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Selective Inhibition of Sweetness by the Sodium Salt of ±2-(4-Methoxyphenoxy)propanoic Acid

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Abstract

The purpose of this study was to determine the degree to which the sodium salt of ±2-(4-methoxyphenoxy)propanoic acid (Na-PMP) reduced sweet intensity ratings of 15 sweeteners in mixtures. Na-PMP has been approved for use in confectionary/frostings, soft candy and snack products in the USA at concentrations up to 150 p.p.m. A trained panel evaluated the effect of Na-PMP on the intensity of the following 15 sweeteners: three sugars (fructose, glucose, sucrose), three terpenoid glycosides (monoammonium glycyrrhizinate, rebaudioside-A, stevioside), two dipeptide derivatives (alitame, aspartame), two *N*-sulfonylamides (acesulfame-K, sodium saccharin), two polyhydric alcohols (mannitol, sorbitol), 1 dihydrochalcone (neohesperidin dihydrochalcone), one protein (thaumatin) and one sulfamate (sodium cyclamate). Sweeteners were tested at concentrations isosweet with 2.5, 5, 7.5 and 10% sucrose in mixtures with two levels of Na-PMP: 250 and 500 p.p.m. In addition, the 15 sweeteners were tested either immediately or 30 s after a pre-rinse with 500 p.p.m. Na-PMP. In mixtures, Na-PMP at both the 250 and 500 p.p.m. levels significantly blocked sweetness intensity for 12 of the 15 sweeteners. However, when Na-PMP was mixed with three of the 15 sweeteners (monoammonium glycyrrhizinate, neohesperidin dihydrochalcone and thaumatin), there was little reduction in sweetness intensity. Pre-rinsing with Na-PMP both inhibited and enhanced sweetness with the greatest enhancements found for monoammonium glycyrrhizinate, neohesperidin dihydrochalcone and thaumatin, which were not suppressed by Na-PMP in mixtures. The mixture data suggest that Na-PMP is a selective competitive inhibitor of sweet taste. The finding that pre-treatment can produce enhancement may be due to sensitization of sweetener receptors by Na-PMP.

Introduction

The chemical structures of molecules that confer a sweet taste are diverse, and include both sugars and nonsugars (Schiffman and Gatlin, 1993). Representative compounds that have sweet tastes include saccharides, diterpene glycosides, polyols, amino acids, dipeptides and other nonsugars. At the present time, the chemical properties of molecules that induce sweet tastes are not well understood. However, recent psychophysical, electrophysiological and biochemical studies suggest that sweet taste perception for these structurally diverse compounds may involve multiple receptor types and transduction mechanisms (Schiffman *et al.*, 1983, 1993, 1994a, 1994b, 1995; DeSimone *et al.*, 1984; Avenet *et al.*, 1988; Tonosaki and Funakoshi, 1988; Striem *et al.*, 1989, 1991; Naim *et al.*, 1991, 1994; Breslin *et al.*, 1996; DuBois, 1997).

One method for investigating the possible multiplicity of sweet receptors has been the use of sweetness inhibitors. A variety of compounds has been found to inhibit sweet taste intensity to varying degrees in either humans or animals or both (Jakinovich and Sugarman, 1989). The modes of action by which inhibitors block sweet taste are not well understood but several mechanisms have been proposed, including competitive inhibition of sweetener receptors, interference with channels and second messenger systems in the taste cell membrane, and nonspecific interactions with taste cell membranes (Jakinovich and Sugarman, 1989; Lindley, 1991; Schiffman *et al.*, 1994b).

One sweetness inhibitor, ±2-(4-methoxyphenoxy)propanoic acid sodium salt (Na-PMP), is reported to exert its suppressive effect on sweet taste by a competitive mechanism (Lindley, 1986, 1991). If this competitive inhibitor were found to block the taste of some sweeteners but not others, this would provide further evidence for receptor subtypes for sweetness. Selective competitive antagonists are used in many areas of pharmacology and biochemistry to study receptor subtypes (Guh *et al.*, 1995; Hegde *et al.*, 1995; Piper and Hollingsworth, 1995). Na-PMP has been reported to block the sweet taste of several compounds, including

carbohydrate sweeteners and high potency sweeteners (Lindley, 1986, 1991; Johnson *et al.*, 1994). However, further studies are required to determine the full range of sweeteners that are inhibited by Na-PMP.

The purpose of the present study was to determine if the inhibition induced by Na-PMP varies across a broad range of sweetener compounds and is thus selective for specific molecular structures. First, 15 sweeteners were mixed with Na-PMP to determine if the degree of inhibition of sweetness intensity varied over sweeteners. In addition, the effect of pre-rinsing with Na-PMP (rather than mixing Na-PMP with the sweetener) on sweetness intensity ratings was determined. It was not known a priori whether a selective competitive inhibitor of sweet taste would still be effective when applied prior to the sweetener rather than mixed with the sweetener.

Materials and methods

Two studies were performed to determine the effect of Na-PMP on the 15 sweeteners. In the first study, each sweetener was presented simultaneously with Na-PMP, i.e. in the same solution. In the second study, Na-PMP was presented prior to tasting a sweetener.

Subjects

For both studies a trained panel of 17 subjects (eight males and nine females) participated in the study. The minimum number of subjects who participated in a given taste session was 10 and the maximum was 13. The mean age of the subjects was 44 ± 18 years. All subjects were from the Duke University or Durham, NC community. Subjects were paid for their participation.

Stimuli

For both studies the following 15 sweeteners were tested: three sugars (fructose, glucose, sucrose), three terpenoid glycosides [monoammonium glycyrrhizinate (MAG), rebaudioside-A, stevioside], two dipeptide derivatives (alitame, aspartame), two *N*-sulfonylamides (acesulfame-K, sodium saccharin), two polyhydric alcohols (mannitol, sorbitol), one dihydrochalcone [neohesperidin dihydrochalcone (neo-DHC)], one protein (thaumatin) and one sulfamate (sodium cyclamate). Each sweetener was tested at concentrations equivalent with 2.5, 5, 7.5 and 10% sucrose according to formulae developed by DuBois and colleagues (DuBois *et al.*, 1991). Neo-DHC, rebaudioside-A, sodium saccharin and stevioside, however, were not tested at concentrations isointense to 10% sucrose since they do not reach this sweetness intensity level. Also, MAG was not tested at concentrations equivalent to either 7.5 or 10% sucrose for the same reason. In study 1, each equivalency level of each sweetener was mixed with two levels of Na-PMP, 250 and 500 p.p.m. (i.e. a given concentration of sweetener was mixed in either a 250 p.p.m. Na-PMP solution or a 500 p.p.m. Na-PMP solution). In study 2, each equivalency level of each sweetener was tested following an oral rinse of a Na-PMP solution at the 500 p.p.m. level. Table 1 gives all the concentrations tested in both studies.

Table 1 Concentrations of sweeteners tested at levels isointense with 2.5, 5, 7.5 and 10% sucrose

Sweetener	2.5% equiv.	5% equiv.	7.5% equiv.	10% equiv.
Acesulfame-K (p.p.m.)	129.12	356.06	859.76	2937.50
Alitame (p.p.m.)	5.79	14.58	29.58	60.87
Aspartame (p.p.m.)	103.70	254.55	494.12	933.33
Fructose (%)	1.94	3.91	5.87	7.84
Glucose (%)	4.20	8.37	12.53	16.70
MAG (p.p.m.)	109.38	456.52	n/a[a]	n/a
Mannitol (M)	0.24	0.43	0.62	0.82
Na-cyclamate (p.p.m.)	894.62	1583.10	2626.10	5591.50
Na-saccharin (p.p.m.)	48.51	112.59	303.06	n/a
Neo-DHC (p.p.m.)	18.15	55.21	172.83	n/a
Rebaudioside-A (p.p.m.)	66.67	200.00	600.00	n/a
Sorbitol (M)	0.31	0.47	0.65	0.87
Stevioside (p.p.m.)	138.51	418.37	1281.30	n/a
Sucrose (%)	2.50	5.00	7.50	10.00
Thaumatin (p.p.m.)	1.18	3.53	10.38	360.00

[a]n/a, not tested because sweetener does not achieve this sweetness intensity according to formulae determined by DuBois *et al.* (DuBois *et al.*, 1991).

Procedure

Study 1

All mixtures of the sweetener–inhibitor combinations of 15 sweeteners and Na-PMP given above were evaluated by the trained panel of subjects. At a given taste session, panelists would typically evaluate solutions of five different sweeteners equivalent in sweetness intensity level (i.e. either 2.5, 5, 7.5 or 10% sucrose equivalent) mixed in a solution of either 250 or 500 p.p.m. Na-PMP. Panelists gave sweetness intensity ratings, in addition to ratings of other taste, odor, or tactile components of each sample. Each sample consisted of ~10–15 ml of solution in a 30 ml plastic medicine cup. Samples were coded with random three-digit numbers for identification.

Before evaluating the samples of sweeteners mixed with Na-PMP, panelists at a given taste session would receive and taste references according to the method used by DuBois *et al.* (1991). Sweet taste references were the following: 2 sweet (2% sucrose), 5 sweet (5% sucrose), 7.5 sweet (7.5% sucrose), 10 sweet (10% sucrose), 12 sweet (12% sucrose) and 15 sweet (16% sucrose). Panelists would also taste bitter references, which were labeled 2.2 bitter (0.02% caffeine) and 4 bitter (0.03% caffeine), and sour references, which were labeled 2.1 sour (0.01% citric acid) and 7.4 sour (0.08% citric acid).

These references were based upon intensity evaluations made previously by the trained panel using sucrose intensities as a standard.

Samples were presented to panelists in a balanced randomized design within a given taste session. Panelists were instructed to swirl each sample around in their mouths for ~10 s and then to expectorate. After tasting a sample, each panelist would give a full 'flavor profile' which included all tastes (sweet, bitter, sour, salty), odors (licorice, vanilla) and tactile sensations (metallic, astringent, cooling, viscous, chalky). Within the designated flavor profile, subjects would indicate which factors were perceived, as well as their intensity. Intensity was noted by making a mark on a 15 cm line scale which was anchored at 0, 5, 10 and 15 cm. Panelists would then measure their marks using a 15 cm ruler. Panelists also noted the time of onset of maximum sweetness intensity by circling either early, middle or late. Between tasting samples during a given taste session, each panelist would thoroughly rinse his/her mouth with deionized water and would wait an appropriate amount of time to ensure the previous taste had dissipated before continuing (~4 min). In addition, panelists had the option of eating unsalted top crackers between samples to rid their mouths of particularly lingering tastes. Subjects also refrained from smoking, eating or drinking anything other than water for 30 min prior to each tasting session.

Subjects also evaluated Na-PMP at 250 and 500 p.p.m. in water in the absence of sweeteners.

Study 2

Fifteen sweeteners at four isointensity levels (with several exceptions described above under Stimuli) were tested either immediately or after a 30 s delay following a pre-rinse with a 500 p.p.m. Na-PMP solution. At a given taste panel, two sweeteners were tested at the same isointensity level both immediately following and 30 s following the Na-PMP rinse. Panelists were asked to give sweetness intensity ratings in addition to ratings of other sensory qualities for every sample at each taste session. Approximately 15 ml of each solution was served in 30 ml plastic medicine cups. Samples were all coded with random three-digit numbers for identification.

Prior to receiving the experimental samples, each subject would receive taste references according the methods used by DuBois and colleagues (DuBois *et al.*, 1991). The sweet taste references were identical to those given in study 1.

During a given taste session, panelists were instructed to swirl the 500 p.p.m. Na-PMP solution in their mouths for 10 s and then to expectorate. Subjects were then told to taste and immediately complete their evaluation of the experimental sample. Subjects then waited for 3 min before continuing to the next Na-PMP rinse and experimental sample. During the 3 min intervals between one experimental sample and the next Na-PMP rinse, subjects would rinse their mouths thoroughly with deionized water and would eat unsalted top crackers to rid their mouths completely of lingering tastes. For the next experimental sample, subjects were instructed to wait 30 s between expectoration of the Na-PMP rinse (also held in mouth for 10 s) and doing the sensory profile of the experimental sample. No rinsing was allowed between Na-PMP rinses and the corresponding experimental samples. In doing a sensory profile of an experimental sample, subjects rated all tastes, odors and tactile factors. Subjects would indicate all factors perceived as well as their intensity. Intensity was noted by making a mark on a 15 cm line scale which was anchored at 0, 5, 10 and 15 cm. Panelists would then measure the mark using a 15 cm ruler. Subjects also indicated the time of maximum sweetness intensity by circling either early, middle or late. In addition, panelists refrained from smoking, eating or drinking anything other than water for 30 min prior to the tasting session.

Results

Study 1

Statistical methods

A mixed-model analysis of variance (ANOVA) was used to analyze each sweetener separately in order to determine the impact of Na-PMP on the sweetness intensity ratings of a trained panel. The ANOVA model looked at the effects of panelist (panelists, random effect), level of Na-PMP (250 or 500 p.p.m., fixed effect), level of sweetener (target sweetness levels equivalent to 2.5, 5, 7.5 or 10% sucrose, fixed effect) and the interaction between level of Na-PMP and level of sweetener (fixed effect). From these analyses, *t*-tests were constructed to test the hypothesis of no inhibition due to the presence of Na-PMP by contrasting the model estimated mean (lsmean) with the expected sweetness values of the mixtures of Na-PMP and sweetener based on the aforementioned formulae (DuBois *et al.*, 1991). The *p*-values for these *t*-tests were adjusted to compensate for the multiple tests with the Bonferroni method. The above analyses were obtained using SAS Institute's (Cary, NC) PROC MIXED procedure (SAS Institute Inc., 1992).

Comparison between experimental responses and expected responses

The sweetness intensity ratings for all the sweeteners, except for MAG, neo-DHC and thaumatin, were significantly inhibited by either 250 or 500 p.p.m. Na-PMP. There was no instance where the sweet ratings of MAG, neo-DHC or thaumatin were significantly suppressed. All other sweeteners were inhibited at every concentration by both levels of Na-PMP at either the 5 or 1% significance level, except for stevioside at the two lower sweetness levels with 250 p.p.m. Na-PMP, which were not statistically significant. Figure 1a–o shows the mean sweetness intensity ratings of all 15 sweeteners, respectively, at 2–4 concentrations, mixed with

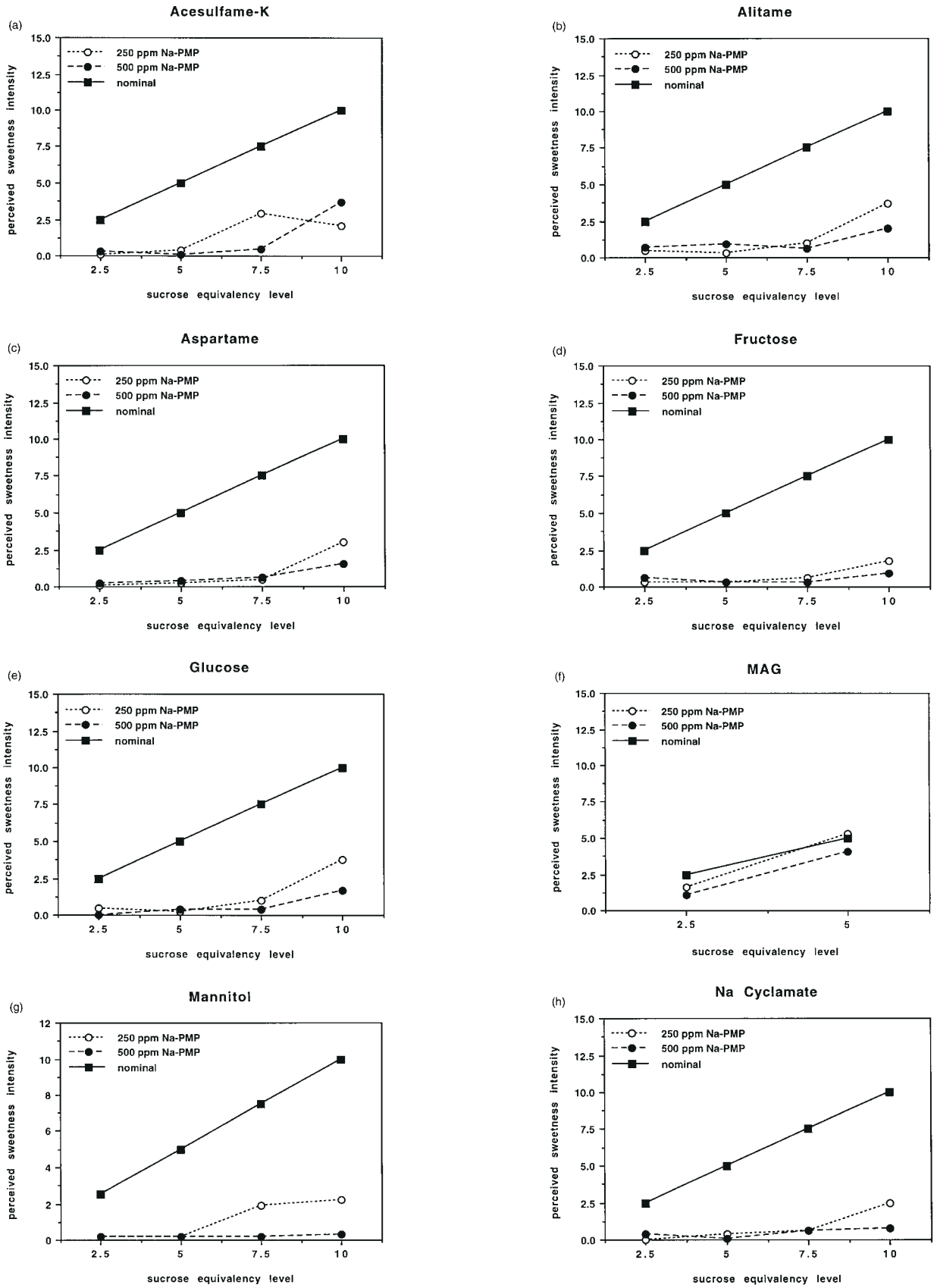
(a)
Acesulfame-K
(b)
Alitame
(c)
Aspartame
(d)
Fructose
(e)
Glucose
(f)
MAG
(g)
Mannitol
(h)
Na Cyclamate
250 ppm Na-PMP
500 ppm Na-PMP
nominal
perceived sweetness intensity
sucrose equivalency level

Figure 1 (a–h)

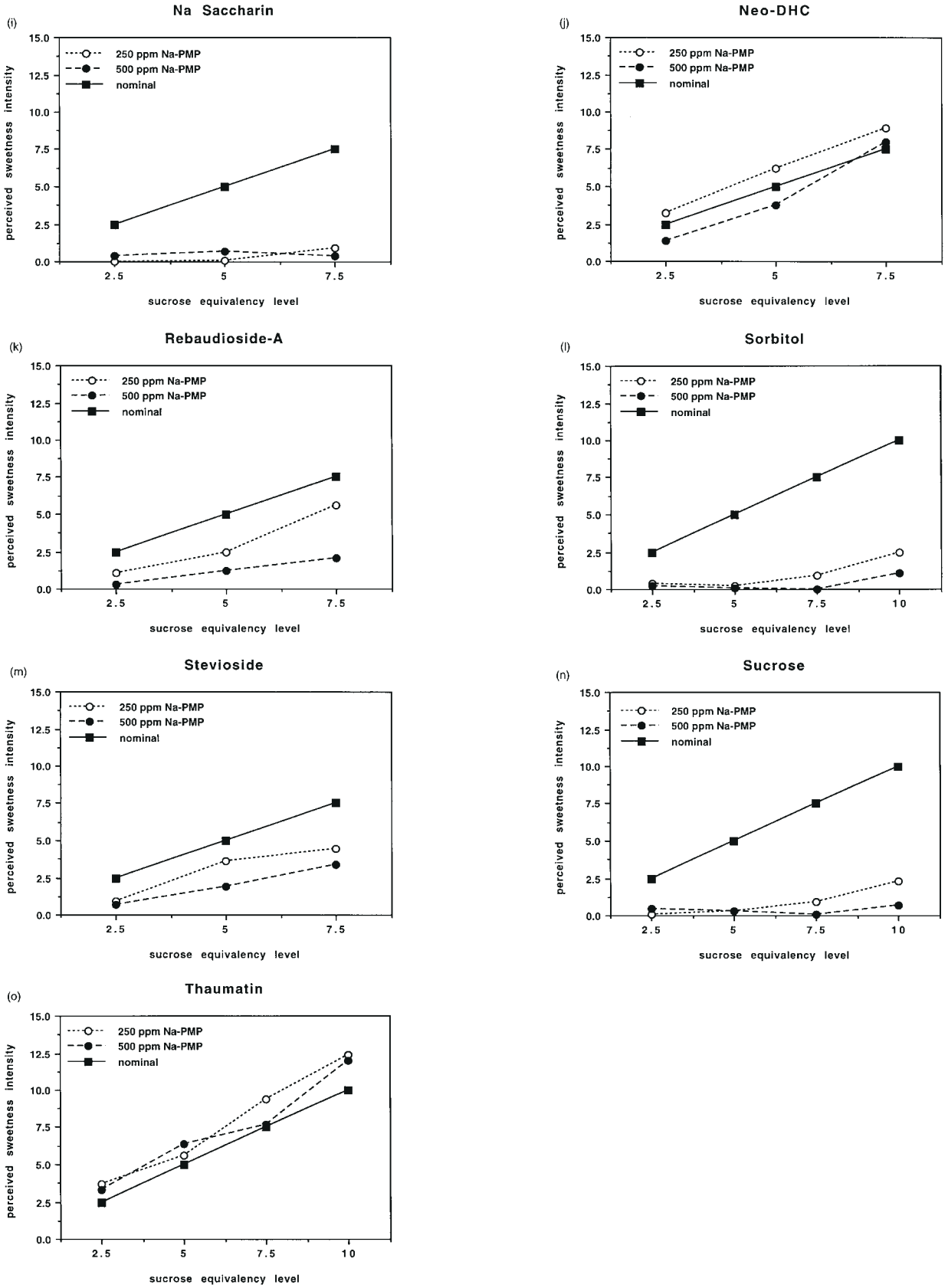

Figure 1 **(a–o)** Mean sweetness intensity ratings of all 15 sweeteners, respectively, at 2–4 concentrations, mixed with 250 and 500 p.p.m. Na-PMP. A line is given depicting the nominal or expected response for each sweetness intensity level.

Table 2 Least square mean perceived sweetness intensities of 15 sweeteners at 4 levels following both an immediate pre-rinse and a 30 s delayed pre-rinse

Sweetener	Target sweetness							
	Immediate pre-rinse				30 s delay pre-rinse			
	2.5%	5%	7.5%	10%	2.5%	5%	7.5%	10%
Acesulfame-K	3.3	3.6	4.4**	4.5**	4.8**	5.9	7.4	6.3**
Alitame	3.4	5.8	8.7	8.0**	5.8**	8.1**	11.1**	10.2
Aspartame	4.0	5.6	7.9	9.2	4.7**	7.6**	10.6**	11.1
Fructose	3.5	5.3	6.8	7.7**	5.3**	8.0**	8.9*	10.1
Glucose	4.0	5.3	7.8	9.7	6.2**	7.7**	9.7*	11.6
MAG	4.2	7.8**	n/a	n/a	5.3**	8.3**	n/a	n/a
Mannitol	2.2	3.3**	5.5*	7.6**	4.4*	6.5	8.6	10.4
Na-cyclamate	5.3**	3.6	3.8**	6.2**	7.4**	5.7	6.2	8.1
Na-saccharin	2.5	3.3	5.0**	n/a	4.5**	6.4*	7.2	n/a
Neo-DHC	4.7**	7.4**	9.3*	n/a	5.8**	9.0**	10.1**	n/a
Rebaudioside-A	4.8**	6.0	7.6	n/a	5.3**	8.1**	8.9	n/a
Sorbitol	2.7	4.0	6.0**	8.4**	5.4**	6.9**	8.4	10.2
Stevioside	5.0**	6.8	7.6	n/a	7.5**	8.1**	8.6	n/a
Sucrose	4.1*	4.9	7.1	8.7	6.7**	7.1**	10.2**	10.9
Thaumatin	3.4	9.8**	10.6*	13.2**	6.2**	9.3**	9.9	13.1**

Statistically significant difference at $*P < 0.05$ and $**P < 0.01$.

250 and 500 p.p.m. Na-PMP. A line depicting the nominal or expected response at each intensity level is also given.

Additional observations

The Na-PMP has a slight bitter-metallic taste of its own, and several subjects detected a faint salty component when asked to rate a Na-PMP solution. However, these qualities did not significantly increase the bitter, metallic or salty ratings of the mixtures of Na-PMP with sweeteners.

Study 2

Statistical methods

A mixed-model analysis of variance (ANOVA) was performed on the sweetness intensity ratings of each sweetener separately (SAS Institute Inc., 1992). The ANOVA model included effects for panelist (panelists, random effect), Na-PMP pre-rinse (immediate or 30 s delay, fixed effect), level of sweetener (2.5, 5, 7.5 and 10% sucrose equivalencies, fixed effect) and the interaction between pre-rinse and sweetener level (fixed effect). For each sweetener ANOVA, the *P*-values were adjusted in order to control the significance level from multiple t-tests by the Bonferroni method (Miller, 1981) of multiplying the *P*-value by the number of tests performed. The ANOVA's also provided contrast *t*-tests of each 30 s evaluation versus its immediate evaluation, by sweetener and sweetener level.

Comparison between pre-rinse evaluations and expected responses

There were both significant inhibition and enhancement of responses (see Table 2). For the immediate evaluations, significant suppression of sweet taste occurred for one or more levels of acesulfame-K, alitame, fructose, mannitol, Na-cyclamate, Na-saccharin and sorbitol. Significant enhancement of sweet responses occurred for one or more levels of MAG, Na-cyclamate, neo-DHC, rebaudioside-A, stevioside, sucrose and thaumatin. For the 30 s delay evaluations, significant suppression of sweet taste occurred for only acesulfame-K at the 10% sucrose equivalency level. Significant enhancement of sweet response occurred for the majority of sweeteners at 2.5 and 5 levels and almost half of sweeteners at the 7.5 level. Only one sweetener, thaumatin, was significantly enhanced at the 10% sucrose equivalency level.

Comparison of immediate pre-rinse versus 30 s delay pre-rinse

Figure 2a–d represents the mean sweetness intensity ratings of all sweeteners following the immediate and 30 s delay pre-rinses at the 2.5, 5, 7.5 and 10% sucrose equivalency levels respectively. A dotted line represents the nominal or expected level of sweetness. At the 2.5% sucrose equivalency level, means for the 30 s delay evaluation were significantly higher than those for the immediate delay evaluation for alitame, fructose, glucose, mannitol, Na-cyclamate, Na-saccharin, sorbitol, stevioside, sucrose and thaumatin. At the 5% sucrose equivalency level responses at the 30 s delay

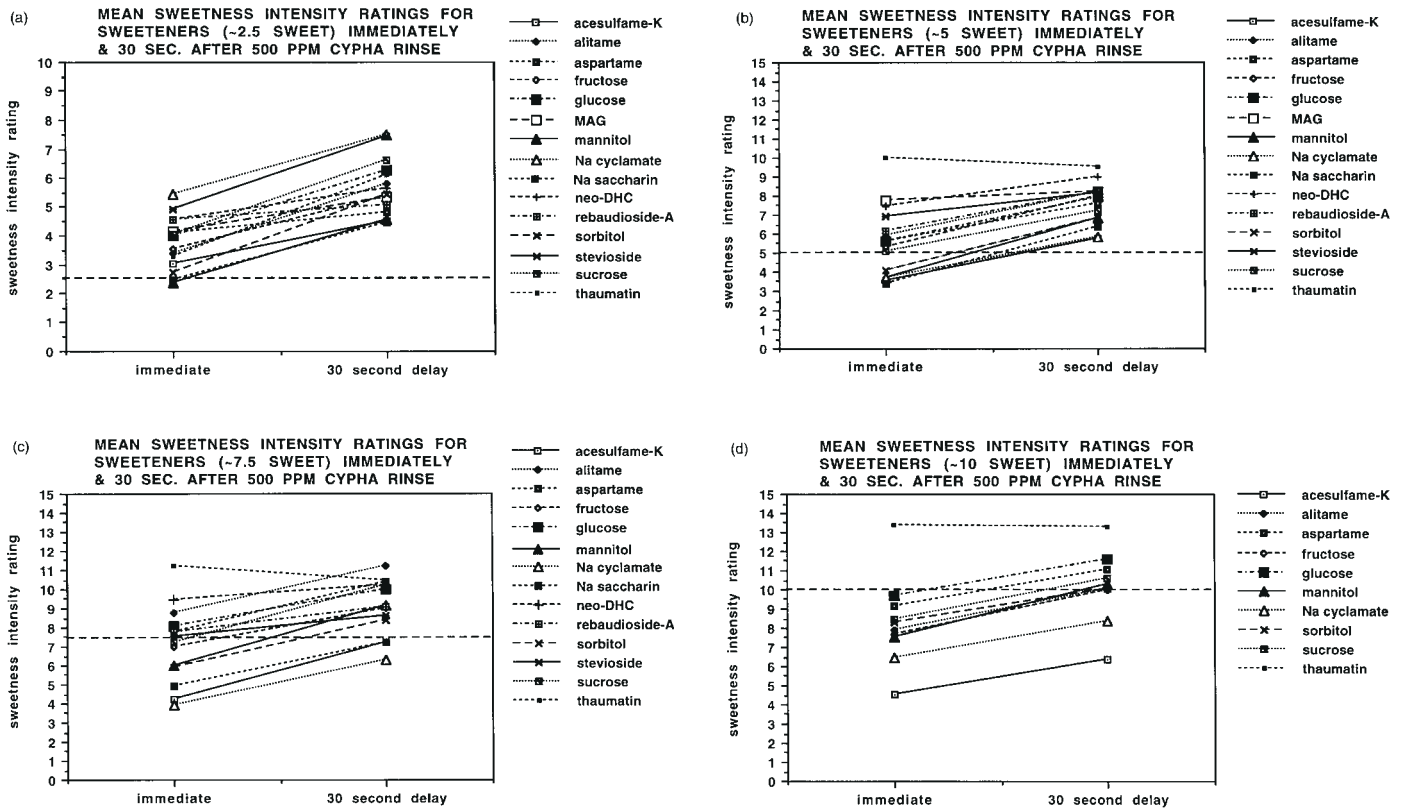

Figure 2 **(a–d)** Mean sweetness intensity ratings of 15 sweeteners at 2.5, 5, 7.5 and 10% sucrose equivalencies, respectively, immediately and 30 s following a Na-PMP pre-rinse. A dotted line represents the nominal or expected sweet intensity response.

were significantly higher than those for the immediate delay for all sweeteners except MAG, stevioside and thaumatin. The mean response for thaumatin was actually slightly lower at the 30 s delay response. At the 7.5% sucrose equivalency level, again, responses at the 30 s delay were significantly higher than those at the immediate delay for all sweeteners except neo-DHC, rebaudioside-A, stevioside and thaumatin. Like the 5% level, the mean response for thaumatin was slightly lower at the 30 s delay level. At the 10% sucrose equivalency level, responses at the 30 s delay were significantly higher for all sweeteners except thaumatin, for which the 30 s delay mean was again slightly lower than the mean at the immediate pre-rinse.

Discussion

The results of study 1 of this experiment suggest that Na-PMP is a selective competitive antagonist of sweet taste, and that at least two sweet receptors or mechanisms are involved in taste transduction for the 15 sweeteners tested. When Na-PMP was mixed with sweeteners ranging widely in chemical structure, it significantly blocked the sweetness intensity of 12 of 15 sweeteners with no effect on three of the 15 sweeteners. Na-PMP at both 250 and 500 p.p.m. significantly suppressed the sweetness of three sugars (fructose, glucose, sucrose), two dipeptide derivatives (alitame, aspartame), two N-sulfonylamides (acesulfame-K, sodium saccharin), two polyhydric alcohols (mannitol, sorbitol) and one sulfamate (sodium cyclamate). Two of the three terpenoid glycosides (rebaudioside-A and stevioside) were also significantly suppressed but to a lesser degree. One terpenoid glycoside (monoammonium glycyrrhizinate), one dihydrochalcone (neohesperidin dihydrochalcone) and one protein (thaumatin) were not suppressed by Na-PMP.

Further support for Na-PMP as a competitive antagonist comes from the finding that very high concentrations of sucrose and sorbitol overcome the inhibition (see Table 3) (Schiffman, 1996). This finding that sweetness suppression by Na-PMP is surmounted by high concentrations of sucrose (and sorbitol) along with the fact that sweetness intensity is reduced when Na-PMP is mixed with some but not all compounds suggest that Na-PMP may act as a selective competitive antagonist of sweet taste. However, because sucrose and sorbitol were used at much higher molar proportions and mass fractions than the other sweeteners, the consequent difference in water activity and solute apparent volume could also possibly account for this finding (Birch, 1994).

The finding that pre-treatment with Na-PMP does not uniformly suppress sweet taste (and in fact greatly enhances some tastes when they follow the pre-rinse by a 30 s delay)

Table 3 Percent reduction in mean sweetness intensity ratings of five concentrated solutions of both sorbitol and sucrose mixed with 250 and 500 p.p.m. Na-PMP solutions, compared with sorbitol and sucrose in water

Sweetener	Concentration (%)	Level of Na-PMP	
		250 p.p.m.	500 p.p.m.
Sorbitol	20	–62.6	–89.3
	30	–48.9	–71.9
	40	–32.3	–55.6
	50	–16.8	–35.2
	60	–8.17	–34.6
Sucrose	20	–41.7	–65.0
	30	–25.7	–44.6
	40	–11.7	–29.8
	50	–14.0	–24.4
	60	–18.9	–25.3

does not refute the conclusion that Na-PMP is a selective inhibitor of sweet tastes. Pretreatment with Na-PMP may sensitize certain sweet receptor subtypes or partially depolarize cells that respond to sweeteners. This finding that Na-PMP blocks sweetness in a mixture but does not block it when used as a pre-rinse is consistent with other putative competitive inhibitors of sweet taste including *p*-nitrophenyl α-D-glucopyranoside (PNP-Glu) and methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (MAD-diCl-Gal), which display the same pattern (Jakinovich, 1983; Vlahopoulos and Jakinovich, 1986). Interestingly, sweet taste inhibitors that are not competitive inhibitors (e.g. gymnemic acid, ziziphins) block sweet taste when used as a pre-rinse.

In conclusion, Na-PMP appears to be a selective competitive inhibitor of sweet taste. In addition, this selectivity provides further evidence for at least two sweet receptors or mechanisms.

References

Avenet, P., Hofmann, F. and **Lindemann, B.** (1988) *Transduction in taste receptor cells requires cAMP-dependent protein kinase*. Nature, 331, 351–354.

Birch, G.G. (1994) *The chemical basis of sweetness perception in beverages*. Food Chem., 51, 359–364.

Breslin, P.A.S., Beauchamp, G.K. and **Pugh, E.N. Jr** (1996) *Monogeusia for fructose, glucose, sucrose, and maltose*. Percept. Psychophys., 58, 327–341.

DeSimone, J.A., Heck, G.L., Mierson, S. and **DeSimone, S.K.** (1984) *The active ion transport properties of canine lingual epithelia* in vitro*. Implications for gustatory transduction*. J. Gen. Physiol., 83, 633–656.

DuBois, G.E. (1997) *New insignts on the coding of the sweet taste message in chemical structure*. In Salvadori, G. (ed.), Olfaction and Taste: a Century for the Senses. Allured Publishing Corp., Carolstream, IL, pp. 32–95.

DuBois, G.E., Walters, D.E., Schiffman, S.S., Warwick, Z.S., Booth, B.J., Pecore, S.D., Gibes, K., Carr, B.T. and **Brands, L.** (1991) *Concentration–response relationships of sweeteners: a systematic approach*. In Walters, D.E., Orthoefer, F.T. and DuBois, G.E. (eds), ACS Symposium Series 450. Sweeteners: Discovery, Molecular Design and Chemoreception. American Chemical Society, Washington, DC, pp. 261–276.

Guh, J.H., Ko, F.N., Yu, S.M., Wu, Y.C. and **Teng, C.M.** (1995) *Pharmacological evaluation of* N*-methyl-actinodaphnine, a new vascular* α*-adrenoceptor antagonist, isolated from Illigera luzonensis*. Eur. J. Pharmacol., 279, 33–41.

Hegde, S.S., Bonhaus, D.W., Johnson, L.G., Leung, E., Clark, R.D. and **Eglen, R.M.** (1995) *RS 39604: a potent, selective and orally active 5-HT4 receptor antagonist*. Br. J. Pharmacol., 115, 1087–1095.

Jakinovich, W. Jr (1983) *Methyl 4,6-dichloro-dideoxy-*α*-D-galacto-pyranoside: an inhibitor of sweet taste responses in gerbils*. Science, 219, 408–410.

Jakinovich, W. and **Sugarman, D.** (1989) *Peripheral mechanisms of mammalian sweet taste*. In Cagan, R.H. (ed.), Neural mechanisms in taste. CRC Press, Boca Raton, FL, pp. 37–83.

Johnson, C., Birch, G.G. and **MacDougall, D.B.** (1994) *The effect of the sweetness inhibitor 2-(4-methoxyphenoxy)propanoic acid (sodium salt) (Na-PMP) on the taste of bitter-sweet stimuli*. Chem Senses, 19, 349–358.

Lindley, M.B. (1986) Method for Inhibiting Sweetness. US Patent 4,567,053, January 28.

Lindley, M.G. (1991) *Phenoxyalkanoic acid sweetness inhibitors*. In Walters, D.E., Orthoefer, F.T. and DuBois, G.E. (eds), ACS Symposium Series 450. Sweeteners: Discovery, Molecular Design and Chemoreception. American Chemical Society, Washington, DC, pp. 251–260.

Miller, R.G. (1981) Simultaneous Statistical Inference. Springer-Verlag, New York.

Naim, M., Ronen, T., Striem, B.J., Levinson, M. and **Zehavi, U.** (1991) *Adenylate cyclase responses to sucrose stimulation in membranes of pig circumvallate taste papillae*. Comp. Biochem. Physiol., 100B, 455–458.

Naim, M., Seifert, R., Nürnberg, B., Grünbaum, L. and **Schultz, G.** (1994) *Some taste substances are direct activators of G-proteins*. Biochem. J., 297, 451–454.

Piper, A.S. and **Hollingsworth, M.** (1995) *The purinoceptors of the guinea-pig isolated taenia caeci*. Eur J Pharmacol., 280, 125–134.

SAS Institute Inc. (1992) SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, Release 6.07. SAS Institute Inc., Cary, NC, pp. 287–368.

Schiffman, S.S. and **Gatlin, C.A.** (1993) *Sweeteners: state of knowledge review*. Neurosci. Biobehav. Rev., 17, 313–345.

Schiffman, S.S., Lockhead, E. and **Maes, F.W.** (1983) *Amiloride reduces the taste intensity of* Na^+ *and* Li^+ *salts and sweeteners*. Proc. Natl Acad. Sci. USA, 80, 6136–6140.

Schiffman, S.S., Pecore, S.D., Booth, B.J., Losee, M.L., Carr, B.T., Sattely-Miller, E., Graham, B.G. and **Warwick, Z.S.** (1994a) *Adaptation of sweeteners in water and in tannic acid solutions*. Physiol. Behav., 55, 547–559.

Schiffman, S.S., Suggs, M.S. and **Losee, M.L.** (1994b) *Effect of modulators of the adenylate cyclase system on sweet electrophysiological taste responses in gerbil*. Pharmacol. Biochem. Behav., 48, 991–998.

Schiffman, S.S., Suggs, M.S., Losee, M.L., Gatlin, L.A., Stagner, W.C. and **Bell, R.M.** (1995) *Effect of lipid-derived second messengers on electrophysiological taste responses in the gerbil*. Pharmacol. Biochem. Behav., 52, 49–58.

Striem, B.J., Pace, U., Zehavi, U., Naim, M. and **Lancet, D.** (1989) *Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes*. Biochemical J., 260, 121–126.

Striem, B.J., Naim, M. and **Lindemann, B.** (1991) *Generation of cyclic AMP in taste buds of the rat circumvallate papillae in response to sucrose*. Cell Physiol. Biochem., 1, 46–54.

Tonosaki, K. and **Funakoshi, M.** (1988) *Cyclic nucleotides may mediate taste transduction*. Nature, 331, 354–356.

Vlahopoulos, V. and **Jakinovich, W., Jr** (1986) *Antagonism of the gerbil's sucrose taste response by* p*-nitrophenyl* α*-D-glucopyranoside and chloramphenicol*. J. Neurosci., 6, 2611–2615.

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