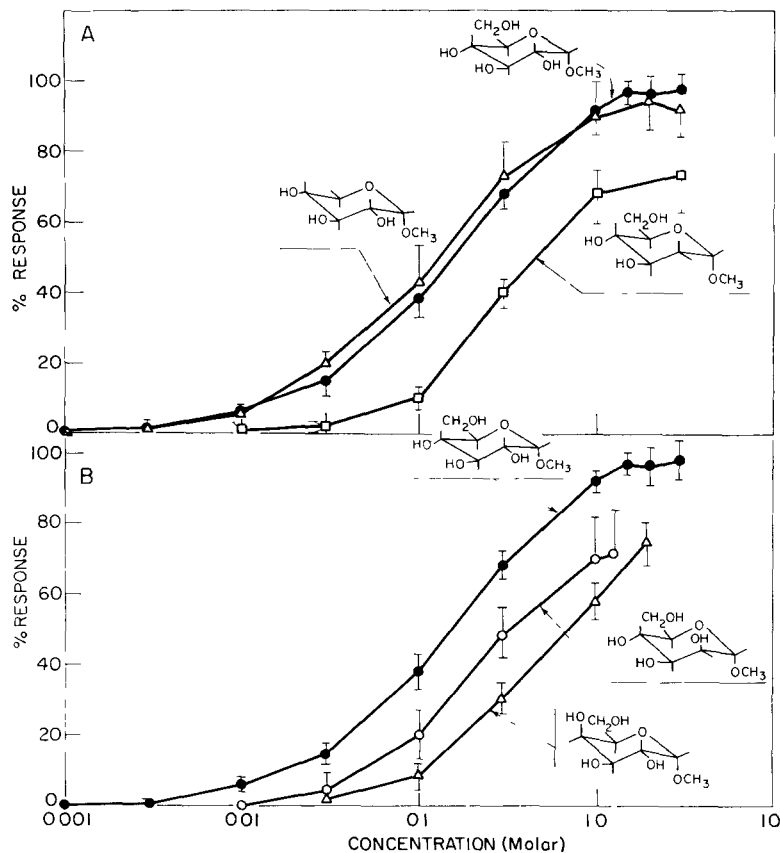


Fig 6 A comparison of integrated chorda tympani nerve responses to methyl  $\alpha$ -D-glucopyranoside ( $\bullet$ )  $N = 15$ , methyl  $\alpha$ -D-xylopyranoside ( $\square$ )  $N = 5$  and methyl 2-deoxy- $\alpha$ -D-arabino-hexopyranoside ( $\triangle$ )  $N = 5$  solutions flowed over the tongue. Bars represent 95% confidence intervals. B taste responses to methyl  $\alpha$ -D-glucopyranoside ( $\bullet$ )  $N = 15$ , methyl  $\alpha$ -D-mannopyranoside ( $\circ$ )  $N = 6$  and methyl  $\alpha$ -D-galactopyranoside ( $\triangle$ )  $N = 5$ . Responses relative to sucrose response of 100%.

monosaccharides tested show linear results in a Beidler plot. In the fly's sugar receptor disaccharides stimulate according to the Beidler taste equation but monosaccharides give anomalous results<sup>29</sup>. These latter findings were interpreted to signify that either a receptor site is composed of two subsites each of which is occupied by a single disaccharide or two monosaccharide molecules or that allosteric transition occurred in the receptor site. The formation of a multimolecular complex between stimulating molecules and the receptor site has been suggested for the sweet receptor of the rat<sup>38,39</sup>. Analysis of the present data on fructose or methyl  $\beta$ -D-fructofuranoside is not inconsistent with these ideas (Fig 4B).

In the earlier report<sup>25</sup> it was suggested that the great stimulatory effectiveness of sucrose over other disaccharides may be due to the presence of one or more of the following types of sites: sucrose,  $\beta$ -D-fructofuranose or  $\alpha$ -D-glucopyranose. On evolutionary grounds one could argue for a sucrose receptor site. Sucrose is the most common soluble sugar found in plants<sup>3,33</sup>, so it is likely natural selection favored animals

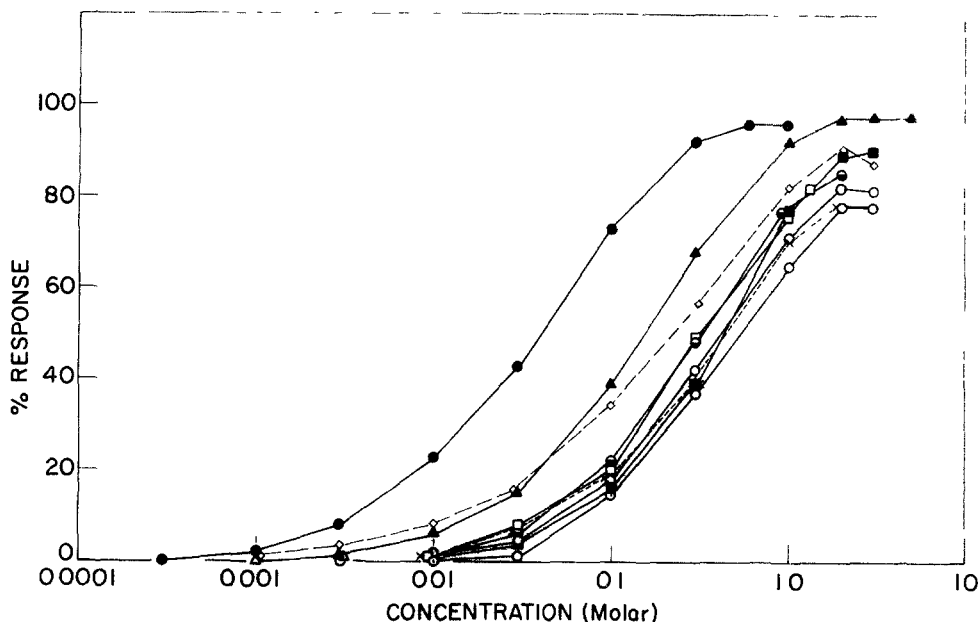


Fig 7 Mean integrated responses of the chorda tympani nerve to different sugars applied to the tongue. Number of animals (N), maximum response (MR) and the molar concentration producing a 50% response ( $CR_{50}$ ) with a 95% confidence interval are given below. Sucrose, ●, (N = 49, MR = 1,  $CR_{50} = 0.045 \pm 0.005 M$ ), methyl  $\alpha$ -D-glucopyranoside, ▲, (N = 14, MR =  $0.99 \pm 0.05$ ,  $CR_{50} = 0.14 \pm 0.02 M$ ), D-xylose, equilibrated, ○, (N = 5, MR =  $0.83 \pm 0.07$ ,  $CR_{50} = 0.19 \pm 0.06 M$ ), D-galactose, equilibrated, □, (N = 5), MR =  $0.80 \pm 0.09$ ,  $CR_{50} = 0.32 \pm 0.07 M$ ), D-mannose, equilibrated, ◻, (N = 6, MR -- not measurable,  $CR_{50} = 0.12 M$  estimated), D-glucose, equilibrated, ■, (N = 15, MR =  $0.92 \pm 0.05$ ,  $CR_{50} = 0.37 \pm 0.03 M$ ), 1,5 anhydro-D-mannitol, ▾, (N = 5, MR -- not measured,  $CR_{50} = 0.12 M$  estimated), fructose, ◇, (N = 6, MR =  $0.93 \pm 0.05$ ,  $CR_{50} = 0.22 \pm 0.08 M$ ), 1,5 anhydro-D-galactitol, x, (N = 4, MR -- not measured,  $CR_{50} = 0.21 M$  estimated). All responses are relative to maximum sucrose response of 100%.

possessing means of detecting it. The extreme sensitivity and ubiquitous nature of 'sucrose receptors' in insects<sup>9,10,37</sup>, the fact that sucrose is one of the sweetest sugars known<sup>7,30</sup>, the ability of sucrose to evoke greater gustatory nerve responses than any other sugar in many mammals<sup>14,17,27</sup> and the universal preference in mammals for sucrose<sup>28,35</sup> suggests there may be a specific sucrose receptor site. The heightened effectiveness of methyl  $\alpha$ -D-glucopyranoside and methyl  $\beta$ -D-fructofuranoside compared to other monosaccharides is consistent with a sucrose receptor composed of two subsites, one for the glucopyranoside and one for the fructofuranoside portion of the molecule. Since the fructose isomers were poor stimuli this would account for the failure of the fructosyl glucopyranosides, turanose and palatinose, which exist in solution as a mixture of isomers, to be very stimulatory reducing sugars<sup>25</sup>. The present results confirm and extend Anderson's *et al*<sup>1,2</sup> proposal that the stimulatory effectiveness of a disaccharide depends upon its constituent monosaccharides. There is no reason to discount two completely separated sites, a glucose and a fructose site. This idea would be consistent with single fiber responses in which fructose is a better stimulus than sucrose or that sucrose is a better stimulus than fructose<sup>34</sup>. These

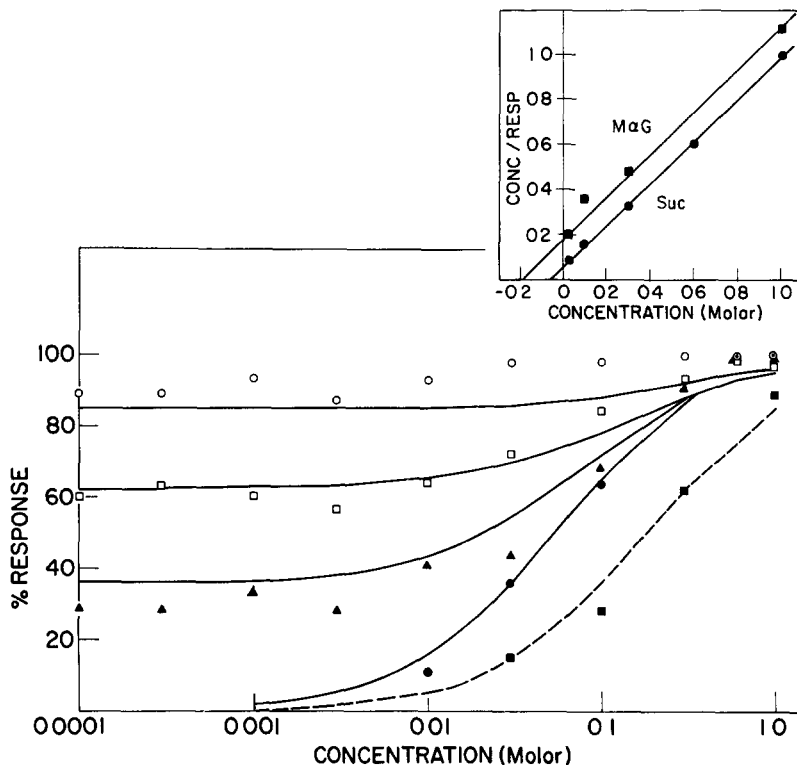


Fig 8 Concentration–response curve of sucrose in the presence of methyl  $\alpha$ -D-glucopyranoside (MaG). The solid lines are theoretical curves obtained from an equation describing the competitive interaction of two substances with a single receptor site (see text). Data points for sucrose alone:  $\bullet$ , sucrose + 0.1 M (MaG),  $\blacktriangle$ , sucrose + 0.3 M (MaG),  $\square$ , sucrose + 1.0 M (MaG),  $\circ$ , MaG alone.  $\blacksquare$  Dashed line is the theoretical curve drawn from the binding equation for MaG.  $K_d$  for sucrose (0.05 M) and  $K_d$  for MaG (0.18 M) were determined from the Beidler plot (see inset).  $CR_{50}$  for sucrose = 0.052 M and for MaG = 0.18 M.

fibers would innervate cells that contained a higher number of one type of site. The rare ‘maltose-fiber’<sup>1</sup> could be explained as the fusion of two glucose sites. It is of interest that, to date, single neuron studies have not revealed lactose, galactose or mannose responding fibers<sup>1</sup>. The present experiment does not exclude the existence of other sugar receptor sites. Their existence is probably the reason the response to the mixture of sucrose and methyl  $\alpha$ -D-glucopyranoside is slightly greater than predicted by the theoretical equation for competitive interaction.

The finding that methyl  $\alpha$ -D-glucopyranoside competes with sucrose for a binding site and results from other glucoside studies permit detailed analysis of the requirements of the  $\alpha$ -D-glucopyranose portion of the receptor site. The C-2 and C-4 positions of the D-pyranose ring are more effective with equatorial hydroxyl groups whereas an axial hydroxyl is favored at C-1. An O-methyl substituent on the axial C-1 hydroxyl enhances activity. Methyl  $\beta$ -D-glucopyranoside is active but at a much reduced level. The equatorial methyl group could result in steric hindrances (*i.e.*, reduced effect caused by a bulky molecular group which blocks access to the relative

site) because  $\beta$ -D-glucopyranose is less effective than either  $\alpha$ -D-glucopyranose or the equilibrium mixture. The value of the axial hydroxyl group at C-1 is established by the reduced effectiveness of 1,5-anhydro-D-glucitol and the elevated effectiveness of  $\alpha$ -D-glucopyranosyl fluoride. The involvement of the oxygen atom at the C-1 position in hydrogen bonding to the site is indicated since the fluorine atom of  $\alpha$ -D-glucopyranosyl fluoride and the hydroxyl group of  $\alpha$ -D-glucopyranose are isosteric<sup>36</sup>. The fluorosugar lacks the proton and is considered to be a strong hydrogen bondor. The slightly elevated response to the fluorosugar over  $\alpha$ -D-glucopyranose may point to a mechanism whereby the strongly bound sugars induce a greater change in the site. These results with the C-1 substituted sugars are taken to indicate that the effectiveness of a sugar to bind to the sucrose receptor site is reduced by the lack of an axial hydroxyl rather than the presence of an equatorial hydroxyl at C-1.

The absence of an equatorial hydroxyl at C-2 drastically reduced the effectiveness of the glycoside. Both methyl 2-deoxy- $\alpha$ -D-arabino-hexopyranoside and methyl  $\alpha$ -D-mannopyranoside show reduced effectiveness because the former lacks a C-2 hydroxyl group and the latter's C-2 hydroxyl is oriented in an axial plane. Similarly 1,5-anhydro-D-mannitol elicits a relatively poor response in part because it lacks the required C-1 hydroxyl but undoubtedly also because it possesses a deleterious axially oriented C-2 hydroxyl group. Further evidence of a rather stringent requirement for a C-2 equatorial hydroxyl group is seen in the failure of methyl  $\alpha$ -D-fructopyranoside and methyl  $\beta$ -D-fructopyranoside to be effective stimuli. In this instance the superposition of the fructopyranosides and the mannopyranoside results in the axial hydroxyl at C-5 of the fructoside being in an equivalent position to the C-2 axial hydroxyl of the mannoside.

Although the 4-deoxy-D-glucose (4-deoxy-D-xylo-hexose) derivative was not available, the requirement for an equatorial C-4 hydroxyl group was established by the reduced effectiveness of 1,5-anhydro-D-galactitol and methyl  $\alpha$ -D-galactopyranoside. The two major configurational differences between these two sugars are that 4-deoxy- $\alpha$ -D-xylo-hexopyranose possesses a preferred C-1 axial hydroxyl and lacks a C-4 hydroxyl group while 1,5-anhydro-D-galactitol lacks the C-1 hydroxyl and possesses an axially oriented C-4 hydroxyl group. However, by inversion the molecules can be superimposed so that the C-1 of the 1,5-anhydro-sugar now is in a position equivalent to the C-4 methyl of 4-deoxy- $\alpha$ -D-xylo-hexopyranoside. Now the preferred C-1 axial and C-2 equatorial hydroxyls are maintained. Methyl  $\alpha$ -D-galactopyranoside resembles the corresponding glucoside except for an axial hydroxyl at C-4.

It appears that substitution of the C-5 hydroxyl methyl group with hydrogen (*e.g.*, methyl  $\alpha$ -D-xylopyranoside) does not dramatically change effectiveness.

The present study has extended our knowledge of substances that activate the taste receptors of mammals. Unlike the sugar receptor of the fly which responds only if the sugar molecules are of the correct configuration<sup>9, 11, 15, 26</sup> the receptor cells of the gerbil respond to every sugar tested. In the gerbil, methyl  $\beta$ -D-fructofuranoside is the most stimulatory fructoside.  $\beta$ -D-Fructofuranose is thought to be the fructose isomer responsible for fly taste receptor stimulation!<sup>16</sup> The finding that equatorial hydroxyls are required at C-2 and C-4 of the glucopyranoside molecule for maxi-

num stimulation is remarkably similar to the requirement of the sugar receptor of the fly. An axial hydroxyl at C-1 promotes the sugar receptor response in the fly and gribil. Residues at the C-5 position in both animals are less important. It would appear that in the evolution of receptor sites both animals have taken advantage of many of the same conformational and configurational characteristics of sugars to detect sucrose.

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