STIMULATION OF THE GERBIL'S GUSTATORY RECEPTORS BY POLYOLS

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SUMMARY

The gustatory responses of the Mongolian gerbil were tested with 12 sugar alcohols. The electrophysiological effectiveness of the linear polyols as gustatory stimulants increased as the length of the carbon chain increased from 2 to 5. Six and 7 carbon acyclic polyols were no more effective than the pentitols. By comparison myo-inositol, a cyclic polyol, was more effective in evoking a response. Responses to mixtures of D-sorbitol and sucrose suggest that these sugars compete for a common receptor site. A sucrose receptor site and a model of it is proposed.

INTRODUCTION

Structural similarities existing between the sugars and the sugar alcohols are thought to account for the sweetness of polyols. The ability of polyols to stimulate taste receptors has been studied electrophysiologically in the dog, monkey, hamster, and rat. In all cases, polyols are less effective than sucrose. Responses of sucrose sensitive fibers to ethylene glycol and glycerol have suggested an interaction with a 'sucrose receptor site' in the dog and monkey. In the hamster the order of stimulatory effectiveness of the polyols is mannitol > glycerol > ethylene glycol. This suggests an increase in taste effectiveness of polyols with length of the carbon chain. Interest in the properties of a putative sucrose-binding site and the importance of polyols as sweeteners has prompted the present electrophysiological experiment in gerbils.

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MATERIALS AND METHODS

Animals
Male and female Mongolian gerbils, Meriones unguiculatus, were used.

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

Sugar solutions
All compounds were dissolved in distilled water and stored in a refrigerator at 2 °C for as long as 7 days before use.

Exposure of nerve, electrophysiology and stimulus presentation
Multi-unit gustatory discharges were recorded from the chorda tympani nerve. This procedure has been previously described.

Concentration–response curve
Dissociation constants (Kd) for each polyol were calculated from a Beidler plot. CR50 (concentration evoking a 50% response) and the maximum response were measured directly from semilogarithmic plots. Theoretical curves for the interaction of two substances with a single receptor site were derived from the following equation:

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where [A] and [B] are the concentrations of the two substances and K_A and K_B are their respective dissociation constants.

RESULTS

Each of the polyols evoked a neural response. The gerbil's taste nerve responded with increased activity to increasing concentrations of sugar–alcohol applied to the tongue. Polyol stimulation resulted in an electrophysiological response similar to that already reported for sucrose.

When plotted on semilog scale the polyol concentration–response curves were sigmoidal (Fig 1). As shown in Fig 1 the concentration–response curves of all the compounds were approximately parallel. Seven additional polyols were tested: D-mannitol, D-galactitol, perseitol, D-sorbitol, D-xylitol, L-arabinitol, and D-arabinitol. Their concentration–response curves lie within the range of those shown in Fig 1. Unlike the disaccharides, most polyol concentration–response curves did not show a reduced response after the maximum response had been reached. However, reduced responses were seen in 40% of the animals tested with either L-arabinitol or D-xylitol and 60% of the animals tested with D-sorbitol. The limited solubility of myo-inositol,
Fig 1 Comparison of mean integrated chorda tympani responses to sucrose (○), myo-inositol (▲), d-ribose (□), erythritol (△), glycerol (●) and ethylene glycol (○). All responses scaled to the maximum sucrose response which is set at 100%. See legend of Fig 2 for N.

Fig 2 Relationship between the number of carbons in sugar alcohols and the concentration (left ordinate) which elicited a 50% response (CR₀) and the maximum response (right ordinate) relative to sucrose, maximum taken as 100%. Bars indicate 95% confidence interval. Ethylene glycol (2C, ▲ N = 5), glycerol (3C, ● N = 5), erythritol (4C, ▲ N = 6), d-ribose (5C, ▼ N = 6), l-arabinol (5C, ▲ N = 6), d-arabinol (5C, ▼ N = 6), d-xylitol (5C, ▼ N = 5), d-sorbitol (6C, ○ N = 5), d-galactitol* (6C, ◇ N = 5), d-mannitol* (6C, ◇ N = 5), myo-inositol* (6C, ○ N = 10), persimol* (7C, ● N = 6), sucrose (●, N = 28) Asterisk indicates sugars whose insolubility prevented direct determination of maximum response. The CR₀ for these compounds was estimated from λ₀.
Fig 3 Comparison of the mean integrated chorda tympani responses to the 4 pentitols D-arabinitol ( ), D-arabinitol ( ), D-riboitol ( ) and D-xylitol ( ). All responses relative to maximum sucrose response of 100%. These values are the mean responses of 5 animals.

perseitol, D-galactitol and D-mannitol prevented direct determination of their maximum response. Ethylene glycol and glycerol evoked a maximum equal to the maximum sucrose response but as the length of the polyol chain increased the maximum response decreased significantly (Fig 2). Similarly, the CR_{50}s were reduced as the chain length increased. CR_{50}s were derived from K_d where solubility limits prevented direct measurements.

As a group, only the pentitols were soluble enough to test over a wide concentration range. The 4 pentitols differed only slightly in their stimulating effectiveness. D- and L-arabinitol being somewhat less effective than D-riboitol or D-xylitol at high concentrations (Fig 3).

On the Beidler plot (Fig 4) and the Hill plot (Fig 4, inset) the concentration-response function for each of the polyols approximated a straight line. The only exception, ethylene glycol, showed a substantial upturn at low concentrations on the Beidler plot. On the Hill plot each one of the polyol concentration-response functions has a slope of nearly one.

Similar to the mixture of sucrose and methyl α-D-glucopyranoside, the mixtures of sucrose and D-sorbitol fit the theoretical curves determined for a competitive interaction of the two substances for a single receptor site (Fig 5). Response to the mixture was not additive at high concentrations and the maximum response for the mixture did not exceed the maximum response evoked by sucrose alone. With D-sorbitol alone, the plot of the data was slightly below the theoretical expectation K_d for this curve was determined from the Beidler plot (Fig 5, inset).
DISCUSSION

The effectiveness of the polyols increases (e.g., CR$_{50\%}$) until the length of the carbon chain reaches 5 carbons (5 carbon cut-off) thus, manntitol, glycerol, ethylene glycol. Hardman$^7$ found the same ranking for the hamster.

The straight lines in the Beidler plot and Hill plot for the polyols are consistent with a monomolecular binding hypothesis$^4,6$ Even with the upturn of the ethylene glycol curve the hypothesis still holds. One of the peculiarities of the reciprocal plot Beidler$^4$ used to support his taste theory is that it is extremely sensitive to measurement errors near threshold. This may account for the failure of ethylene glycol, fructose and glycine to fit the Beidler plot$^7,14,15$

As seen in Figs 1 and 2 the maximum response of the polyols decreases as chain length increases. The most probable reason for the decreased effectiveness of the longer polyols is that larger molecules cannot fit into as many types of receptor sites. Alternatively, they may fit and bind yet fail to vigorously activate the receptor. For example, Ariens$^2$ has referred to 'intrinsic activity' to account for the fact that many

Fig 4 Beidler plot of gerbil mean integrated taste response to various polyols Ethylene glycol (●), glycerol (○), erythritol (□), d-ribose (■), L-arabinose (▲), d-arabinose (▲), myo-inositol (▲), persentol (▲), d-mannitol (○) Inset Hill plot of gerbil mean integrated taste response to various polyols Ethylene glycol (●) n = 1 2, glycerol (○) n = 1 0 4, erythritol (□) n = 1 0 8, d-ribose (▲) n = 1 0 8, d-sorbitol (▲) n = 1 5 For the meaning of Resp/Resp$_{max}$ — Resp and n see discussion
Fig 5 Concentration-response curve of sucrose in the presence of D-sorbitol (Sorb). The solid lines are the theoretical curves obtained from the equation describing the competitive interaction of two substances with a single receptor site (see text). Data points for sucrose alone (●), sorbitol alone (▲), sucrose 0.03 M (Sorb) (□), sucrose 0.1 M (Sorb) (▲), sucrose + 0.3 M (Sorb) (○). Dashed line (— — — —) is the theoretical curve drawn from the taste equation for sorbitol alone. Dotted line ( ) is the theoretical curve for sorbitol where the intrinsic activity of sorbitol is 0.87. See discussion section for details. \( K_a \) for sucrose 0.015 M, \( K_a \) for sorbitol 0.29 M. Dissociation constants were determined from the Biedler plot (inset).

Drugs had high affinities (low \( K_a \)) but were unable to evoke a response. These drugs could bind yet presumably possessed a group that somehow turned a highly effective drug into an ineffectual one. Decreased ‘intrinsic activity’ could also account for the decreased maximum response of the gerbil’s taste nerve to some disaccharides and monosaccharides.

In the development of the binding equation one of the assumptions was that response = \( k [SR] \) (see ref 9). If one were to assume that at \( \text{Resp}_{\text{max}} \) all the receptor sites are filled then the size of maximum response is an index of ‘intrinsic activity’. In this case assuming all the sucrose sites are filled by sorbitol but are only able to evoke a maximum response equal to 87% that of sucrose. Therefore, from this experiment (Fig 5) \( k_{\text{sucrose}} \neq k_{\text{sorbitol}} \) but rather \( k_{\text{sucrose}} = 1 \) and \( k_{\text{sorbitol}} = 0.87 \). The constant \( k \) represents the intrinsic activity. The binding equation then becomes
Fig 6 Proposed model for the 'sucrose site' in the membrane of the gerbil gustatory cell. See text for explanation.

![Proposed model for the 'sucrose site'.](image)

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\frac{\text{Resp}}{\text{Resp}_{\text{max}}} = \frac{k_{\text{sugar}}}{1 + \frac{K_d}{[S]}}
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With recalculation, using decreased intrinsic activity, the theoretical curve for D-sorbitol in Fig 5 fits the data.

In both flies and gerbils the cyclic hexitol, myo-inositol, is a better stimulant than the linear polyols. This may result from myo-inositol's close resemblance to the effective stimulants \(\alpha\)-D-glucopyranose and methyl \(\alpha\)-D-glucopyranoside.

Previous work on gerbils with disaccharides showed sucrose was the most stimulatory sugar. Methyl glycosides, such as methyl \(\alpha\)-D-glucopyranoside and methyl \(\beta\)-D-fructofuranoside, which have structural features in common with sucrose were the most effective monosaccharides for eliciting a neural response. Also, the lengths of superimposed Dreiding models of a pentitol and sucrose almost perfectly coincide. Response to mixtures of methyl \(\alpha\)-D-glucopyranoside and sucrose, and D-sorbitol and sucrose suggest that these sugars act at a common receptor site. Based on these results the presence of a model sucrose receptor site as shown in Fig 6 is suggested.

The 'sucrose site' in Fig 6 would operate with a sugar molecule occupying it. Presuming that the competition of the polyol for the sucrose receptor site the identical lengths of sucrose and the pentitols, and the unimproved effectiveness of linear polyols beyond 5 carbons together suggest the proposed sucrose receptor site is about as long as a sucrose molecule. The \(\beta\)-fructofuranosyl portion is tentatively considered to occupy a 'deep' subsite and the hydroxy methyl group at C-5 of the glucopyranoside is expected to stick out into the solution. The deep subsite is associated with a high degree of specificity as evidenced by the failure of the two fructosyl glucosides, turanose (3-O-\(\alpha\)-D-glucopyranosyl fructose) and palatinose (6-O-\(\alpha\)-D-glucopyranosyl fructose) to be as stimulatory as sucrose. Monosaccharide response data support the proposed binding of hydroxyl groups at positions C-1, C-2, and C-4 of the glucose moiety. D-Pyranosides which have equatorial substituents at C-2 and C-4 and the C-1 axial substituent were the most effective monosaccharides. A C-5 hydroxy methyl binding position is not required. This supports a model with the C-6 hydroxyl group.
protruding into the surrounding solution. The enhanced activity of methyl \( \alpha \)-d-glucopyranoside over \( \alpha \)-d-glucopyranose\(^10\) points to a hydrophobic region in the site. This concides with the relatively hydrophobic carbon atom of the fructose moiety shown in Fig. 6. This model would account for the relatively weak responses with the other disaccharides as gustatory stimulants. Large bulky substituent groups at C-1 would block effective interaction between the sugar and the site. Similarly, this model would account for the elevated effectiveness of the \( \beta \)-galactopyranoside containing disaccharides such as \( \beta \)-lactose and lactitol, compared with the \( \alpha \)-galactopyranoside disaccharides, melibiose and melibutol\(^9\). By inverting these molecules, hydroxyl groups at C-4, C-3, C-2 and C-1 of the galactose moiety can be superimposed over the \( \alpha \)-glucopyranoside portion of the receptor site. The C-4 hydroxyl group of the \( \alpha \) and \( \beta \) galactosyl portions of these disaccharides are axial and similar to the C-1 substituent of the \( \alpha \)-d-glucopyranoside portion of sucrose. Axial hydroxyls at C-3 and C-2 correspond to respective hydroxyls at C-2 and C-3 of the \( \alpha \)-d-glucoside. The galactoside's C-1 substituent would be superimposed over the C-4 of the glucopyranoside. Melibiose and melibutol possess axial substituents at this position. Only the \( \beta \)-galactopyranosyl disaccharides, lactose and lactitol possess an axial group at this position which is similar to the \( \alpha \)-d-glucopyranosyl portion of sucrose.

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