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# STIMULATION OF THE GERBIL'S GUSTATORY RECEPTORS BY DISACCHARIDES

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SUMMARY

The gustatory responses from the chorda tympani nerve of the Mongolian gerbil, *Meriones unguiculatus*, were tested with 13 disaccharides Sucrose was the most stimulatory sugar

The ability of fructosyl glycosides to stimulate may depend upon the linkage between fructose and the glycoside Disaccharides possessing 1 → 3, 1 → 4, or 1 → 6 linkages were poor stimuli compared to sucrose which has a 1 → 2 linkage Glucopyranosyl disaccharides with an $\alpha$-linkage were better stimuli than the $\beta$-anomers, while galactopyranosyl disaccharides possessing a $\beta$-linkage were better than their $\alpha$-anomers

## INTRODUCTION

There have been several attempts to account for the sweetness of structurally diverse sweet chemicals[9,25,28,31 37] Electrophysiological recordings from single receptor cells[26,31] or primary afferent fibers[12 14 15,29,32,33] in mammals have not found receptor cells or taste nerve fibers which respond more or less exclusively to chemicals that are sweet to humans Biochemical investigations with sugar-binding protein preparations[5,6,20] of tongue epithelium suffer from a scarcity of physiological data from which inferences concerning the identity of sweet receptor sites can be drawn

Sugar-receptor site specificity was examined by analyzing electrophysiological responses of the gerbil's chorda tympani nerve to disaccharides Both $\alpha$ and $\beta$ anomers such as maltose and cellobiose, melibiose and lactose, and their chemically reduced derivatives, the alditols, were compared since anomeric linkages play an important role in determining the effectiveness of a disaccharide as a gustatory stimulant[7 16] The alditol disaccharides were synthesized from their parent sugars to see if sub-

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stantial changes in the molecule would have an effect on the stimulatory ability of the disaccharide Subsequent papers will deal with monosaccharides and polyol

## MATERIALS AND METHODS

### *Animals*

The Mongolian gerbil, *Meriones unguiculatus*, was used because of its demonstrated sensitivity to sucrose[22,24] Animals used in these experiments were obtained from a closed colony (J Hasenau, Northville, Mich ) and were less than 1-year-old

### *Sugars*

The sugars were obtained from Pfanstiehl Laboratories, Waukegan, Ill Sigma Chemical Co , St Louis, Mo , or were synthesized Cellobiitol, lactitol maltitol and melibiitol were prepared by the reduction of the respective disaccharide with $NaBH_4$ $Na^+$ was removed with Amberlite IR-120 ($H^+$) followed by Amberlite IR-45 ($OH^-$) to remove the borate ion The purity of the disaccharide alditols was evaluated by thin-layer chromatography on activated Silica Gel G coated plates using *n*-butanol–acetic acid–ethyl acetate–water (9 6 3 2) as the solvent Components were visualized by spraying with 50% (v/v) ethanol–sulfuric acid followed by charring in an oven at 120 C

### *Taste solutions*

All compounds were dissolved in distilled water and were stored at 2 C for 0–14 days before use Solutions of reducing sugars were prepared a day before use and were allowed to reach mutarotational equilibrium at room temperature Solutions of $\beta$-lactose were used immediately after preparation

### *Electrophysiological*

The animal was anesthetized with an intramuscular or intraperitoneal injection of sodium pentobarbital (35 mg/kg) One-half the initial dose was given at 30-min intervals until the animal was completely anesthetized During the experiment doses of similar magnitude were given to maintain the level of anesthesia

The procedures for exposing the chorda tympani nerve in the middle ear and for chemically stimulating the tongue have been described[22,24] Electrical activity was recorded by touching the nerve with a nichrome electrode which was led to a differential amplifier (Grass P-511) The integrated discharge of the whole chorda tympani nerve was used because it represents a summation of activity from many receptor cells[26] A response was defined as the difference between the integrated spontaneous activity and the greatest integrated potential elicited by a given solution applied to the tongue The integrator (Grass Model No 73PA) time constant was set at 0 5 sec, full wave rectification An indifferent electrode was placed on a nearby piece of moist tissue within the auditory bulla

The temperatures of the distilled water rinse and the taste solution were identical (25 ± 1 °C) Solutions were presented in increasing series of approximately 0 5 log molar concentration steps (*e g* , 0 0001 *M*, 0 0003 *M*, 0 001 *M* 0 1 *M*, 0 3 *M*)

Responses were calculated from at least two complete series of test solutions per animal. A standard solution (0.3 *M* sucrose) was presented frequently between test solutions. Whenever the standard 0.3 *M* sucrose solutions elicited responses that varied more than ± 10% all interjacent responses were rejected.

*Concentration–response curves*

All concentrations of sugars are expressed in molarity and plotted on a logarithmic scale. Each animal was presented with a concentration series of sucrose which allowed computation of the response of any other sugar as a percentage of the maximum (saturation level) sucrose response. In the 5 animals, out of 59, in which the maximum response was not attained within the sucrose concentration range tested, the response to 1.0 *M* sucrose was considered 100%.

The effectiveness of sugars is indicated by their $CR_{50}$s. This value, the concentration that evokes a response 50% of maximum, is similar but not identical to the dissociation constant of Beidler's taste model[3]. Unlike the dissociation constant of Beidler's taste theory which is measured from the reciprocal plot, the $CR_{50}$ is not linked to theoretical assumptions about the nature of sugar–receptor interactions.

$CR_{50}$s were compared in those sugars which were sufficiently soluble to permit a maximum response to be reached. The dissociation constant, obtained from the reciprocal plot, was used as the index of relative stimulating effectiveness for those sugars that were too viscous or insoluble to use at high concentrations. The dissociation constant was calculated from the summated response data which reflects the interaction of the stimulus, S, upon the receptor sites, R, as represented by the following equation

$$S + R \leftrightharpoons SR \tag{1}$$

The dissociation constant, $K_d$, is equal to the reciprocal of the association constant ($K_a$) of Beidler's taste theory[3]

$$K_d = \frac{[S]\,[R]}{[SR]} \tag{2}$$

Assuming that (a) the response (Resp) measured was linearly related to the number of stimulus molecules bound (Resp $\propto$ [SR]) and (b) at a high concentration of S the maximal response ($Resp_{max}$) was reached and then

$$\frac{Resp}{Resp_{max}} = \frac{\text{sites filled}}{\text{total sites}} = \frac{[SR]}{[R] + [SR]} \tag{3}$$

Then by substitution of equation (2) into equation (3)

$$\frac{Resp}{Resp_{max}} = \frac{1}{1 + \dfrac{K_d}{[S]}} \tag{4}$$

By rearrangement of equation 4 we get

$$\frac{[S]}{Resp} = \frac{[S]}{Resp_{max}} + \frac{K_d}{Resp_{max}} \quad (5)$$

Since $K_a = 1/K_d$ this equation is Beidler's taste equation[3] At $Resp/Resp_{max} = 1/2$ it follows that $CR_{50} = K_d$ $[S] = 1/K_a$ If more than one stimulus molecule combined with a receptor site then

$$nS + R \rightleftharpoons RS_n \quad (6)$$

and the response would be proportional to the amount of $RS_n$ formed Equation 4 would become

$$\frac{Resp}{Resp_{max}} = \frac{1}{1 + \frac{K_d}{[S]^n}} \quad (7)$$

By rearranging equation 7 and taking the log we get

$$\log \frac{Resp}{Resp_{max} - Resp} = n \log [S] - \log K_d \quad (8)$$

This is identical to the Hill plot[21] A similar equation was used by Tateda and Hidaka[36] for the analysis of neural response by a rat to glycine

## RESULTS

When any effective chemical was flowed onto the tongue there was an initial rapid rise of neural activity which was dependent upon concentration Fig 1 is a typical series of recordings A slight upward deflection of the baseline at threshold can be seen at 0 003 *M* The weakest effective stimulus in the concentration series was defined as the threshold concentration Sometimes at high concentrations, shown in Fig 1 for 0 3 *M* and 1 0 *M*, after the neural discharge reached its initial peak it declined and then recovered to its maximum level The dip phenomenon only occurred at high concentrations of sugars The second peak was not a water rinse response since it occurred before the water rinse Generally the response declined gradually after the initial response Upon rinsing with distilled water the discharge dropped rapidly to the prestimulation level 'Off' discharges of the type seen with divalent cation stimulation[24] were rarely observed with the disaccharides

On semilogarithmic coordinates, the concentration–response function for sugars was always sigmoidal (Fig 2) Of all the disaccharides tested, sucrose was the best stimulus (Table I), it gave the greatest response, had the lowest $CR_{50}$ and was detected at the lowest concentration All other sugars tested had a threshold 10–30 times higher than sucrose

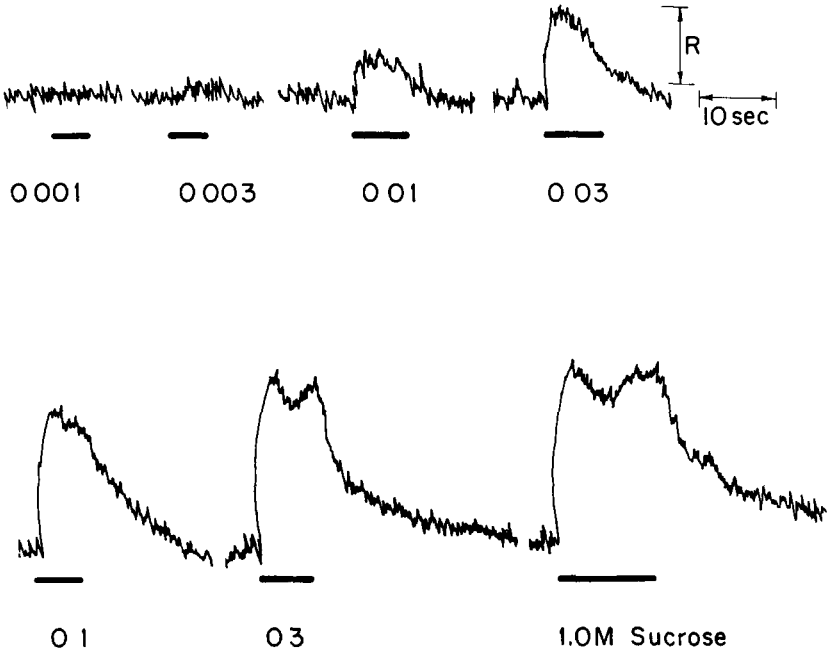


Fig 1 Integrated neural discharge from the gerbil's chorda tympani nerve in response to a series of increasing concentrations of sucrose applied to the tongue The solid bar under the records indicate stimulus duration, R is the measure of response

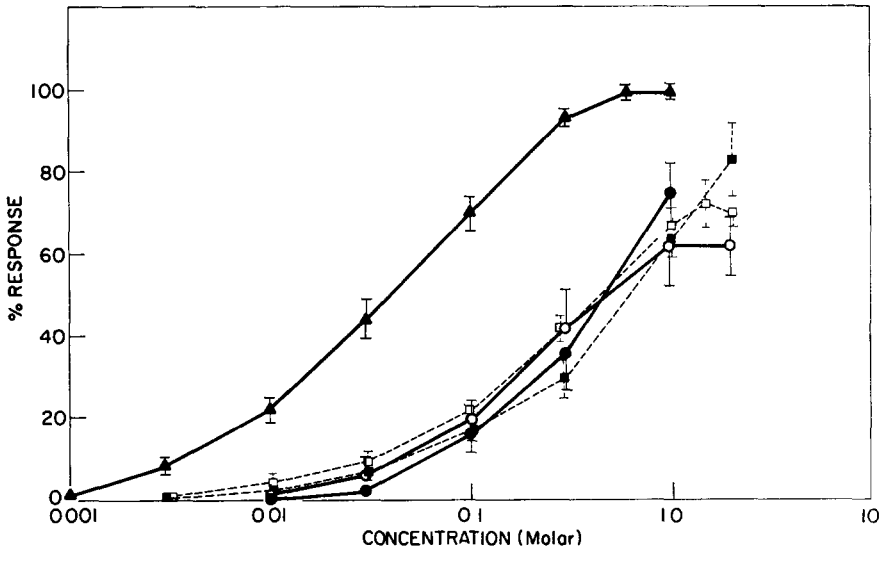


Fig 2 Comparison of mean integrated response of 5 disaccharides as gustatory stimulants The maximum response of sucrose is considered to be 100% and the response of the other sugars are compared to the maximum sucrose response Sucrose, ▲ (N = 37), maltose, □ (N = 5), maltitol, ■ (N = 5), palatinose, ● (N = 4), and turanose, ○ (N = 5) Bars indicate 95% confidence intervals

TABLE I

STIMULATING EFFECTIVENESS OF SOME DISACCHARIDES (MEAN VALUES)

| *Sugar* | *Structure** | *Threshold** (molar)* | *$CR_{50}$ (molar)* | *$K_d$ (molar)* | *n* | *Maximum response* | *N* |
|---|---|---|---|---|---|---|---|
| Sucrose | Glu $\alpha$(1←→2) Fru | 0 001 | 0 042 ± 0 005§ | 0 037 | 0 96 | 1 0 | 32 |
| Turanose | Glu $\alpha$(1→3) Fru | 0 03 | 0 23 ± 0 02 | 0 30 | 1 14 | 0 69 ± 0 08 | 5 |
| Palatinose | Glu $\alpha$(1→6) Fru | 0 03 | - | 0 49 | 1 0 | | 4 |
| Maltose | Glu $\alpha$(1→4) Glu | 0 01 | 0 24 ± 0 05 | 0 29 | 1 00 | 0 75 ± 0 06 | 5 |
| Cellobiose | Glu $\beta$(1→4) Glu | 0 01 | | 0 33 | 1 2 | | 5 |
| Maltitol | Glu $\alpha$(1→4) GluOH | 0 03 | -- | 0 34 | 0 86 | | 5 |
| Cellobiitol | Glu $\beta$(1→4) GluOH | 0 03 | | 0 50 | 1 27 | | 5 |
| Trehalose | Glu $\alpha$(1←→1) Glu | 0 03 | 0 21 ± 0 03 | 0 26 | 1 22 | 0 83 ± 0 10 | 5 |
| Lactulose | Gal $\beta$(1→4) Fru | 0 01 | 0 18 ± 0 02 | 0 23 | 0 98 | 0 88 ± 0 08 | 5 |
| $\beta$-Lactose | Gal $\beta$(1→4) Glu | 0 01 | - | 0 31 | 0 89 | - | |
| Melibiose | Gal $\alpha$(1→6) Glu | 0 03 | 0 18 ± 0 03 | 0 37 | 1 09 | 0 68 ± 0 27 | 5 |
| Lactitol | Gal $\beta$(1→4) GluOH | 0 01 | - | 0 26 | 1 02 | | 5 |
| Melibiitol | Gal $\alpha$(1→4) GluOH | 0 03 | | 0 23 | 1 00 | | |

* Abbreviations Glu, glucose, Gal, galactose, Fru, fructose, GluOH, glucitol

** Threshold is defined as the lowest concentration tested which elicited a measurable response in 50% of the animals (see text)

n = No of molecules per receptor site

N = No of animals

§ 95% confidence intervals are indicated

Thirteen disaccharides were tested Three (cellobiose, $\beta$-lactose and palatinose) were not soluble enough to test at high concentrations and 4 (cellobiitol, lactitol, maltitol and melibiitol) were too viscous to flow through the system at high concentrations The remaining 6 sugars were soluble and not viscous at high concentrations They had concentration–response curves which reached a maximum value (Fig 2, Table I) Frequently, when the maximum had been reached, a higher concentration of sugar applied to the tongue resulted in a significantly smaller response This reduced response (5–15% less) was not an adaptation effect since it occurred regardless of the concentration or recency of the previous stimulus Consequently, high concentrations must have been inhibitory in some way After the maximum had been obtained at 0 3 *M* or 0 6 *M* sucrose, 25% of the animals tested showed a decrease of response Of the other sugars tested a decrease at high concentrations was never observed in lactulose but was observed in 60% of the animals for trehalose, 80% for melibiose, 80% for maltose and 60% for turanose All of these sugars except sucrose were tested with a maximum concentration of 2 0 *M*

The mean response to a sugar plotted on a double reciprocal plot or the Beidler plot (Fig 3A) approximated a straight line The dissociation constant was determined from the x-intercept and the $CR_{50}$ from the concentration–response curve As seen in Table I there is a close correspondence between the $CR_{50}$ and the dissociation constant Melibiose was an exception The Hill plot of the data fitted a straight line with a mean slope of nearly one in every case For those sugars in which a maximum re-

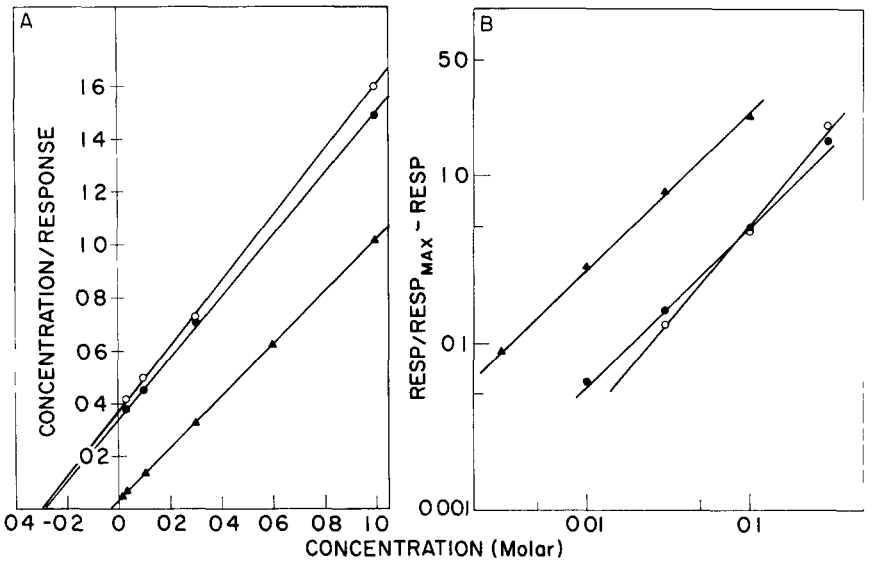


Fig 3 A Beidler plot of data from Fig 2 Resp = response (height of integrated nerve response) slope is $1/Resp_{max}$ $Resp_{max}$ being the maximum response and $K_d/Resp_{max}$ is the y-intercept $K_d$ is the dissociation constant and $-K_d$ is the x-intercept Sucrose (▲), turanose ( ), and maltose (●) are presented B Hill plot of data from A The slope is n, n being the number of molecules interacting with each receptor site Sucrose, ▲, n = 0 96, turanose, , n = 1 14, maltose, ●, n = 1 00

sponse could not be attained the $Resp_{max}$ from the Beidler plot was used

In a comparison of $K_d$ values maltose and maltitol were slightly better stimuli than the $\beta$-anomers, cellobiose and cellobiitol ($\alpha$-glucopyranosides > $\beta$-glucopyranosides) Reduction of maltose to maltitol resulted in a slightly less effective molecule The same is true of cellobiose and cellobiitol The $\beta$-galactopyranoside containing disaccharides, $\beta$-lactose and lactitol, were generally better stimuli than the $\alpha$-anomers, melibiose and melibiitol ($\beta$-galactopyranosides > $\alpha$-galactopyranosides)

## DISCUSSION

In conformity with the results from other mammals, such as the rat[17 19], hamster[19], two species of macaque monkeys[30] and the human[10], the gerbil's sucrose concentration–response curve is sigmoid with a semilog plot Electrophysiologically the gerbil's taste threshold for sucrose (0 001 *M*) is slightly better than that of the hamster (0 005 *M*) and much better than that of the rat (0 01 *M*), macaque monkeys (0 01–0 03 *M*), human (0 02 *M*), calf (0 10 *M*)[4] or squirrel monkey (0 01 *M*)[30] The order of stimulatory effectiveness (sucrose > maltose = lactose) in the Mongolian gerbil, when compared over a single concentration, is generally consistent with the effectiveness of these disaccharides in other mammals tested For example, as a taste stimulant sucrose > maltose = lactose for both the rat and hamster[19], sucrose lactose ≥ maltose in the human[10,11] and sucrose > maltose lactose in the dog[1 2]

When Beidler[3] formulated his taste theory he envisioned a single stimulus

molecule binding to a single receptor site to form a stimulus–receptor complex He assumed that the integrated chorda tympani nerve response was proportional to the number of interactions or complexes formed between the stimuli and the receptor site The data from the gerbil's chorda tympani nerve response to disaccharides are consistent with this simple monomolecular binding hypothesis because they fit a straight line in the reciprocal plot and have a slope of one in the Hill plot Additional support for Beidler's taste theory was derived from the fact that the $K_d$ values whose computation assumes a one-to-one binding mechanism are identical to the $CR_{50}$s which do not depend on this assumption

The present study extends the number of sugars tested electrophysiologically and provides information on sugar–receptor interaction Effectiveness of these disaccharides as stimuli must be explained by their different chemical or physical properties

Whether a sugar is reducing or non-reducing probably does not play an important role in stimulation since neither type is necessarily more effective Maltose, cellobiose, lactose, turanose, palatinose, lactulose and melibiose are all reducing sugars and are poor stimuli compared to sucrose, a non-reducing sugar On the other hand, the non-reducing disaccharides maltitol, cellobiitol, melibiitol, lactitol and trehalose are no more effective than their reducing counterpart

Increasing water solubility has been associated with increasing sweet taste of sugars and their stimulating ability[1,2] Palatinose, cellobiose and lactose all poor stimuli, are barely soluble compared to sucrose But cellobiose, a poorly soluble sugar, has a lower threshold than its extremely soluble derivative cellobiitol By comparison turanose possesses better solubility characteristics than sucrose but is a poorer stimulant than sucrose, therefore, solubility cannot be the sole determinant of effectiveness

The dominant effectiveness of sucrose suggests the presence of a sucrose receptor site The other disaccharides may fit into this same site but not perfectly as witnessed by their failure to stimulate well A highly specific sucrose site is suggested because the two fructosyl glucosides (the constitutional isomers of sucrose), turanose and palatinose, were much less effective stimuli than sucrose Comparison of Dreiding models show they are slightly longer than sucrose This may prevent access to the receptor site Unlike sucrose they are reducing sugars, they mutarotate, and exist as a mixture of isomers A paucity of one type of isomer which would be complementary to the site could also account for the poor response

Alternately the effectiveness of the disaccharides could be explained by the presence of monosaccharide receptor sites Sucrose, an α-D-glucopyranoside, may be effective because it is binding in a glucopyranoside receptor site The presence of such a site on the blowfly and flesh fly taste receptor cell has been postulated[8,27,35] Failure of turanose, palatinose, maltose and maltitol — all α-D-glucopyranosides — to be as effective as sucrose could be attributable to steric hindrance involving the substituents at position C-1 of the glucopyranoside ring A strict anomeric configurational requirement for an α-glycoside bond as in fly taste receptors[7,13,18,23] appears to be absent in the gerbil's taste response Maltose and cellobiose, the α and β anomers, respectively,

were equal in threshold Maltose is a slightly better stimulant ($K_d$) An $\alpha$-glucopyranoside receptor site is possible, maltitol, the $\alpha$-glucoside, is a better stimulant ($K_d$ = 0 34 $M$) than cellobiitol ($K_d$ = 0 50 $M$), the $\beta$-glucoside Failure of the galactosides to be effective stimulants could be attributed to their failure to fit the glucopyranoside site well Sucrose could also be fitting into a separate fructose site In this case the substitution of a bulky substituent at C-3 (turanose), C-4 (lactulose) or C-6 (palatinose) of fructose would reduce the effectiveness of the molecule by steric hindrance Sucrose which can be considered a fructose derivative, with a bulky substituent at C-2, is unaffected The 3 fructose containing disaccharides are reducing sugars, unlike sucrose, and would exist in solutions as a mixture of furanose and pyranose isomers Should the fructose site require a $\beta$-fructofuranose type of molecule, sucrose would be the most stimulatory

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