Concentration–Response Relationships of Sweeteners

A Systematic Study

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Sweetness intensity ratings were made by a trained panel for a range of concentrations of nineteen sweeteners. Panelists were trained to make sweetness ratings relative to six sucrose standards (2%-16%). The shapes of the concentration-response plots were sweetener-dependent. Sugars and sugar alcohols yielded linear concentration-response relationships for intensities up to that of a 16% sucrose standard. High-potency sweeteners including aspartame, acesulfame-K and alitame yielded hyperbolic concentration-response plots.

The purpose of this study was to utilize a systematic approach determining concentration-response relationships for a bro range of sweeteners relative to a sucrose reference. Train panelists evaluated sugars (sucrose, glucose, fructose, fruc oligosaccharide sweetener), sugar alcohols (maltitol, lactit isomalt), terpenoid glycosides (monoammonium glycyrrhizina stevioside, rebaudioside-A), dipeptide derivatives (aspartan alitame), a sulfamate (sodium cyclamate), a protein (thaumatin) chlorodeoxysugar (sucralose), two N-sulfonyl amides (sodi saccharin, acesulfame-K), a dihydrochalcone (neohesperic dihydrochalcone, and an amino acid (glycine). Althou concentration-response relationships have previously been obtain for some of these sweeteners (1-6), the present methodolc expands upon prior work in that: 1) a wider range of structu types is evaluated; 2) the intensity ratings are referenced standard sucrose concentrations by a trained taste panel; and 3) t mathematical forms of the concentration-response relationship a examined.

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Method

Subjects: Screening and training. The subjects were screened for normal taste and odor acuity and the ability to recognize and discriminate among various taste stimuli (7). The final panel participants were selected for their ability to: correctly identify the tastes sweet (2.0% sucrose), salty (0.2% NaCl), sour (0.07% citric acid), and bitter (0.07% caffeine); accurately rank order a series of four concentrations of each taste stimulus; successfully identify the odd sample in a series of triangle tests; and recognize and describe the aroma of six common odorants.

Five female and thirteen male students and employees of Duke University, Durham, N.C., (mean age 36.5 years ± 13.3) were trained across seven weeks (17 one-hour sessions) in a modified Spectrum™ descriptive flavor profile method (8). The purpose of the training was to familiarize the panel with taste profiles of sweeteners. The panel was trained in techniques to recognize, describe and quantify the tastes and aromatic characteristics of sweeteners.

The training began with an overview of basic taste and olfactory physiology as well as the psychophysical principles of vocabulary development and scaling. Through the use of flavor attribute references (e.g., metallic = .0003 g Ferrous sulfate in 150 mL 500 ppm aspartame solution; licorice = 1 drop McCormick anise extract in 100 mL 500 ppm aspartame solution), the panel learned standard flavor vocabulary to describe tastes and odors of sweeteners. Six concentrations of sucrose (2%, 5%, 7.5%, 10%, 12%, and 16%) were used to standardize sweetness intensity ratings on a 15 cm line scale (Figure 1). These sucrose standards were assigned the intensity values 2, 5, 7.5, 10, 12, and 15, respectively. Two concentrations of caffeine were used to standardize bitterness intensity ratings: 0.05% (assigned a value of 2 bitter) and 0.08% (assigned a value of 5 bitter). The panel evaluated seven sweeteners at various concentrations during the training: acesulfame-K, aspartame, sodium cyclamate, sodium saccharin, sucrose, and thaumatin. Two mixtures were also used in training: aspartame plus caffeine, and sucrose plus caffeine.

Stimuli. The stimuli, classification, dilution range, and number of concentrations tested are given in Table I. Samples were dissolved in deionized water at room temperature within twelve hours of evaluation. Concentrations are reported on a weight/volume basis, correcting the weight for analyzed purity of the sample (e.g., Na-saccharin, Sigma, lot 76F0079, contained 14.3% water according to the supplier). Panelists received 20 mL aliquots of test stimuli, served at room temperature in 30 mL odor-free plastic cups coded with randomly selected three-digit numbers. The panel was conducted under natural lighting in a quiet, odor-free room.

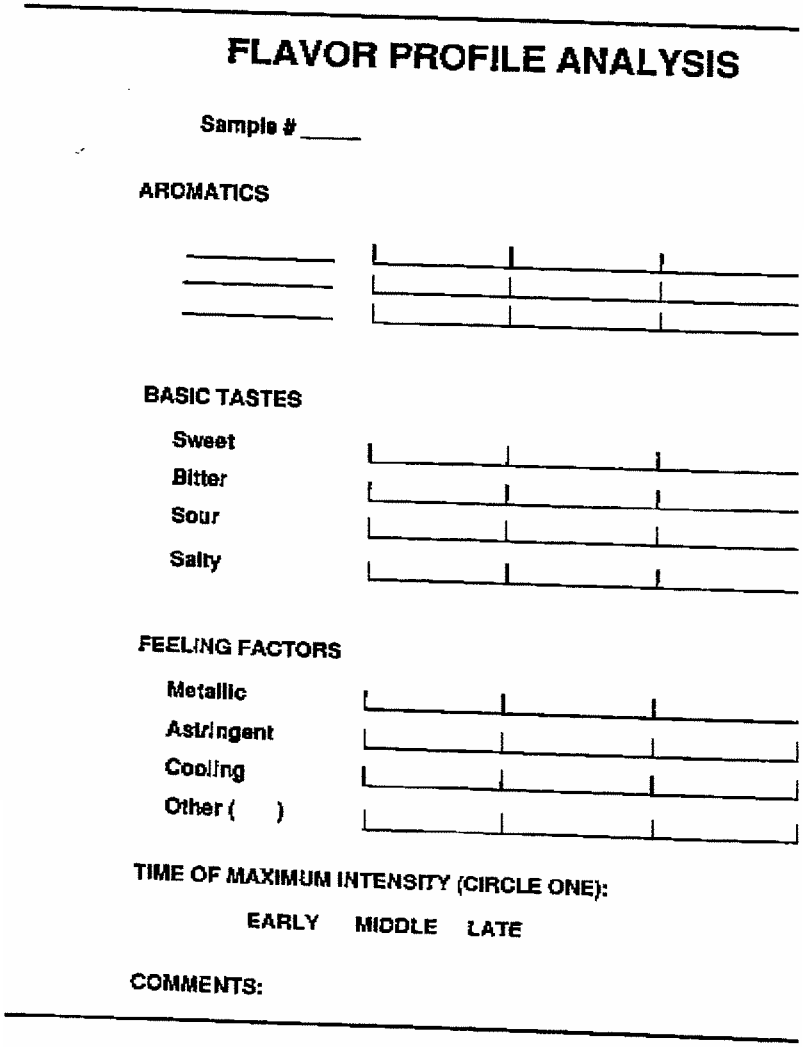


Figure 1. Panelist response sheet.

Table I. Sweeteners used in this study

Compound	Classification	Dilution Range (ppm)	No. of concs.
Acesulfame-K	N-Sulfonyl amide	100-1560	10
Alitame	Dipeptide	5-200	10
Aspartame	Dipeptide	60-3000	28
Fructo-oligosaccharide sweetener	Polyol*	13,000-100,000	6
Fructose	Polyol	6,400-35,000	11
Glucose	Polyol	9,600-50,000	10
Glycine	Amino acid	13,000-200,000	7
Isomalt	Polyol†	13,400-300,000	8
Lactitol	Polyol	20,000-500,000	10
Maltitol	Polyol	6,500-500,000	10
Monoammonium glycyrrhizinate	Terpenoid glycoside	200-3000	7
Neohesperidin dihydrochalcone	Polyketide	32-875	9
Rebaudioside-A	Terpenoid glycoside	70-1500	13
Sodium cyclamate	Sulfamate	500-9000	8
Sodium saccharin	N-Sulfonyl amide	20-1200	17
Stevioside	Terpenoid glycoside	70-1500	12
Sucralose	Chlorosugar	19-1200	11
Sucrose	Polyol	16,000-160,000	53
Thaumatin	Protein	2.8-60	18

* Fructo-oligosaccharide sweetener is a mixture of kestose, nystose, and 1-O-β-D-fructofuranosyl-nystose.

† Isomalt is a 1:1 mixture of 6-O-α-D-glucopyranosyl-sorbitol and 6-O-α-D-glucopyranosyl-mannitol

Deionized water and unsalted crackers were available to clear palate between stimuli.

Procedure. The concentration-response data were obtained presenting four concentrations of a sweetener in one session. fifth sample containing sucrose or aspartame was presented a control. In each session, the panelists first tasted their sweet a bitter references: 2%, 5%, 7.5%, 10%, 12% and 16% sucrose, a 0.05% caffeine. They then tasted the first of five test samp followed by a water rinse. The panelists were instructed to ta the samples, holding and swirling in the mouth for ten secon and to rate the maximum intensity each attribute reached bef discarding the sample. Intensity scores were recorded or response sheet individually coded for each sample. Panelists rin with water three times and waited approximately 60 seconds, until all taste sensation subsided, before proceeding to the n sample. Evaluation of the four remaining samples was conducte a similar fashion. The order of the five stimuli was randomi across subjects.

Statistical treatment. Each data point on the concentrati response plots represents the average of all panelist inten responses within a session. While only sweetness and bitter data are presented here, panelists provided a full sensory profil each sweetener sample. This included quantification of additional tastes that were detected, such as salty, sour, or meta Aromatic notes and "feeling factors" such as burning, viscou smooth were also rated for intensity. In addition, panelists jud the time of maximum sweetness intensity as early, middle or lat onset.

The sweet intensity panel means for each sweetener were te for their fit to three different mathematical models: (a) linear Beidler equation; (c) extended Beidler equation. Equation 1 is Beidler equation (9;), which is analogous to the Michaelis-Me equation for enzyme-substrate interaction. This equation has c been used to fit concentration-response data for tastants. The t model we used (equation 2) is a modification of the Bei equation which is equivalent to the Hill equation (10) for a rece with multiple sites. The Hill-type equation, when the exponent 1, reduces to the Beidler equation.

$$R = \frac{R_m \cdot C}{1/K + C}$$

where R is the observed response; R_m is the maximal respo C is the sweetener concentration; and 1/K = concentra which yields half-maximal response, equivalent to the recipr of the receptor-sweetener association constant.

$$R = \frac{R_m \cdot C^n}{(1/K')^n + C^n} \quad (2)$$

where n is the apparent number of binding sites per receptor molecule, and 1/K' is still the concentration which yields half-maximal response, but it is no longer directly related to the binding efficiency as it is in equation 1.

If we assume that the observed response to sucrose increases with concentration according to a Beidler-type relationship, then the panelists are being trained to linearize a hyperbolic function. The result of this is that compounds with a maximal response comparable to that of sucrose will produce a linear concentration-response relationship (with slope greater than 1 if the potency is greater than that of sucrose, and less than 1 if the potency is less than that of sucrose). Compounds with maximal response less than that of sucrose will produce a hyperbolic curve (of the same form as the Beidler model, equation 1). A manuscript showing the mathematical derivation of these relationships is in preparation (K. Gibes).

Concentration-response data were analyzed by nonlinear regression modeling techniques using SAS Institute's PROC NLIN (*11*), which uses least squares as a fitting criterion. To test whether the Beidler model (equation 1) was sufficient (i.e. exponent=1 in equation 2), an F-test was performed by the "extra sum of squares" principle (*12*). In addition, for aspartame and sucrose, sufficient concentration replications were done to allow for an F-test for "lack of fit" of the model (*13*). Finally, for sucrose an additional t-test was performed to determine whether the slope of the linear fit was equal to 1 as would be expected since sweetness was measured on the sucrose equivalency scale.

Results

Sugars and sugar alcohols. Sucrose gave a linear response with concentration ($p = 0.1043$, lack of fit F-test). It can be seen in Figure 2 that the slope of this line is close to 1.0 ($p = 0.2833$, t-test for equality with 1.0). In other words, the mean panel responses closely matched the actual concentrations of the samples. Since panelists had been trained to make their intensity judgments based on a sucrose-referenced scale, this one-to-one correspondence of sucrose concentration and intensity rating confirms the reliability of the scaling methodology employed in this study.

The other sugars and sugar alcohols were best fit to the linear model over the concentration ranges studied. The graph for fructose has an initial slope that is greater than one, consistent with its increased potency relative to sucrose (Figure 3a). Glucose and fructo-oligosaccharide sweetener, however, exhibit slopes of less than one; this is indicative of sweetness potencies lower than

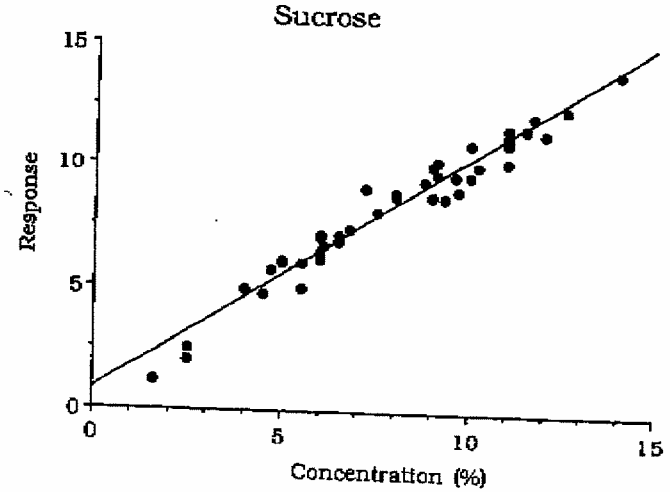
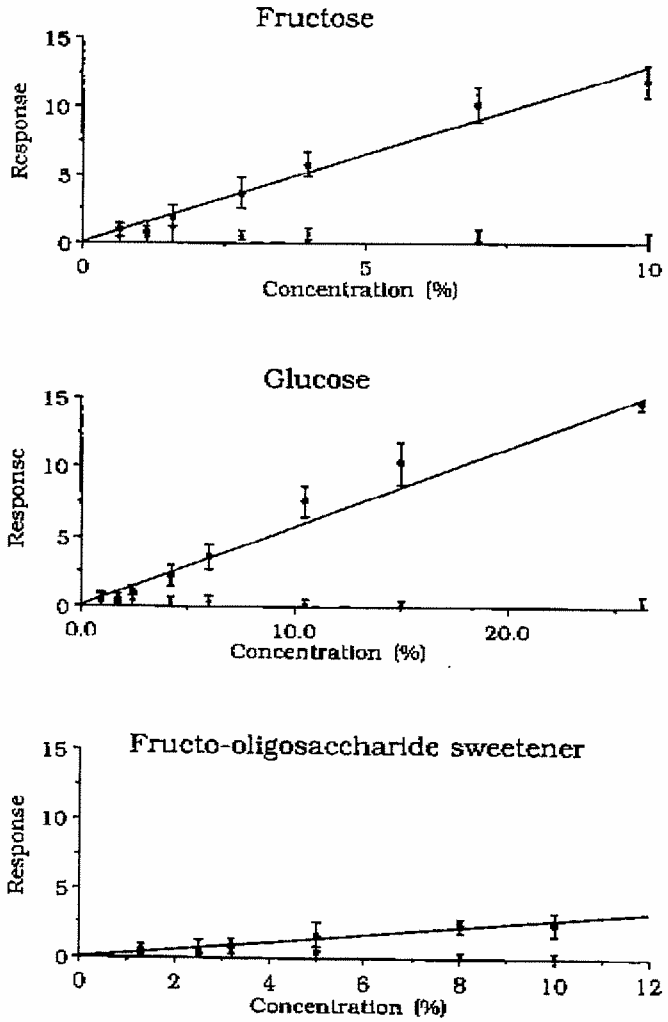


Figure 2. Concentration-response data for sucrose. equation for least-squares fit of a straight line to the . $R = 0.80 + 0.94(C)$; $R^2 = 0.96$.



igure 3. Concentration-response data for fructose, glucose, and ructo-oligosaccharide sweetener. For figures 3-8, large circles ndicate sweetness response and small circles indicate itterness response; error bars are Least Significant Difference, 2•std.dev.)($\sqrt{2/n}$)], where n = no. of panelists. (a) Fructose, R = .04 + 1.27 C; R^2 = 0.973. (b) Glucose, R = -0.02 + 0.60 C; R^2 = .974. (c) Fructo-oligosaccharide sweetener, R = -0.03 + 0.27 C; ^{2} = 0.949.

that of sucrose (Figures 3b and 3c). Sugar alcohols were also foun to be less potent than sucrose. Isomalt, lactitol and maltitol all hav slopes of substantially less than one (Figure 4).

High potency sweeteners. The shape of the concentration response functions obtained for high potency sweeteners differ substantially from those obtained for the polyols discussed above Aspartame exhibited a concentration-response function which wa hyperbolic on the sucrose reference scale (p = 0.7832, lack of f F-test).

The other high potency sweeteners evaluated in this stud behaved similarly, asymptotically approaching maximal responses The Beidler equation (equation 1) gave the best fit to th experimental data except in the cases of sodium cyclamate, sodiur saccharin, and sucralose. For these three compounds, the Hill-typ equation (equation 2; n = 1.8, 1.4, 1.4, respectively) gave a slightl better fit, but in each case a single data point was responsible fc the better fit of the Hill-type equation. While we cannot rule ou the possibility that a Hill-type equation may be required for som compounds, our data indicate that the standard Beidler equation i an adequate model of the sweetener-receptor interactions for th high-potency compounds. As shown in Figure 5, the highes maximal responses were observed for the dipeptide sweetener aspartame and alitame (16.0 and 14.6, respectively) and fc sucralose (13.0). These three compounds have the lowes incidence of non-sweet tastes among the high-potency sweeteners The remaining high-potency sweeteners (Figure 6-8) had R_m value less than 12. These compounds all had significant concentration dependent non-sweet tastes (acesulfame-K, sodium cyclamate monoammonium glycyrrhizinate, neohesperidin dihydrochalcone rebaudioside-A, sodium saccharin, stevioside) or limited solubilit (glycine) which may have prevented attainment of higher sweetnes intensities. Table II lists the values for maximal response (R_m) an apparent receptor-sweetener association constants (1/K) for all c the high-potency sweeteners obtained from fitting to the Beidle equation.

Discussion

The linearity of the sucrose response with concentration is a resu of the panel training. The linearity observed for the other sugar and sugar alcohols indicates that they should exhibit a maxime sweetness similar to that of sucrose. In some cases (e.g., fructc oligosaccharide sweetener and isomalt) the potency and/o solubility is too low to ever actually achieve the same sweetnes intensity as that of a concentrated sucrose solution. The ability c the Beidler equation to fit concentration-response data for th high-potency sweeteners is consistent with a one-to-on sweetener-to-receptor interaction for these compounds. It is les clear how to interpret the apparently improved fit of the Hill-typ

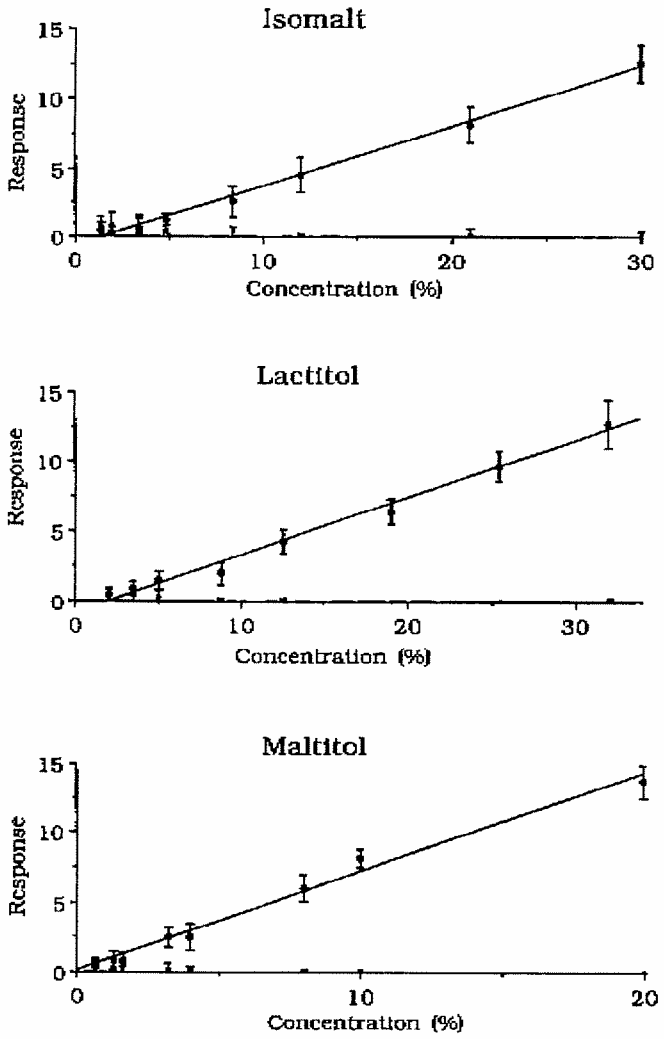


Figure 4. Concentration-response data for isomalt, lactitol, and maltitol. (a) Isomalt, R = -0.63 + 0.43 C; R^2 = 0.996. (b) Lactitol, R = -0.82 + 0.41 C; R^2 = 0.990. (c) Maltitol, R = 0.05 + 0.71 C; R^2 = 0.990.

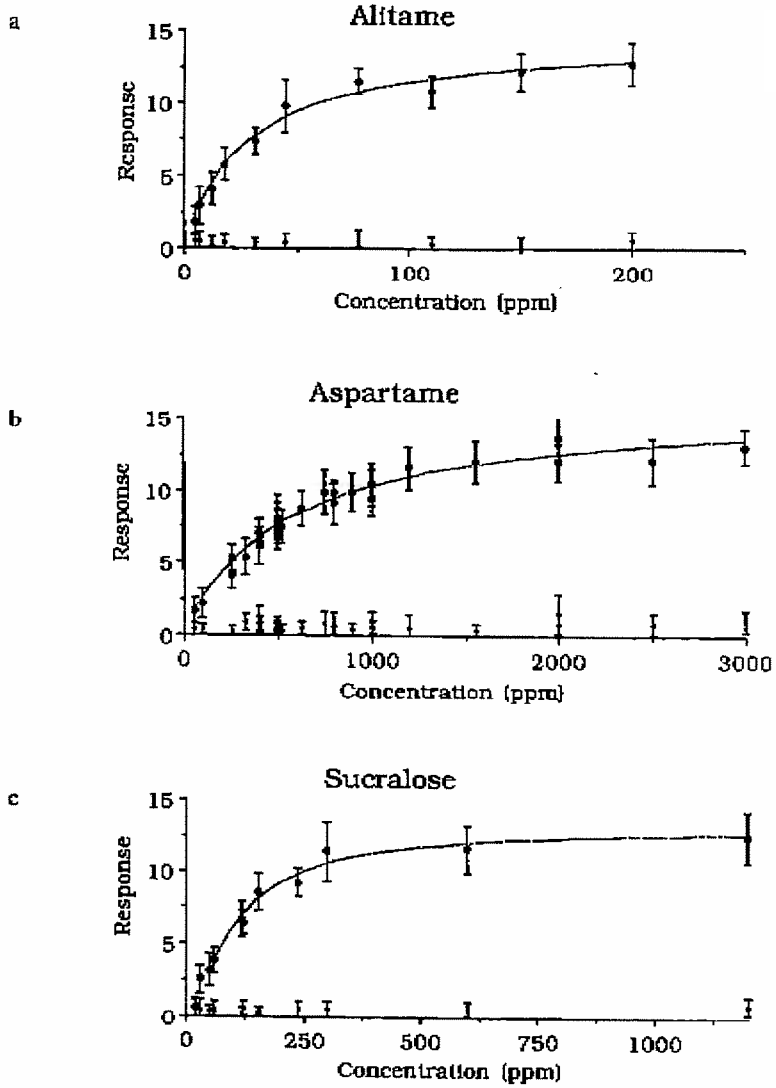


Figure 5. Concentration-response data for alitame, aspartame, and sucralose (Hill-type equation). For figures 5-8, the calculated Beidler (or Hill-type) curve are shown.

(a) alitame: $R = \frac{(14.6)(C)}{28 + C}$; (b) aspartame: $R = \frac{(16.0)(C)}{560 + C}$;

(c) sucralose: $R = \frac{(13.0)(C)^{1.4}}{110^{1.4} + C^{1.4}}$

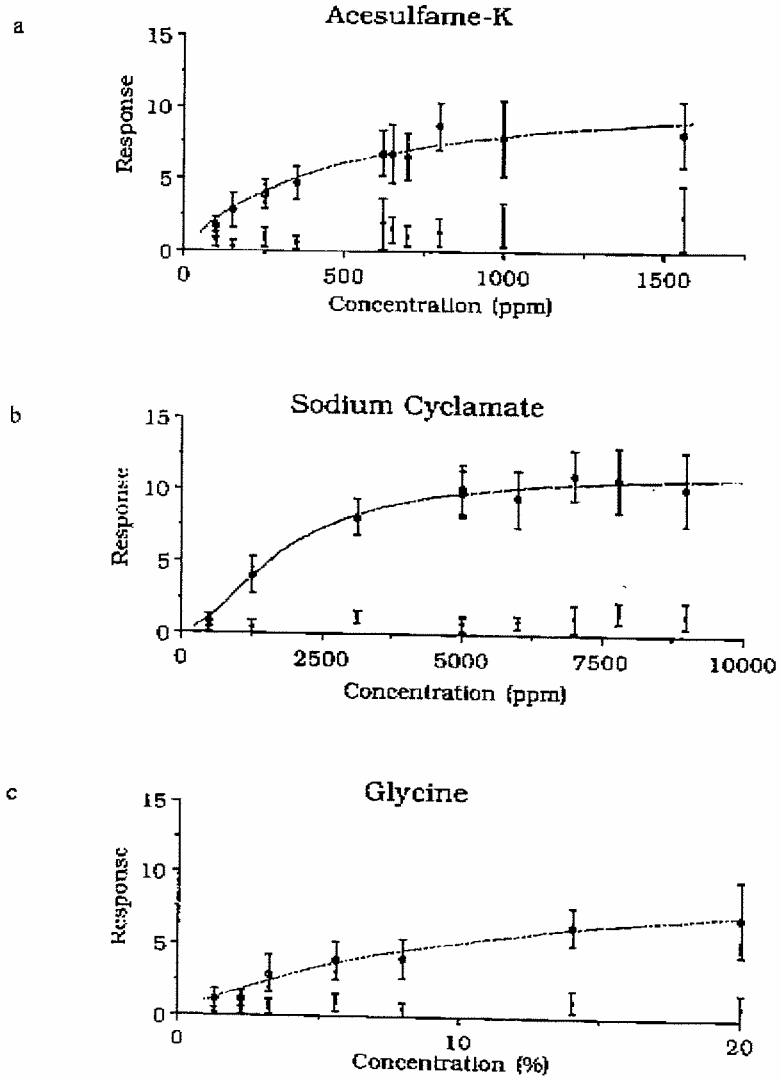


Figure 6. Concentration-response data for acesulfame-K, sodium cyclamate (Hill-type equation), and glycine. (a) acesulfame: $R = \frac{(11.6)(C)}{470 + C}$; (b) cyclamate: $R = \frac{(11.3)(C)^{1.8}}{1800^{1.8} + C^{1.8}}$; (c) glycine: $R = \frac{(11.3)(C)}{12.0 + C}$

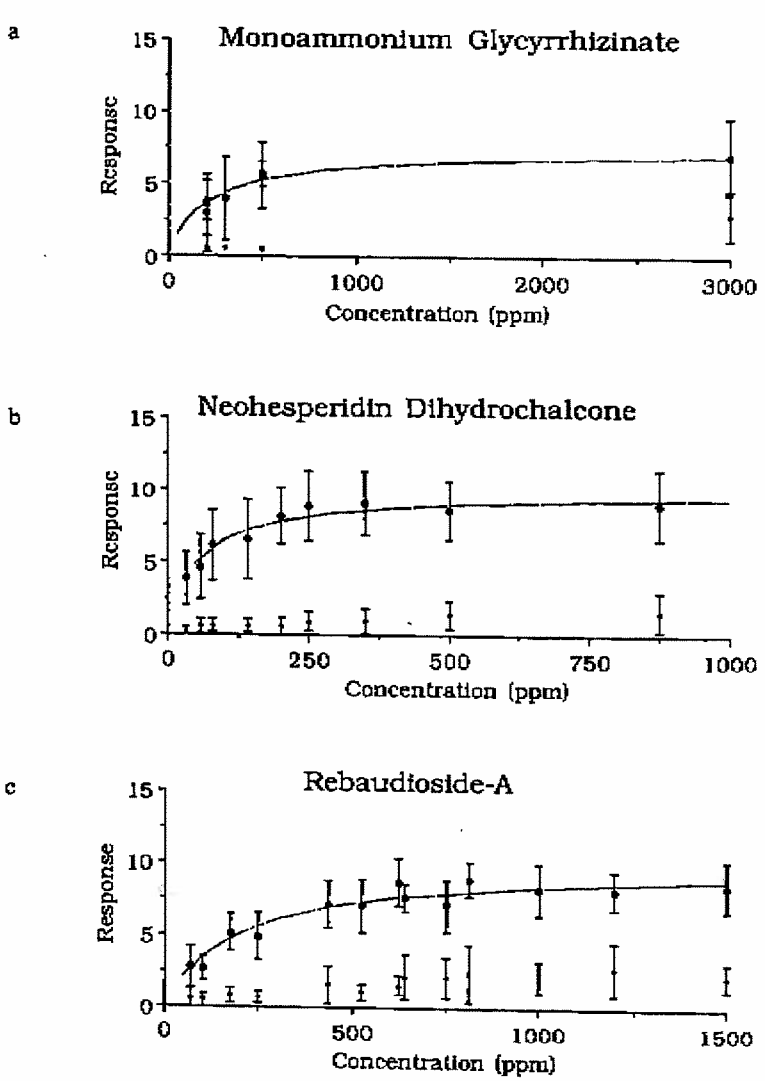
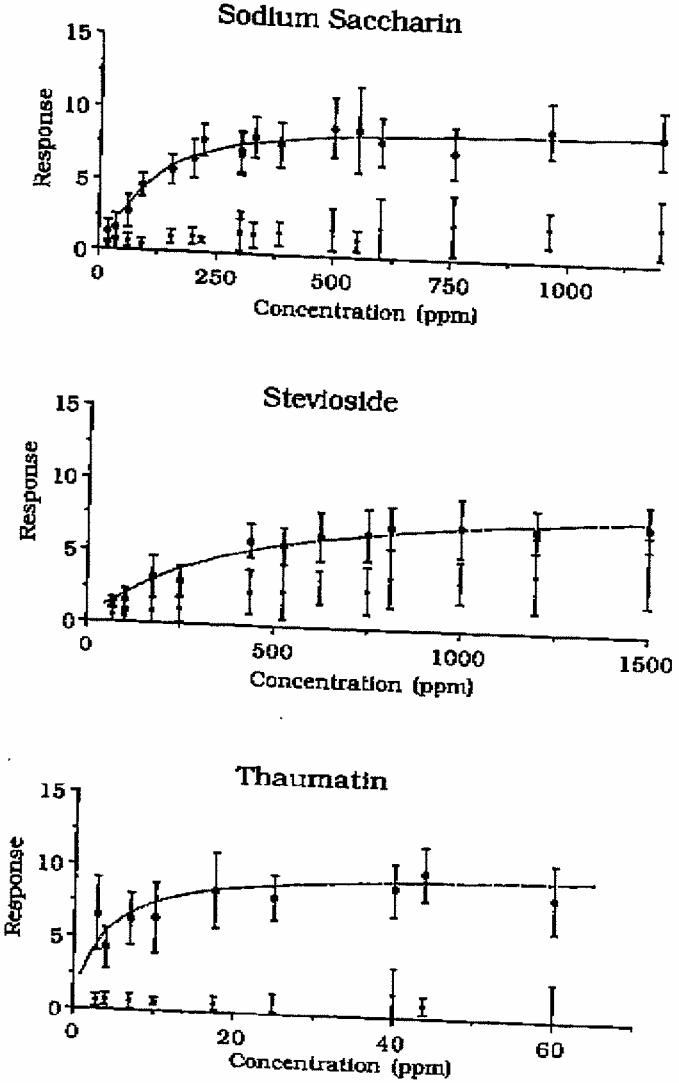


Figure 7. Concentration-response data for monoamm glycyrrhizinate, neohesperidin dihydrochalcone, rebaudioside-A. (a) monoammonium glycyrrhiz $R = \frac{(7.3)(C)}{210 + C}$; (b) neohesperidin dihydrochalcone: $R = \frac{(9.}{5}$ (c) rebaudioside-A: $R = \frac{(10.0)(C)}{200 + C}$



e 8. Concentration-response data for sodium saccharin ype equation), stevioside, and thaumatin.

ccharin: $R = \frac{(9.0)(C)^{1.4}}{96^{1.4} + C^{1.4}}$; (b) stevioside: $R = \frac{(9.9)(C)}{410 + C}$

aumatin: $R = \frac{(10.1)(C)}{3.6 + C}$

Table II. Beidler equation (or Hill-type equation) parameters derived from the concentration-response data

Compound	1/K	R_{max}
Acesulfame-K	470 ppm	11.6
Alitame	28 ppm	14.6
Aspartame	560 ppm	16.0
Glycine	12%	11.3
Monoammonium glycyrrhizinate	210 ppm	7.3
Neohesperidin dihydrochalcone	53 ppm	9.8
Rebaudioside-A	200 ppm	10.0
Sodium cyclamate	1800 ppm[a]	11.3
Sodium saccharin	96 ppm[a]	9.0
Stevioside	410 ppm	9.9
Sucralose	110 ppm[a]	13.0
Thaumatin	3.6 ppm	10.1

[a] Value listed is for 1/K' (equation 2) rather than 1/K.

equation (with non-integral exponents) for three of the compounds. Ariëns points out that interactions which appear to have a higher order than one-to-one are often the result of a sequential series of interactions (14). Multiple receptor types and receptor cooperativity are hypotheses which could be considered.

All of the sugars and sugar alcohols have in common a large number of hydroxyl groups (hydrogen bond donors/acceptors) and a requirement for high concentrations in order to elicit sweet taste. It is conceivable that these compounds activate receptor cells in some non-specific way (e.g., by osmotic or conformational perturbation of cell membranes) rather than by direct interaction with receptor protein(s). High-potency sweeteners, on the other hand, might interact specifically with some subset of the receptor population. This would account for the lower maximal sweetness levels for these compounds. Alternatively, high potency sweeteners and polyol sweeteners may all activate the same receptor protein where the polyols are full agonists and the high potency sweeteners are partial agonists. Mediation of sweet taste by more than one receptor protein or more than one cellular activation system cannot be ascertained by analysis of the data presently in hand. It appears clear, however, that polyol sweeteners as a group are distinct in their behavior from high potency sweeteners. Thus it is tentatively concluded that at least two routes to receptor cell activation must exist.

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