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Gustatory responses of pigs to various natural and artificial compounds known to be sweet in man

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Abstract

The gustatory preferences in pigs towards 33 compounds known to be sweet in humans were evaluated through a specific two-choice preference method. All the 14 carbohydrates tested are preferred over water, sucrose being the most effective. Sucrose and fructose response intensities are identical in pigs and humans but lactose, maltose, D-glucose and D-galactose are two times less efficient in pigs. The molar order of effectiveness is sucrose > D-fructose > maltose = lactose > D-glucose > D-galactose, roughly similar to humans. As in humans, D-glucose, L-glucose and methyl α-D-glucopyranoside display equal potency, while methyl β-D-glucopyranoside is ineffective. The 7 polyols tested are attractive; xylitol is the preferred one, being as effective as sucrose. Out of 12 intense sweeteners tested, 7 are ineffective (aspartame, cyclamate, monellin, NHDC, P-4000, perillartine, thaumatin), and 5 are attractive (acesulfame-K, saccharin, alitame, dulcin, sucralose), but with a much weaker efficiency (acesulfame, 18×less; saccharin, 65×less) than with humans.

1. Introduction

The aim of the present study was to investigate how the pig (*Sus scrofa domesticus*) responds to various compounds known to be sweet in man via a method derived from the well-known 'two-bottle preference' test originated by Richter (see Richter, 1942). By means of this behavioural method, with some modifications to adapt the test to the pig, we were able to determine, semi-quantitatively, the preference of this animal for various compounds, and, at least for the compounds described as sweet by man, and to infer that the compounds which are clearly attractive to the pig should also be perceived as 'sweet' to this animal. Thanks to the Richter-type drinking test, it is in fact already known that pigs exhibit, over water, a strong preference for aqueous solutions of sucrose (the most strongly preferred sugar by pigs), glucose (Baldwin, 1976; Kare, Pond & Campbell, 1965; Kennedy & Baldwin, 1972), lactose (Kare et al., 1965), and sodium saccharin (Baldwin, 1976; Kennedy & Baldwin, 1972), but not for aqueous solutions of sodium cyclamate (Baldwin, 1976; Kennedy & Baldwin, 1972). Further, through electrophysiological measurements, it has been shown that several other compounds tasting sweet to humans, such as monellin, thaumatin (Hellekant, 1976), aspartame or superaspartame (Hellekant & Danilova, 1996), do not elicit any significant neural responses in the chorda tympani nerve of pigs. From these data, it was concluded that these compounds do not taste sweet to pigs (Hellekant & Danilova, 1996). The purpose of the current work was to go deeper into our understanding of the responses of pigs to various compounds sweet to humans, by analysing the gustatory behaviour of pigs towards 15 carbohydrates, seven polyols, and 12 various natural or artificial compounds and some commercially used as sweetening agents for humans.

2. Animals, method and materials

2.1. Animals

Seventy-five pigs (39 males and 36 females, 2–4 months old) were used for this study. Experiments were

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carried out over a period of 2 years with eight different groups. During the test periods, pigs were housed in individual cages (2 m×3 m), each of them being equipped with an automatic water supply freely accessible.

2.2. Method

The method employed is an adapted Richter-type drinking test, derived from the two-bottle preference test previously used by Kare et al. (1965) and Kennedy and Baldwin (1972) in pigs. Two containers — one containing plain water, the other the compound to be tested dissolved in water — are supplied to the animal. The consumption of the tested solution is then measured relative to that of water. In order to test the greatest number of compounds, the problem encountered with pigs is the volume of the solutions ingested (several litres per diem for a strongly preferred solution), these compounds often being very expensive.

To overcome these technical and financial obstacles, the original Richter procedure was modified by carrying out a preliminary training session before the testing session proper, so that the pigs would acquire the habit of sampling before drinking. Every naive pig was thus trained to make a choice between two containers (buckets), one with tap water, the other with a 100 g/l sucrose solution which is highly attractive to pigs. This training is easily performed thanks to the innate preference of pigs for sugar and to their aptitude for being easily conditioned. In fact, pigs are very quick to locate the sweet-tasting solution (by using a few licks, without drinking, to evaluate the taste quality) and to drink its total volume (250 ml) (in less than 1 min), while the volume of the water control remains practically unchanged. To avoid any forced choice and favour a real preference, the animals always had free access to their usual automatic water supply, even during the testing sessions. As previously observed with primates (Steiner & Glaser, 1984, 1995), various other behavioural clues (postural positions, movements of the head, frequency of licking, etc.) were also observed in association with consumption of the sweet solution. The main taste-induced hedonic behaviour expressions elicited in pigs by the sweet-tasting solution are the head oriented towards the stimulus, eager drinking, a quick swallow and sucking-smacking, as illustrated in an available video tape (Glaser, Tinti, Nofre & Wanner, 1997); with a bitter-tasting solution (a quinine hydrochloride solution at a concentration of 49 mg/l), pigs show a typical behaviour of rejection: no consumption of the solution and several behavioural clues associated with the bitter taste, such as the head withdrawn from the stimulus and head shake, as illustrated in the same video tape (Glaser et al., 1997).

Thus, after the training session, each pig knows that one of the two buckets may contain an attractive 'sweet' substance. It was observed that a time generally of about 10–20 s is sufficient for a trained pig to make a rapid choice, through two or three licks, between the two options, and to drink the preferred fluid greedily, or to move away definitively (with no further interest in the experiment) if an appealing solution has not been detected. The standard duration for each tasting experience was thus fixed at only 1 min. Moreover, this brief-exposure procedure, which minimizes the fluid consumption, also has the advantage of avoiding any possible postingestional factor, such as caloric regulation or physiological aversion. The consumption differences between the water control and the preferred sapid solution are always important: generally a few millilitres for the water control versus the total volume (250 ml) for the preferred fluid. The responses to ascending concentrations of the tested substances (the concentrations usually progressed such that each level was twice as great as the one before) are denoted by a '+' sign (strong preference) if the tested solution represents at least 80% of the 'percentage intake' (volume of test solution consumed/volume of total fluid consumed from both test solution and water control×100), or by a '−' sign in the other cases, which can then denote a weak preference for, an indifference to, or a rejection of the test solution (see Fig. 1).

To validate the results, each experiment was generally duplicated with two different groups out of the eight groups of pigs used during the 2-year period of this study, except for some expensive and/or weakly effective compounds which, to avoid excessive costs, were tested on only one group of pigs and/or on a limited number of animals.

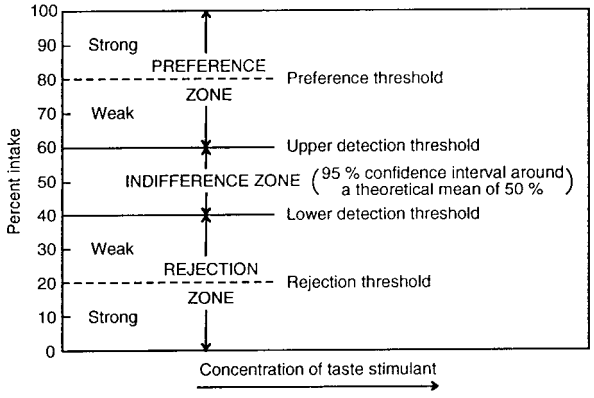

Fig. 1. Data analysis: a summarized diagrammatic presentation of the relationships between taste stimulant concentrations and gustatory responses (adapted from Goatcher & Church, 1970). Note that 'percentage intake' means:

$$\frac{\text{Test solution intake}}{\text{Total fluid intake}} \times 100.$$

The gustatory responses are considered as positive (+) for a percentage intake of 80–100% (in the zone of strong preference), and negative (−) below 80% (in the zones of weak preference, indifference or rejection).

2.3. Materials

Special stainless steel containers, with a conical shape, were designed to allow the pig full access to the water control and to the tested solution. This specific shape makes it possible to reduce the quantity of the ingested solutions to an acceptable volume (250 ml), which limits the pig's insatiability towards 'sweet' solutions and the cost of trials with expensive compounds. The two containers are attached with brackets to a wall of the cage, in a random left–right position to prevent choices that could be based on the place of containers. Experiments started in the morning (at about 9.00 a.m.) and lasted about half-an-hour. During this period, no more than two or three trials were carried out with each animal. Animals were then fed with their usual commercial pelleted food (Hokovit-2150 Natura).

All the chemicals tested were of commercial origin (see footnotes 'a' in Tables 1, 2, 3 and 5 below), except for alitame and P-4000 which were synthesized as by Brennan and Hendrick (1981) and Verkade, Van Dijk and Meerburg (1946), respectively.

3. Results

Pigs have a gustatory preference for all the 15 carbohydrates tested over water (Tables 1 and 2); sucrose is the most preferred carbohydrate (Table 2). Pigs also have a marked preference for all the seven polyols examined versus water; xylitol is the most preferred polyol, being approximately as effective as sucrose on a molar basis (Table 3).

A comparison (on a molar basis with regard to sucrose) between the sweetness potencies of these compounds in humans and their preferences in pigs shows that their relative effectiveness order in pigs closely parallels their relative potencies in humans, except for xylitol which shares the first place with sucrose, and for sorbitol, D-galactose, D-xylose and D-ribose which appear to be in a higher rank in pigs (Table 4). In humans as in pigs, D-fructose is, on a molar basis, half as potent as sucrose (Table 4). Further, the D- and L-enantiomeric forms of glucose display an equal effectiveness, both in humans and in pigs (Table 4).

However, the results obtained with 12 artificial or natural compounds known to be sweet in humans are more disparate (Table 5). Only five compounds — i.e. acesulfame-K, alitame, dulcin, saccharin and sucralose — are able to elicit a preference in pigs; the seven others — i.e. aspartame, cyclamate, monellin, NHDC, P-4000, perillartine and thaumatin — do not elicit any appeal in pigs, even for solutions several tens of times more concentrated than needed to induce an explicit sweet perception in humans (except for P-4000 which is too poorly soluble to test concentrated solutions).

Table 1
Gustatory responses of pigs to seven monosaccharides known to be sweet in humans

Carbohydrates[a]	Concentrations		Number of pigs	Gustatory responses[b]
	mmol/l	g/l		
Pentoses				
D-Ribose	233.79	35.10	4	4+
	150.00	22.52	2	2−
D-Xylose	116.89	17.55	2	2+
	58.44	8.77	2	2−
Hexoses				
D-Fructose	29.14	5.25	10	10+
	15.54	2.62	12	8−,4+
	7.27	1.31	16	7−,9+
	3.60	0.65	4	4−
D-Galactose	116.56	21.00	10	10+
	87.42	15.75	10	3−,7+
	58.28	10.50	4	4−
D-Glucose	116.56	21.00	12	12+
	87.42	15.75	10	4−,6+
	58.28	10.50	13	10−,3+
	29.14	5.25	15	12−, 3+
	14.57	2.62	2	2−
L-Glucose	116.56	21.00	5	5+
	87.42	15.75	2	2−
	58.28	10.50	6	6−
D-Mannose	116.56	21.00	2	2+
	58.28	10.50	2	2−

[a] L-Glucose, D-mannose, D-ribose and D-xylose are compounds from Fluka; D-fructose, D-galactose and D-glucose, from Merck.

[b] + Indicates a preference when the tested solution represents 80% or more of total fluid intake from both test solution and water control; −, an indifference or a rejection in the other cases.

Note that the acesulfame and saccharin molecules, which share common molecular structural features, are both preferred in pigs, but that alitame and aspartame induce opposite taste responses in pigs, alitame being undoubtedly attractive, aspartame not being so. For all the compounds attractive to pigs, the comparison (always on a molar basis relative to sucrose), between their preferences in pigs and their sweetness potencies in man, shows that their effectiveness in pigs is markedly lower (from ~25 times for sucralose to ~120 times for dulcin) than that necessary in humans for matching the sweetness level of a 2% (58.4 mmol/l) sucrose solution (Table 6).

4. Discussion

The present data highlight several basic similarities between the gustatory responses of pigs and of humans to various carbohydrates and polyols.

Thus, the lowest concentration of sucrose clearly preferred in all the animals tested (~14 mmol/l) (Table 2) is

very close to the detection and recognition thresholds of sucrose in humans, which are about 10 and 17 mmol/l, respectively (Amerine, Pangborn & Roessler, 1965a).

The relative molar order of the carbohydrate effectiveness in pigs roughly mirrors the relative molar sweetness potency order in humans (Table 4), except for D-galactose, D-xylose and D-ribose. Thus, if we consider the main nutritive carbohydrate sweeteners, the order of effectiveness, on a molar basis, is for humans: sucrose > D-fructose > maltose = lactose > D-glucose > D-galactose (Table 4), while this order for pigs is: sucrose > D-fructose > maltose = lactose > D-glucose = D-galactose (Table 4). Note that in rats, this order is somewhat different: maltose > D-fructose = lactose > sucrose > D-glucose > D-galactose (Richter & Campbell, 1940; Tonosaki & Beidler, 1989).

Amazingly, D-glucose (in fact, D-glucopyranose) has the same effectiveness as its enantiomeric form, L-glucose (L-glucopyranose), both in pigs and in humans (Table 4), which is in favour of a similar *symmetrical* arrangement of the recognition sites which bind both these chiral (dissymmetrical) mirror-image molecules in the porcine receptor as well as in the human receptor. As a result, as postulated for the human sweetness receptor by two of us (Nofre & Tinti, 1996), the porcine receptor interaction sites of these two molecules with opposite handedness are possibly a lysine residue NH_3^+ group associated with two aspartate or glutamate residue

Table 2
Gustatory responses of pigs to seven oilgosaccharides and to one heteroside known to be sweet in humans

Carbohydrates[a]	Concentrations		Number of pigs	Gustatory responses[b]
	mmol/l	g/l		
Disaccharides				
Lactose.H_2O	100.00	36.03	2	2+
	58.42	21.05	10	2−,8+
	43.71	15.75	10	8−,2+
	29.14	10.50	4	4−
Maltose.H_2O	100.00	36.03	24	24+
	58.42	21.05	24	10−,14+
	43.71	15.75	12	9−,3+
	29.14	10.50	12	12−
Melibiose	116.85	40.00	4	4+
	58.42	20.00	4	2−,2+
	43.82	15.00	4	2−,2+
	29.21	10.00	4	3−,1+
	14.60	5.00	4	4−
Sucrose	14.60[c]	5.00	12	12+
	7.30[c]	2.50	18	8−,10+
	5.84	2.00	12	12−
Trehalose	116.85	40.00	4	4+
	87.64	30.00	6	4−,2+
	43.82	15.00	4	4−
Trisaccharides				
Melezitose.H_2O	58.37	30.50	6	6+
	29.18	15.25	6	3−,3+
Raffinose.$5H_2O$	116.05	69.00	4	4+
	58.02	34.50	12	3−,9+
	29.01	17.25	4	1−,3+
	14.63	8.70	4	3−,1+
	7.23	4.30	4	4−
Heteroside[d]				
Methyl α-D-gluco-pyranoside	116.78	22.68	5	5+
	58.39	11.34	2	2−

[a] Lactose, maltose, sucrose, trehalose, raffinose and methyl α-D-glucopyranoside are compounds from Fluka; melibiose and melezitose, from Sigma.

[b] + Indicates a preference when the tested solution represents 80% or more of total fluid intake from both test solution and water control; −, an indifference or a rejection in the other cases.

[c] Note that these values in pigs are close to the detection and recognition thresholds of sucrose as obtained in humans, which are about 10 and 17 mmol/l respectively (Amerine et al., 1965a). This observation argues in favour of a roughly similar mechanism in the interaction of sucrose with the pig and the human sweetness receptors, and substantiates our choice of adopting sucrose as the sweetness standard preference in pigs.

[d] Note that methyl β-D-glucopyranoside, which is unsweet in humans (unlike its α anomer), does not elicit any preference in pigs, these animals being indifferent towards concentrations of 58.39 to 116.78 mmol/l of this compound.

Table 3
Gustatory responses of pigs to seven polyols known to be sweet in humans

Polyols[a]	Concentrations		Number of pigs	Gustatory responses[b]
	mmol/l	g/l		
Tetrols				
DL-Threitol	116.27	14.20	6	6+
	87.20	10.65	2	2−
Erythritol	234.19	28.60	4	4+
	175.64	21.45	2	2−
Pentols				
D-Arabitol	233.97	35.60	4	4+
	175.48	26.70	2	1−,1+
	116.98	17.80	2	2−
Ribitol (adonitol)	233.97	35.60	4	4+
	175.48	26.70	2	2−
Xylitol	14.60	2.22	20	20+
	7.30	1.11	16	11−,5+
Hexols				
Mannitol	233.84	42.60	4	4+
	175.38	31.95	2	1−,1+
	116.92	21.30	2	2−
Sorbitol	58.46	10.65	5	5+
	48.85	8.90	17	7−,10+
	29.20	5.32	12	12−

[a] DL-Threitol, D-arabitol, ribitol, xylitol and sorbitol are compounds from Sigma; erythritol, from Fluka; mannitol, from Merck.

[b] + Indicates a preference when the tested solution represents 80% or more of total fluid intake from both test solution and water control; −, an indifference or a rejection in the other cases.

Table 4
Comparison (on a molar basis relative to sucrose) between the sweetness potencies in humans and the preferences in pigs for 23 various polyhydroxy compounds (carbohydrates and polyols)

Carbohydrates and polyols	Potencies in humans[a]	Preferences in pigs[c]
Sucrose	1.00	1.00
D-Fructose	0.50	0.50
Melezitose	0.35	0.25
Lactose	0.33	0.146
Maltose	0.33	0.146
Xylitol	0.30	1.00
D-Glucose	0.25	0.125
L-Glucose	0.25	0.125
D-Mannose	0.25[b]	0.125
Melibiose	0.25	0.125
Trehalose	0.25	0.125
Raffinose	0.25	0.125
Methyl α-D-glucopyranoside	0.25	0.125
DL-Threitol	0.25	0.125
Erythritol	0.25	0.062
D-Arabitol	0.25[b]	0.062
Ribitol	0.25	0.062
Mannitol	0.25	0.062
Sorbitol	0.25	0.25
D-Galactose	0.20[b]	0.125
D-Xylose	0.20	0.125
D-Ribose	0.15[b]	0.062
Methyl β-D-glucopyranoside	0.00[b]	0.00

[a] The approximate sweetness potencies in humans (on a molar basis relative to a 2% sucrose solution) were evaluated (or re-evaluated) by six trained panellists of our laboratory through the paired-comparison (two-sample) test (see Amerine, Pangborn & Roessler, 1965b).

[b] Compound with an unpleasant taste (or off-taste) in humans, described as a 'chemical' or a 'metallic' taste (or off-taste) by our panellists.

[c] The approximate relative preferences in pigs were estimated from the lowest concentration of sucrose able to induce a preference in all the animals of the same experimental group (14.60 mmol/l) divided by the lowest concentration of the tested compound which is able to induce a preference in all the animals of the same group (e.g. 29.14 mmol/l for D-fructose).

Table 5
Gustatory responses of pigs to 12 compounds described as sweeteners in humans

Compounds[a]	Concentrations		Number of pigs	Custatory responses[b]
	mmol/l	g/l		
Acesulfame-K	1.73	0.35	4	4+
	0.24	0.05	4	2−,2+
	0.04	0.01	4	4−
Alitame	0.30	0.10	4	4+
	0.15	0.05	4	4−
Aspartame	16.98	5.00	5	5−
	10.19	3.00	4	4−
	5.09	1.50	4	4−
Cyclamate	99.39	20.00	4	4−
(sodium salt)	49.69	10.00	4	4−
	24.84	5.00	4	4−
Dulcin	13.31	2.40	4	4+
	6.65	1.20	4	3−,1+
	3.32	0.60	4	4−
Monellin	0.018	0.20	4	4−
Neohesperidin dihydrochalcone (NHDC)	0.097	0.60	4	4−
5-Nitro-2-propoxy-aniline (P-4000)	0.25	0.05	4	4−
Perillartine	15.13	2.50	4	4−
Saccharin	4.36	0.80	4	4+
	2.18	0.40	4	2−,2+
	1.09	0.20	4	4−
Sucralsoe	0.31	0.125	5	5+
	0.15	0.062	6	2−,4+
Thaumatin	0.009	0.20	4	4−

[a] All the compounds cited are described in *The Merck Index* (12th ed.) as sweeteners. Aspartame, monellin, neohesperidin dihydrochalcone, perillartine and thaumatin, are compounds from Sigma; acesulfame-K, from Supelco; cyclamate, from Merck; dulcin, from Interchim, France; Saccharin, from Fluka; sucralose from Redpath, Canada; alitame and P-400 were synthesized after Brennan and Hendrick (1981) and Verkade et al. (1946), respectively.

[b] + Indicates a preference when the tested solution represents 80% or more of total fluid intake from both test solution and water control; −, an indifference or a rejection in the other cases.

CO_2^- groups symmetrically arranged in space relatively to the lysine ammonium group (Fig. 2).

Another interesting analogy between the porcine and the human responses towards carbohydrates is that pigs have an equal preference for D-glucose (Table 1) and for methyl α-D-glucopyranoside (Table 2), a heterosidic carbohydrate (Fig. 3a); likewise, both these molecules have similar sweetness potencies in humans (Table 4). Moreover, pigs are indifferent towards methyl β-D-glucopyranoside (Fig. 3b), the anomeric form of methyl α-D-glucopyranoside; similarly, methyl β-D-glucopyranoside is unsweet to humans (see Table 2, footnote 'd', and Table 4). The unsweetness of methyl β-D-glucopyranoside can be explained, both in man and in pigs, as the result of a 'steric clash' between the methyl group of its β-methoxy substituent and the methyl group of a threonine residue (denoted Thr-6) of the receptor, leading to a misfitted adaptation of the molecule to the receptor. With methyl α-D-glucopyranoside, this steric hindrance does not occur owing to the different spatial orientation of its α-methoxy group, which allows a suitable docking of the molecule into the receptor, in the same way as D-glucopyranose (Fig. 2a). Although the relative sequence of the responses in pigs towards carbohydrates mirrors the sequence of their potencies in humans, their relative response intensities (by comparison with sucrose, our standard reference) are very different (between the porcine and the human responses) for all the carbohydrates tested (except for D-fructose and sucrose) (see Table 4 and Fig. 4). For example, lactose

Table 6
Comparison (on a molar basis relative to sucrose) between the sweetness potencies in humans and the preferences in pigs for various compounds described as sweeteners in humans

Compounds	Potencies in humans[a]	Preferences in pigs[b,c]	Ratio potency in humans/ preference in pigs[d]
Monellin	100 000	–	
Thaumatin	100 000	–	
Neohesperidin dihydrochalcone (NHDC)	3600	–	
5-Nitro-2-propoxyaniline (P-4000)	2300	–	
Alitame	1900	48.66	40
Sucralose	1160	47.09	25
Perillartine	370	–	
Saccharin	215	3.34	65
Aspartame	155	–	
Acesulfame-K	150	8.43	18
Dulcin	130	1.09	120
Cyclamate (Na)	17.6	–	

[a] The approximate sweetness potencies in humans (on a molar basis relative to a 2% sucrose solution) were evaluated (or re-evaluted) by six trained panellists of our laboratory through the paired-comparison (two-sample) test (see Amerine et al., 1965b).

[b] The approximate relative preferences in pigs were estimated from the lowest concentration of sucrose able to induce a preference in all the animals of the same experimental group (14.60 mmol/l) divided by the lowest concentration of the tested compound which is able to induce a preference in all the animals of the same group (e.g. 4.36 mmol/l for saccharin).

[c] - Indicates an indifference or a rejection.

[d] The value of the ratio indicates how many times, on a molar basis, the studied compound is approximately less 'sweet' in pigs than in humans.

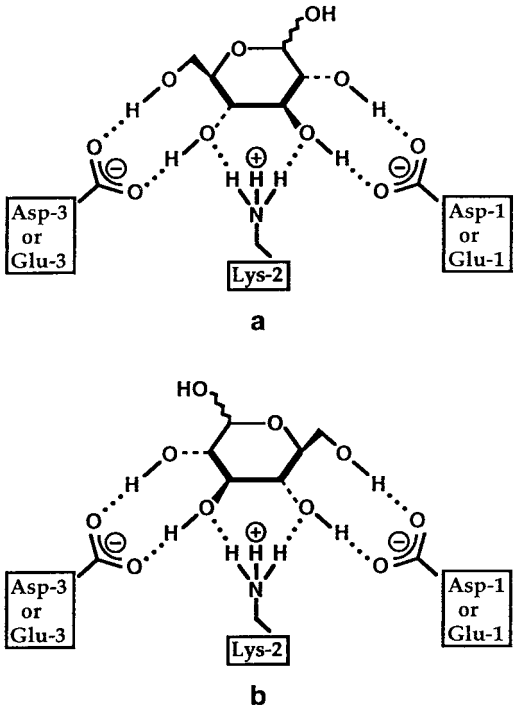

Fig. 2. The six dominant electrostatic interactions (through six ionically-assisted hydrogen bonds, indicated by dotted lines) between the human sweetness receptor and the molecules of (a) D-glucopyranose and (b) L-glucopyranose, as postulated by Nofre and Tinti (1996). Note that the functional groups of the three ionic recognition sites (the so-called 'ionic triad', denoted Asp-1 or Glu-1, Lys-2, and Asp-3 or Glu-3), which are assumed to be implicated in these interactions, are *symmetrically* arranged in space; this enables us to understand why the D- and L-enantiomers of glucopyranose elicit similar responses in humans (Nofre & Tinti, 1996), and, by inference, in pigs.

and maltose are approximately six times less appreciated by pigs than sucrose, while in man both these compounds display a sweetness potency which is about one third that of sucrose; likewise, D- and L-glucose, D-mannose, D-galactose, melibiose, trehalose, raffinose and methyl α-D-glucopyranoside are approximately eight times less preferred by pigs than sucrose, while in man all these compounds exhibit a sweetness potency which is about a quarter that of sucrose.

Concerning the polyols (Tables 3 and 4), xylitol is the most potent of these compounds in pigs and in humans; but, while xylitol is, in humans, about one third less sweet than sucrose on a molar basis (~0.30×sucrose on a molar basis, ~0.70×on a weight basis according to our own assessment), it is roughly as preferred as sucrose on a molar basis in pigs (see Fig. 4). Sorbitol is, just after xylitol, the most favoured polyol in pigs; it is approximately four times less preferred in pigs than sucrose or xylitol, but about twice as preferred as D-glucose, while in man sorbitol is isosweet with D-glucose. Note that sorbitol is common in many fruits (see Wang & van Eys, 1981), often at a concentration of about 10–30 g/l of fresh fruit juice (see Dwivedi, 1986). Although slowly absorbed by the intestine, this polyol may be considered as an effective energetic sweetener, being metabolically converted into D-fructose at the hepatic level (see, e.g. Dwivedi, 1986; Sicard, 1982). DL-Threitol, a tetrol, is isosweet with D-glucose in pigs and in humans. For the other polyols — namely, erythritol, D-arabitol, ribitol and mannitol — these compounds are about 16 times less preferred than sucrose or xylitol and twice less than D-glucose in pigs, while they are approximately four times less sweet than sucrose and are isosweet with D-glucose in man.

Among these results on the carbohydrates and polyols, we particularly highlight the amplification of the human response to D-glucose by comparison with the porcine response, and, conversely, the reduction of the human response to xylitol compared with the porcine response (Fig. 4). From a phylogenetic point of view, the difference between the responses of pigs and humans towards D-glucose and xylitol is possibly a consequence of an evolutionary adaptation of the human (and, more generally, of the catarrhine) sweetness receptor to a keener detection of D-glucose, a highly-energetic free carbohydrate which is common, with sucrose and D-fructose,

in various foods of plant origin (see, e.g. Astrup & Raben, 1996; Frostell, 1980; Guesry & Secrétin, 1991). Concerning xylitol, which is also rather common in various fruits and vegetables (see Wang & van Eys, 1981) at concentrations of about 0.1–0.4 g per kg of fresh weight (see Mäkinen & Söderling, 1980), note that this compound possesses a weak physiological interest as a result of a slow and incomplete intestinal absorption (approximately one-third of the ingested portion of xylitol is absorbed, the rest being actively metabolized by intestinal flora) and of a dual metabolic pathway in the liver through relatively secondary routes (see, e.g. Bär, 1986; Levine, 1986; Schiffman & Gatlin, 1993; Sicard, 1982). The minor interest of xylitol in mammals might explain why the free access to the sweetness receptor of this molecule — which, through its sweetness, should normally interfere with the food selection — is partly hindered in the most 'advanced' receptors, such as in the catarrhine ones.

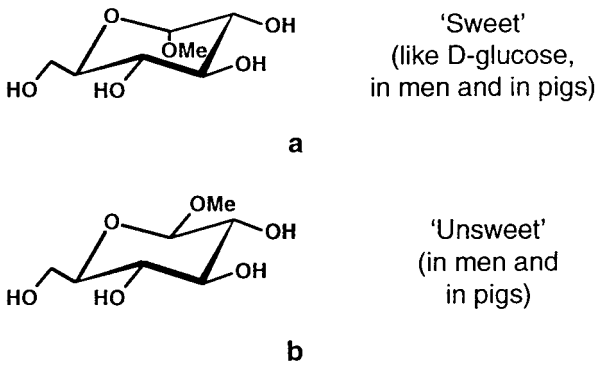

Fig. 3. (a) Methyl α-glucopyranoside, which matches D-glucose both in humans and in pigs, and (b) methyl β-glucopyranoside, which is ineffective both in humans and in pigs. Note that the unsweetness of methyl β-D-glucopyranoside in humans is assigned to a 'steric clash' between the methyl substituent of the equatorially-oriented methoxy group of this heteroside and the side chain of a threonine residue of the receptor, Thr-6 (see Fig. 6 hereafter for further details), which induces a misfit of the ligand into the receptor; by inference, we assume that the pig disinterest in this compound could be due to the same steric hindrance between this molecule and the porcine receptor, and that Thr-6 is consequently retained in the porcine receptor. On the other hand, the methyl substituent of the axially-oriented methoxy group of methyl α-glucopyranoside does not collide with the Thr-6 residue according to a simulated molecular interaction of this molecule with the Nofre/Tinti model of the sweetness receptor, which should explain why this molecule is isosweet with D-glucose in humans as in pigs, since it is able to interact with the receptor through the same dominant electrostatic interactions as those postulated for D-glucose (see Fig. 2a).

Out of the 12 additionally-tested compounds which are also well known to taste sweet to man (Table 5), pigs show no preference for seven of them, namely aspartame, sodium cyclamate, monellin, NHDC, P-4000, perillartine and thaumatin (see Fig. 5).

The indifference of pigs towards aspartame is not surprising as all the mammals tested so far — with the only exception of Catarrhini (Old World primates, including man) (Glaser, Tinti & Nofre, 1995) — do not give any explicit 'sweet' gustatory responses to aspartame, as observed in hamsters (Danilova, Hellekant, Roberts, Tinti & Nofre, 1998; Nowlis, Frank, Pfaffman, 1980), gerbils (Jakinovich, 1981), rats (Nowlis et al., 1980, Hellekant & Walters, 1992), dogs, cows and horses (Glaser, Tinti & Nofre, unpublished results), Prosimii (prosimians) and Platyrrhini (New World monkeys) (Glaser et al., 1995; Glaser, Tinti & Nofre, 1996). According to the multipoint attachment (MPA) theory as proposed by Nofre and Tinti (1996), the human sweetness receptor appears to be formed of at least eight recognition ('binding') sites arranged around the central cavity of the receptor; these sites are assumed to be made up of: an aspartate or a glutamate residue (termed Asp-1 or Glu-1), a lysine residue (Lys-2), another aspartate or glutamate residue (Asp-3 or Glu-3), four threonine residues (Thr-4, Thr-5, Thr-6, Thr-7), and a serine residue (Ser-8) (Fig. 6). As no difference in the gustatory responses of diverse nonhuman catarrhine primates towards various artificial sweeteners has been detected so far, it has been inferred that these primates hold the same key recognition sites in their sweetness receptors as those of humans (Glaser et al., 1996; Nofre,

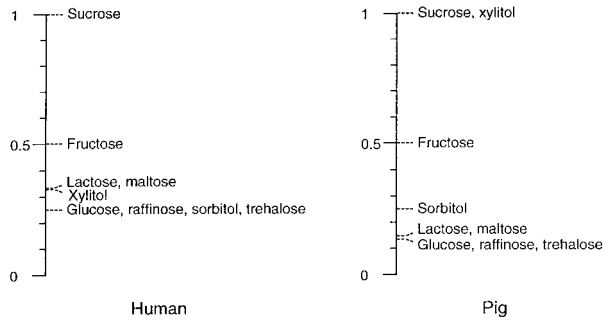

Fig. 4. The relative effectiveness in pigs (on a molar basis) of the main carbohydrates and polyols found in foods compared to the relative sweetness potencies (on a molar basis) of the same compounds in humans.

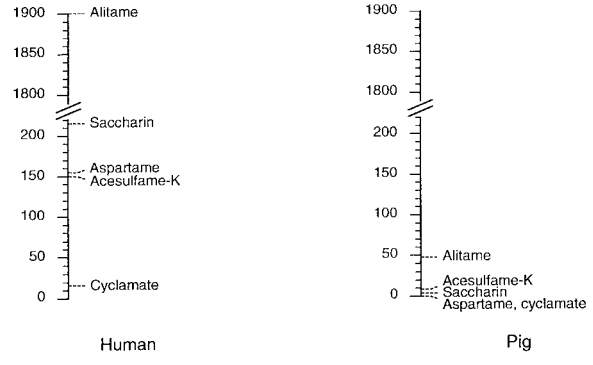

Fig. 5. The relative effectiveness in pigs (on a molar basis) of the main artificial sweeteners compared to the relative sweetness potencies (on a molar basis) of the same compounds in humans.

Tinti & Glaser, 1996). For prosimian and platyrrhine primates, which do not taste aspartame, unlike catarrhine primates (Glaser et al., 1995), it has been proposed, from structure–activity relationships, that this distinctive character between these primates could be due to the presence, in the noncatarrhine sweetness receptors, of a serine or alanine residue (Ser-5 or Ala-5) in place of the Thr-5 site of the catarrhine receptors (Glaser et al., 1996; Nofre et al., 1996). This substitution of Ser-5 (or Ala-5) for Thr-5 makes impossible an effective steric fit of the phenyl ring of aspartame between Thr-5 and Thr-7, this steric fit being apparently crucial for the activation of the receptor by aspartame (Nofre & Tinti, 1996; Nofre et al.). By analogy with the noncatarrhine primates, we infer that the indifference to aspartame of pigs (and, more generally, of all the noncatarrhine mammals) is the result of the replacement of Thr-5 by Ser-5 (or Ala-5) (Fig. 7), which makes this sweetener ineffective.

Just as aspartame, sodium cyclamate, which is sweet to all the catarrhine primates tested until now (Nofre et al., 1996), is 'unsweet' to pigs (Kennedy & Baldwin, 1972; Baldwin, 1976; Glaser et al. in the present work), and to all the mammals studied so far, such as hamsters (Danilova, Hellekant, Roberts et al., 1998; Danilova, Hellekant, Tinti & Nofre, 1998; MacKinnon, Frank & Rehnberg, 1996; Rehnberg, Hettinger & Frank, 1990), gerbils (Jakinovich, 1981), rats (Murray, Wells, Kohn & Miller, 1953), cats (Bartoshuk, Jacobs, Nichols, Hoff & Ryckman, 1975, Beauchamp, Maller & Rogers, 1977), tree shrews, and noncatarrhine primates (Nofre et al., 1996). According to a recent improvement of the MPA theory (Nofre & Tinti, unpublished work), it appears that the sweet stimulus induced by cyclamate in man may be partly due (in addition to several electrostatic interactions between the $NHSO_3^-$ group of cyclamate and some recognition sites of the receptor) to a steric fit of the cyclamate cyclohexyl group between Thr-6 and a valine residue (provisionally termed Val-10, as indicated in the caption of Fig. 6) located behind Thr-4 (and under Thr-5) in the MPA model (see Fig. 6). From this re-examined version of the model, it is argued that, in the porcine receptor (and possibly in all the noncatarrhine mammalian sweetness receptors), Val-10 could be replaced by an Ala residue, which should suppress any possibility of activation of the receptor through a steric fit of the cyclamate cyclohexyl group (Fig. 8).

For the five other compounds sweet to humans but 'unsweet' to pigs (monellin, NHDC, P-4000, perillartine and thaumatin), we believe that the indifference of pigs towards these various compounds is also the result of the absence of one (or more) steric interaction(s) or steric fit(s) between these molecules and the porcine receptor, as has been postulated for aspartame or cyclamate.

The five other artificial sweeteners tested in the present study (acesulfame-K, saccharin, alitame, dulcin, and sucralose) elicit clear 'sweet' responses in pigs

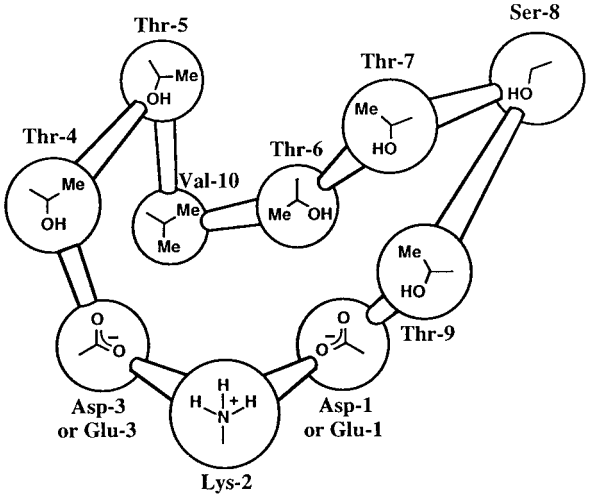

Fig. 6. Model of the human sweetness receptor according to the multipoint attachment (MPA) theory (Nofre & Tinti, 1996). The spheres of the model represent the approximate spatial positions of the different functional groups that may be involved in the interactions of the human receptor with various natural or artificial sweeteners. Note that the MPA model has recently been re-examined (Nofre & Tinti, unpublished work); particularly, it has been inferred, from a comprehensive structure–activity relationship study, that an additional Thr recognition site (denoted Thr-9 in the diagram) must exist above Asp-1/Glu-1 and before Thr-7, and a valine site (denoted Val-10 in the diagram) behind Thr-4 and under Thr-5.

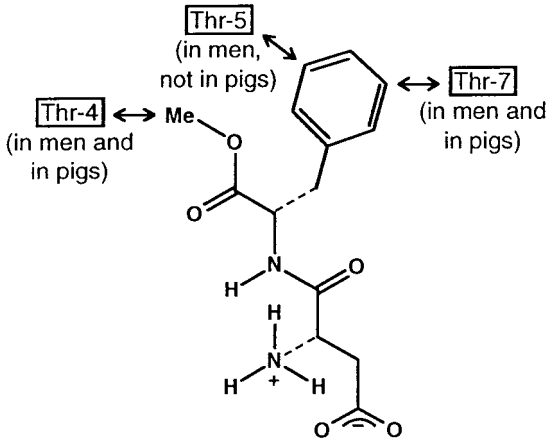

Fig. 7. Aspartame (150×sucrose in man on a molar basis, but not 'sweet' to pigs) and its putative main steric interactions (indicated with double-headed arrows) with the human sweetness receptor, according to the MPA theory (Nofre & Tinti, 1996), or with the porcine receptor, as inferred from detailed structure–activity studies on primates (Glaser et al., 1996; Nofre et al., 1996); from these studies, we suggest, by analogy, that the presumed Thr-5 recognition site of the human receptor could be replaced by a Ser-5 or an Ala-5 residue in the porcine receptor. For clarity, the putative electrostatic interactions between aspartame and the receptor have not been indicated in this diagram; from the MPA theory, it is assumed that these interactions mainly occur between, on the one hand, the CO_2^-, NH_3^+ and $COOCH_3$ groups of aspartame, and, on the other hand, the ionic triad and Thr-4 of the receptor.

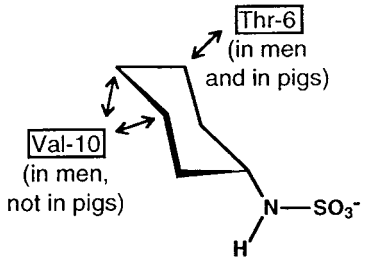

Fig. 8. Cyclamate (17.6×sucrose in man on a molar basis, but not 'sweet' to pigs) and the putative steric fit (indicated with double-headed arrows) of its cyclohexyl moiety between Thr-6 and Val-10 (see the caption of Fig. 6) of the human receptor. As Thr-6 appears to be retained in pigs (see the caption of Fig. 3), only Val-10 should be changed, possibly into an alanine (Ala-10) residue. For clarity, the electrostatic interactions between cyclamate and the receptor have not been represented; from the MPA theory, it is assumed that these interactions take place between the $NHSO_3^-$ part of cyclamate and the ionic triad of the receptor.

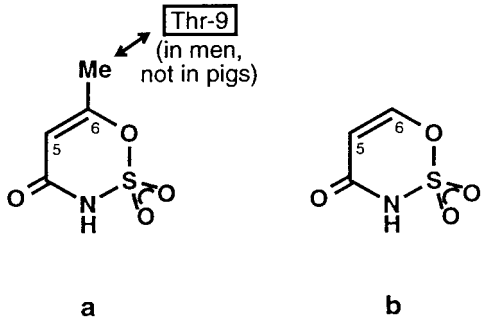

Fig. 9. (a) Acesulfame (6-methyloxathiazinone dioxide): the sweetness potency of this compound is in humans ~150×sucrose, and its effectiveness in pigs ~10×sucrose on a molar basis; (b) unsubstituted oxathiazinone dioxide: its sweetness potency in humans is about 10×sucrose on a molar basis (Clauss & Jensen, 1973). These values indicate that the steric interaction of acesulfame, as assumed in the human sweetness receptor (indicated by a double-headed arrow) and assigned to a putative Thr-9 residue (see the caption of Fig. 6), does not exist in the porcine receptor. As a consequence, the porcine receptor must behave with acesulfame just as the human receptor with the unsubstituted oxathiazinone dioxide, i.e. without formation of a steric interaction between the receptor and the acesulfame methyl group. For clarity, the electrostatic interactions have not been represented in the diagram; these interactions involve (i) the acesulfame $CONHSO_2$ moiety and (ii) the receptor ionic triad and the Thr-6 residue according to the MPA theory.

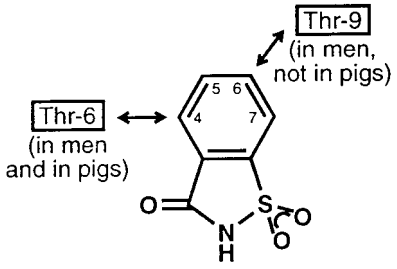

Fig. 10. Saccharin: the sweetness potency of this compound is in humans ~215×sucrose, and its effectiveness in pigs ~3.3×sucrose on a molar basis. It is assumed that saccharin interacts with the human receptor through two steric interactions (represented by two double-headed arrows in the diagram): one between the Thr-6 site and the 4-position of the benzo ring of saccharin, the other between the Thr-9 site and the 6-position of the benzo ring. This generates an efficient steric fit of the molecule of saccharin onto the receptor. In the porcine receptor, while Thr-6 looks retained (see the caption of Fig. 3), Thr-9 appears to be missing, as inferred from the pig responses to acesulfame (see Fig. 9), which prevents any steric fit possibility of the saccharin molecule. For clarity, the electrostatic interactions have not been represented.

(Table 5), but much weaker than in humans (from ~18 to ~120 times less intense according to the sweetener employed) (Table 6). The weakness of the pig responses is attributed to the lack of some steric interaction (or steric fit) aptitudes of the porcine receptor with regard to the steric interaction (or steric fit) capabilities of the human receptor. To illustrate this view, we shall take two examples, acesulfame-K and saccharin, on account of their importance as commercial sweeteners. For convenience, the other less-known sweeteners will be the subject of separate publications.

The sweetness potency of acesulfame (Fig. 9a) is in humans ~150 times that of sucrose and its effectiveness in pigs is ~10 times that of sucrose on a molar basis (see Fig. 5 and Table 6). According to data from structure-activity relationship studies (Nofre & Tinti, unpublished work), it is assumed that acesulfame should interact with the human receptor, in addition to several electrostatic interactions, through one steric interaction which occurs between the 6-methyl group of acesulfame and the assumed Thr-9 recognition site (see the caption of Fig. 6). Furthermore, it is known that the unsubstituted oxathiazinone dioxide ring (Fig. 9b) is only ~10 times sweeter than sucrose on a molar basis in humans (Clauss & Jensen, 1973); the low potency of this compound is interpreted, through the views of the MPA theory, by the impossibility, for this molecule, of contracting a steric interaction with the Thr-9 recognition site. As acesulfame has a relative effectiveness of ~10 times sucrose in pigs (just as the unsubstituted oxathiazinone dioxide ring in humans), it is inferred that Thr-9 is not retained in pigs (see Fig. 9a), and that this residue could be, for example, an alanine (Ala) or a serine (Ser) residue in the porcine receptor.

Concerning saccharin (Fig. 10), which is justly regarded as a very close structural analogue of acesulfame, its sweetness potency is in humans of ~215 times that of sucrose, and its effectiveness in pigs of ~3.3 times that of sucrose on a molar basis (see Fig. 5 and Table 6). From structure–activity relationship studies (Nofre & Tinti, unpublished work), it is now assumed that saccharin should interact with the human sweetness receptor, in addition to several electrostatic interactions, through the steric fit of its benzo aromatic ring between the methyl groups of (i) Thr-6 (via the 4-CH of the saccharin benzo ring) and (ii) Thr-9 (via the 6-CH of the

saccharin benzo ring) (Fig. 10). Through the concepts of the MPA theory, since it appears that Thr-6 should be maintained in pigs (see the caption of Fig. 3) but not Thr-9 (see the caption of Fig. 9), the steric fit of the saccharin molecule, which is highly efficient in the human receptor, should be missing in the porcine receptor. This could explain why saccharin is about 65 times less effective in pigs than in humans.

If the presence or absence of Thr-9 in receptors is really the source of the disparities between species in their gustatory responses to saccharin (or acesulfame), it may be supposed that its presence or absence in a receptor could also be at the origin of the substantial individual variations often encountered with these sweeteners *within* species (e.g. through erratic results in the gustatory responses, through tendencies towards bimodal distributions of the sweetened fluid intake, or, in rodents, via animals selectively bred for high versus low saccharin consumption). Such individual variations have been observed, e.g. in rats (Badia-Elder, Kiefer & Dess, 1996; Dess, 1993; Giza, McCaughey, Zhang & Scott, 1996; Nachman, 1974), guinea pigs (Jacobs, 1978), Virginia opossums (Pressman & Doolittle, 1966), hedgehogs (Ganchrow, 1976), squirrel monkeys (Dua-Sharma & Smutz, 1977; Fisher, Pfaffmann & Brown, 1965), or even in pigs (Kare et al., 1965).

The genetic origin of these within-species variations in the responses to saccharin (or acesulfame) has been particularly well documented in mice, in which clear-cut dichotomous differences have been demonstrated between various inbred mouse strains (Beauchamp et al., 1998; Capretta, 1970; Fuller, 1974; Lush, 1989; Lush, Hormigold, King & Stoye, 1995; Ninomiya, Higashi, Katsukawa, Mizukoshi & Funakoshi, 1984; Pelz, Whitney & Smith, 1973; Ramirez & Fuller, 1976). For example, it is recognized that C57BL/6 mice strongly prefer saccharin solution to water, while DBA/2 mice show a much lower preference for this sweetener (Capretta; Fuller; Lush); this effect is even more marked with acesulfame (Lush; Lush et al.). This strain difference appeared to be due to a single gene called Sac, the C57BL/6 allele having been designated Sac^b, the DBA/2 allele, Sac^d (Fuller); these findings were confirmed by Lush, who localized this gene on mouse chromosome 4 (Chr 4), mapping it near the telomeric end of the chromosome, between the D4Smh6b and Tel4q regions, at 8.1 ± 3.4 cM distal to Nppa (Lush; see Mock & Hirano, 1998, for the latest report on mouse chromosome 4).

From these findings, it is tempting to speculate that the molecular difference among the animals having a strong preference for saccharin or acesulfame and those having a weak preference for these sweeteners lies only in the presence or in the absence of a threonine residue in their sweetness receptors.

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