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PII: S0308-8146(96)00023-4

Food Chemistry, Vol. 56, No. 3, pp. 263–274, 1996

0308-8146/96 $15.00 + .00

Sweetness reception in man: the multipoint attachment theory

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(Received 29 August 1995; revised version received 20 November 1995; accepted 20 November 1995)

The multipoint attachment (MPA) theory assumes the presence, in the human sweetness receptor (probably a seven-pass transmembrane receptor), of at least eight fundamental recognition sites, namely, the B-, AH-, XH-, G1-, G2-, G3-, G4- and D-recognition sites. The B-recognition site is assumed to be a Lys residue; the AH- and XH-recognition sites, Asp (or Glu) residues; the G1-, G2-, G3- and G4-recognition sites, Thr residues; and the D-recognition site, a Ser (or Thr) residue. These eight recognition sites are able to interact with eight sweetener interaction sites, namely, the B, AH, XH, G1, G2, G3, G4 and D interaction sites. The number of interaction sites in a given sweetener may be equal to or lower than eight. Apart from D, the interaction sites are made up of two elementary interaction sites (interaction points or subsites). These interaction points (namely, the B1, B2, AH1, AH2, XH1, XH2, G1, E1, G2, E2, G3, E3, G4, E4 and D points) operate with the receptor through three types of elementary interactions, namely, ionic, H-bonding and steric interactions. B (B1, B2) is an anionic group (CO_2^-) or an H-bond acceptor atom (Cl, Br, O); AH1, AH2, XH1, XH2 are H-bond donor groups (NH^+, NH, OH); E1, E2, E3, E4 are H-bond acceptor atoms (N, O, Cl, Br); G1, G2, G3, G4 are steric interaction subsites (CH_3, CH_2, CH, halogens); and D is an H-bond acceptor group (CN). Several examples are given to illustrate the theory. For example, according to the MPA theory, D-glucose is a B1, B2, AH1, AH2, XH1, XH2-type sweetener, D-fructose an E1, E2, E3, E4-type sweetener, and sucrose a B1, B2, AH1, AH2, XH1, XH2, G1, E1, G2, E2, G3, E3, G4, E4-type sweetener. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Among the theories that have been put forward to explain the interaction of sweeteners with the human sweetness receptor, three types are noteworthy, namely, a two-point, a three-point and a multipoint attachment theory.

The two-point attachment theory (Shallenberger & Acree, 1967) assumes that any sweetener interacts with the sweetness receptor by means of two concerted hydrogen bonds; one is donated to the receptor by a donor group (AH) of the sweetener, the other is accepted from the receptor through an acceptor atom (B) of the sweetener, B and H being approximately 0.3 nm apart (Fig. 1a).

Owing to the impossibility of explaining the important differences in potency between sweeteners by the AH, B theory alone, Kier (1972) supposed the existence of a third interaction point. This three-point attachment theory assumes that the potent sweeteners must interact with the sweetness receptor through three interactions, two by means of hydrogen bonding according to Shallenberger's theory, and the third by means of dispersion (van der Waals) forces, now often considered as a hydrophobic interaction. The location of this interaction (noted X) on the sweeteners was estimated to be at about 0.35 nm from A and at about 0.55 nm from B, A and B being approximately 0.26 nm apart (Fig. 1b).

The invention, by our team, of several series of high-potency sweeteners (Nofre & Tinti, 1987*a,b*, 1990, 1993*a,b*, 1994, Nofre *et al.*, 1989, 1990) and the detailed analysis of their structure–activity relationships enabled us to conclude that the sweetness receptor must be provided with a set of several recognition sites able to interact with several sweetener interaction sites. In its present form, we name our theory the multipoint attachment (MPA) theory to differentiate it from the former two-point or three-point theories. Preliminary accounts of the theory were disclosed at the 199th National Meeting of the American Chemical Society

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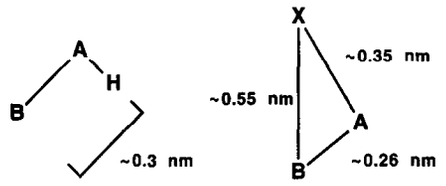


Fig. 1. (a) The B, AH unit of a sweetener according to Shallenberger & Acree (1967). (b) The tripartite B, AH, X unit of a sweetener according to Kier (1972). B is a hydrogen-bond acceptor atom; AH is a hydrogen-bond donor group; X is an atom or a group interacting with the receptor by means of dispersion forces.

held in Boston in 1990 (Tinti & Nofre, 1991) and at the ECRO Symposium on 'Sweet-Taste Chemoreception' held in Reims in 1991 (Nofre & Tinti, 1993*c*). In the present work, we will give a revised and updated version of the theory.

REVISED ACCOUNT OF THE THEORY

Sweetener interaction sites and subsites

The MPA theory, in its revised form, assumes the presence, in the human sweetness receptor, of at least eight fundamental recognition sites able to interact with at least eight fundamental interaction sites ('binding sites') of sweeteners. These eight interaction sites are designated as sites B, AH, XH, G1, G2, G3, G4 and D (see Table 1).

The number of fundamental interaction sites in a given sweetener may be equal to or lower than the number of fundamental latent recognition sites of the receptor. Although certain low-potency sweeteners can exceptionally interact with the receptor via only three sites (such as glycine or 1,2-ethanediol), the majority of them operate via more than three different sites. The relative spatial arrangement of these eight sweetener interaction sites is given in Fig. 2.

With the exception of the D site, each interaction site is made up of two elementary interaction sites, also designated as interaction points or interaction subsites. Two subsites of the same interaction site can work independently or cooperatively, but they are assumed, as a result of their very close interaction, to operate with only one recognition site of the receptor. Thus, B is composed of subsites B1 and B2, AH of subsites AH1 and AH2, XH of subsites XH1 and XH2, G1 of subsites G1 and E1, G2 of subsites G2 and E2, G3 of subsites G3 and E3, and G4 of subsites G4 and E4. Site D probably operates through only one interaction point.

Consequently, as the theory currently stands, there are 15 fundamental interaction points (see Table 1). For example, we assume that glycine or 1,2-ethanediol interact with the receptor through three interaction sites (B, AH and XH) and, more precisely, through four interaction points (B1, B2, AH1 and XH1).

Table 1. Sweetener interaction sites, interaction points, elementary interactions, and presumed nature of the receptor recognition sites

Interaction sites	Interaction points	Elementary interactions	Recognition sites
B	B1	Ionic and/or	Lys
	B2	H-bonding	
AH	AH1	Ionic and/or	Asp/Glu
	AH2	H-bonding	
XH	XH1	Ionic and/or	Asp/Glu
	XH2	H-bonding	
G1	G1	Steric	Thr
	E1	H-bonding	
G2	G2	Steric	Thr
	E2	H-bonding	
G3	G3	Steric	Thr
	E3	H-bonding	
G4	G4	Steric	Thr
	E4	H-bonding	
D	D	H-bonding	Ser/Thr

Elementary interactions

The sweetener interaction points work by means of essentially three types of elementary interaction: (i) reinforced ionic interactions (reinforced by hydrogen bonding), (ii) hydrogen-bonding interactions and (iii) intermolecular steric interactions (van der Waals contacts).

In the present revised MPA theory, site B (B1, B2) is an anionic group (such as a CO_2^-, an SO_3^- or a CN_4^- tetrazolyl group) or H-bond acceptor atoms (such as halogen or oxygen atoms).

Subsites AH1, AH2, XH1 and XH2 are H-bond donor groups (such as NH^+, NH or OH).

Subsites E1, E2, E3 and E4 are essentially H-bond acceptor atoms (such as a nitrogen, an oxygen or a halogen atom). These subsites are often able to work initially as an H-bond donor group (such as NH^+, NH or OH) in the early stage of the interaction (initial attack), or even to operate through a weak intermolecular steric interaction. If necessary, we can differentiate the nature of the prevailing or initial interaction

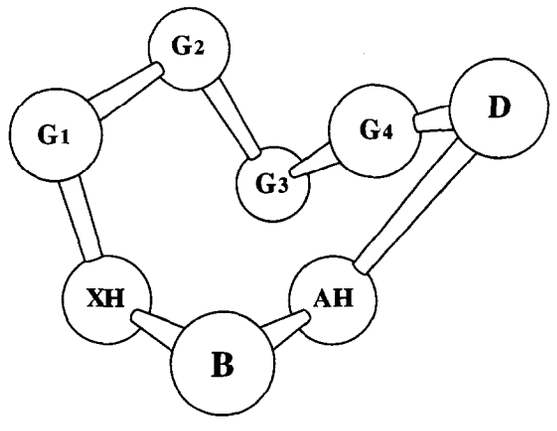


Fig. 2. Spatial arrangement of the eight interaction sites.

by the symbols a (for acceptor), d (for donor) and s (for steric); for an E1 interaction, for example, we can specify the dominant (or initial) interaction by the symbols E1a, E1d or E1s.

Subsites G1, G2, G3 and G4 are assumed to act on a recognition site by means of a punctual intermolecular steric interaction (Gs-type interaction). The groups involved are often non-polar or weakly polar small groups or atoms, such as a CH_3, a CH_2 or a CH group, or a fluorine atom. Polarizable large atoms, such as Cl or Br, can also operate effectively by acting on a recognition site through both a Gs and an Ea interaction. Note that, in the revised MPA theory, we consider the hydrophobic interaction concept as no longer valid to explain the interaction of sweeteners with the sweetness receptor. In our revised theory, the physicochemical concept of hydrophobic interaction is replaced by the structural, mechanical concept of a steric fit. We will therefore substitute for the term hydrophobic site with the more appropriate term steric interaction site.

Finally, site D, which is often a 4-cyanophenyl group, is considered to act essentially with the receptor via its H-bond acceptor group (CN); the hydrogen-bonding interaction is possibly reinforced with a π-stacking interaction of its electron deficient aromatic ring with the receptor.

Receptor recognition sites and subsites

According to the present revised MPA theory, the human sweetness receptor (which is very probably a seven-pass transmembrane receptor coupled to a G protein) is provided with eight fundamental recognition sites, namely, the recognition sites of the B, AH, XH, G1, G2, G3, G4 and D interaction sites, consisting of 15 fundamental recognition points, namely, the recognition points of the B1, B2, AH1, AH2, XH1, XH2, G1, E1, G2, E2, G3, E3, G4, E4 and D interaction points.

The B-recognition site is assumed to be the ϵ-ammonium group of the side-chain of a lysine residue; the B1- and B2-recognition subsites are two of the hydrogen atoms of the lysine ϵ-NH_3^+ group.

The AH- and XH-recognition sites are each considered to be the β-(or γ-)carboxylate group of an aspartate (or a glutamate) residue; the AH1- and AH2-recognition subsites are the two oxygen atoms of the bidentate carboxylate group of the AH-recognition site, and the XH1- and XH2-recognition subsites, the two oxygen atoms of the bidentate carboxylate group of the XH-recognition site.

The G1-, G2-, G3- and G4-recognition sites are assumed to consist of the side-chains (CH_3CHOH) of four threonine residues; in this hypothesis, the four β-OH groups of the threonine residues are the E1-, E2-, E3- and E4-recognition subsites, and the four methyl groups, the G1-, G2-, G3- and G4-recognition subsites.

Concerning the D-recognition site, we believe that it could be the side-chain of a serine (CH_2OH) or a threonine (CH_3CHOH) residue, the β-OH group being able to play the role of an H-bond donor group.

Activation of the receptor: a conformational change

We assume that the sweetness receptor, in its resting state (R state), must be in a contracted conformation (C conformation) as the result of reinforced ionic and H-bonding interactions occurring between several recognition sites (Fig. 3).

When a sweetener interacts with the receptor, this association must lead to the splitting of several of the reinforced ionic and H-bonding interactions connecting the recognition sites. This splitting triggers a profound conformational change of the receptor which passes from a C conformation to a more open expanded conformation (E conformation) regarded as the activated state (R* state) of the receptor (Fig. 4).

The approximate relative distances between the recognition sites in the expanded conformation of the receptor are given in Table 2.

In Table 2, we observe that distances AH–B, B–XH, XH–G1, G1–G2, G2–G3, G3–G4 and G4–AH are

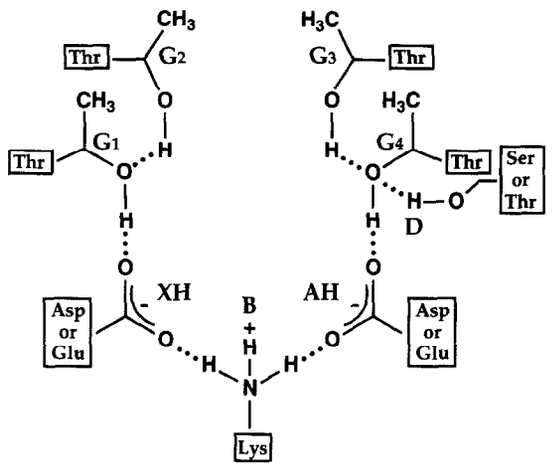


Fig. 3. Schematic representation of the sweetness receptor in its resting state.

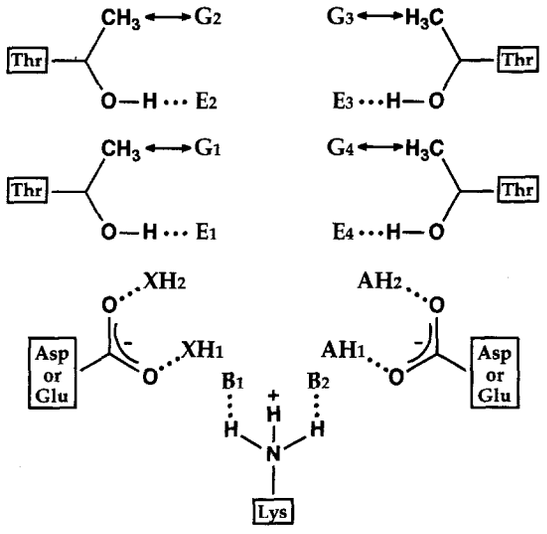


Fig. 4. Activated state of a part of the sweetness receptor after its interaction with a sweetener through 14 elementary interactions (sucrose for example).

Table 2. Approximate values (in nm) of distances between the recognition sites of the human sweetness receptor in its expanded conformation when sucrose is anchored to the receptor[a]

Sites	AH	XH	G1	G2	G3	G4	D
B	0.65	0.65	1.1	1.3	1.2	1.1	1.4
AH		0.75	1.2	1.1	0.65	0.65	0.95
XH			0.65	1.0	0.9	1.2	1.6
G1				0.65	1.0	1.1	1.55
G2					0.65	0.8	1.25
G3						0.65	1.1
G4							0.45

[a]The distances have been evaluated on a constructed CPK molecular model consisting of the putative side-chains of the recognition sites when they are in interaction with sucrose. The distances were measured between the centre of the ϵ-NH_3^+ nitrogen of lysine for the B-recognition site, of the CO_2^- carbon of aspartate (or glutamate) for the AH- and XH-recognition sites, and of the threonine (or serine) b-carbon for the G1-, G2-, G3-, G4- and D-recognition sites. In this working model, the location of the D-recognition site was only estimated, as sucrose does not have a D interaction site.

approximately equal (0.65 nm). These seven basic recognition sites (which are the recognition sites of the natural sweet carbohydrates) are arranged in space according to a skew heptagon with sides of about 0.65 nm (Fig. 5). In our laboratory, we name this arrangement the 'sweetness heptagon'. Sucrose, for example, is anchored to the receptor in accordance with this geometry.

The activation of the sweetness receptor by a sweetener is therefore the result of a concerted split of several intramolecular H-bonding or reinforced ionic interactions which bind together the recognition sites of the receptor in its resting state. The H-bond split is the consequence of an intermolecular transfer of the hydrogen bonds to acceptor sites of the sweetener. This transfer can also be induced or assisted by steric interactions of the sweetener on certain recognition sites of the receptor. For example, high-potency sweeteners generally function because of the presence (i) of high-affinity interaction sites (such as the CO_2^-, CN, ammonium or guanidinium groups) which are able to easily disrupt the hydrogen bridges linking the recognition sites, and (ii) of a rigid group (often a cycle) which is able, like a molecular wedge, to fit into the recognition sites (steric fit) and to push them apart.

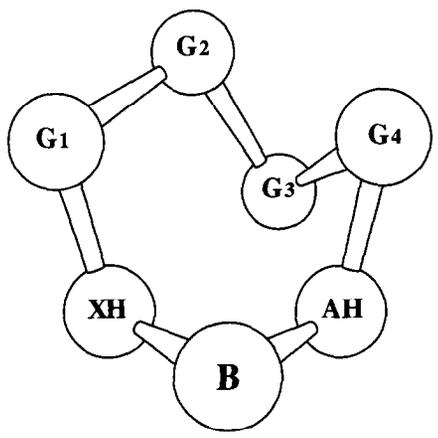


Fig. 5. Heptagonal arrangement ('sweetness heptagon') of the seven recognition sites (basic recognition sites) able to interact with sucrose.

Following the receptor activation (through its E conformation) and the concomitant sweet-taste triggering effect (through the coupled G protein and the associated biochemical cascade), the receptor expels the sweet molecule and returns to its resting state (C conformation) as a result of the electrostatic driving forces existing between the polar or ionic moieties of the recognition sites, which act as a molecular return spring.

ILLUSTRATION OF THE THEORY

Exploring and mapping the sweetness receptor

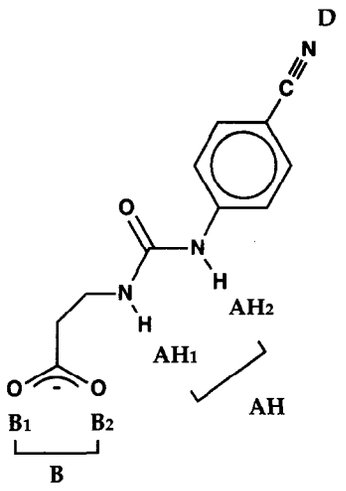
To explore and to map the different regions of the receptor, we will use selected sweeteners, all provided with two highly characteristic points, namely, site D made up of a 4-cyanophenyl group, and site B made up of a carboxylate group. These two chemically unambiguous points will be employed as 'molecular landmarks'. From these two reference points, we will be able to accurately locate the relative positions of the other previously identified or newly discovered points.

The B, AH region

To explore the B, AH region, with regard to site D, which is our main datum point, we will use cyanosuosan (**1**), a compound which is about 650× sweeter than sucrose on a weight basis relative to a 2% sucrose solution (Tinti *et al.*, 1981). In the present work, the sweetness potencies will always be given on a weight basis relative to a 2% sucrose solution, except where otherwise indicated.

This sweetener, which requires two free NH groups, is, according to our sweetener systemization, a typical B, AH, D-type sweetener, or more precisely a B1, B2, AH1, AH2, D-type sweetener. It must be remarked that, in the present theory, the ureido group interacts with the receptor in a Z, Z conformation which is considered as being the active conformation.

Besides its role of an H-bond acceptor group, the CN group also plays here the role of an electron-



650 x

1

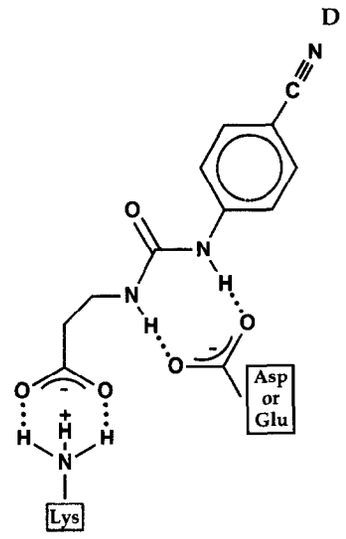
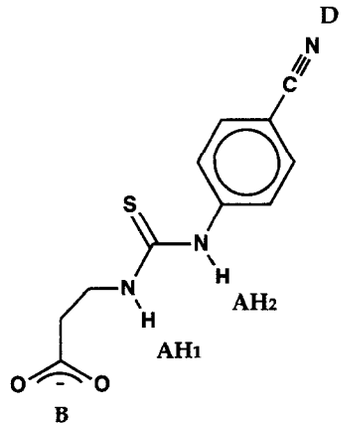


Fig. 6. Interactions of cyanosuosan with the B- and AH-recognition sites of the receptor.

withdrawing substituent which enhances the acidity of the two ureido-NH groups. Moreover, if we replace the oxygen atom of the ureido group by an electron-withdrawing substituent such as a sulphur atom, the resulting thiocyanosuosan (**2**) has a sweetness potency of about 2900× sucrose (Nofre & Tinti, 1993*a*). This activity enhancement is due to an additional increase in the acidity of the two H-bond donor subsites (AH1 and AH2), which favours their binding affinity to the receptor.



2 900 x

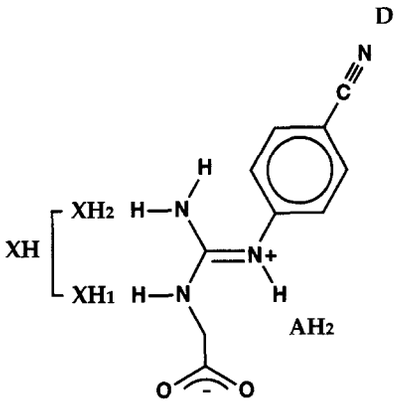
2

The AH-recognition site of the ureido (or thioureido) group of these sweeteners is a bidentate H-bond acceptor group, in other words a β- or γ-carboxylate group of a side-chain of an aspartate or a glutamate residue.

As we have already seen, the B-recognition site of the carboxylate group (site B) of these sweeteners is probably the ϵ-ammonium group of a lysine residue (Fig. 6).

The B, XH region

To explore this region, we will use 4-cyanophenylguanidineacetic acid (**3**) whose potency is about 2700× sucrose (Nofre *et al.*, 1989).



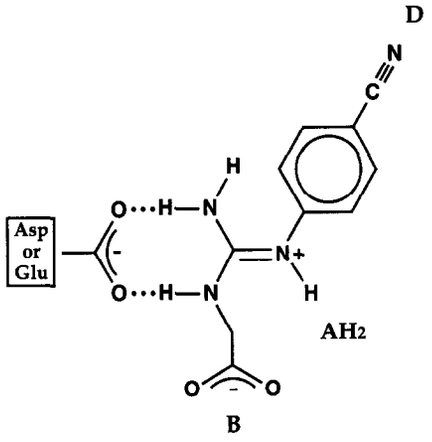
2 700 x

3

According to the conformation given in the above formula, which very probably represents the active conformation, this compound must interact with the AH-recognition site of the receptor by means of only one subsite (AH2), through a reinforced ionic interaction.

On the other side of the molecule, the interaction, which needs two free NH groups (XH1 and XH2), must logically use, here too, an XH-recognition site consisting of a carboxylate group belonging to an aspartate or a glutamate residue (Fig. 7).

In the resting (contracted) state of the receptor, the B-, AH- and XH-recognition sites form an ionic triad made up of a cationic ammonium group inserted between two anionic carboxylate groups, the whole linked together by means of reinforced ionic interactions (see Fig. 3).



B

Fig. 7. Interaction of 4-cyanophenylguanidineacetic acid with the XH-recognition site of the receptor.

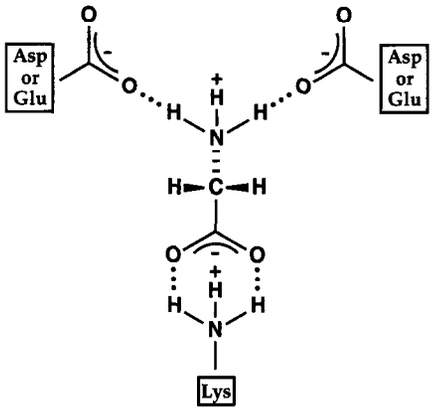
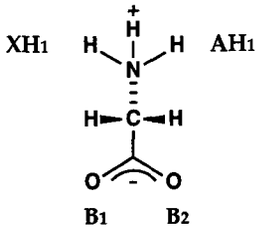


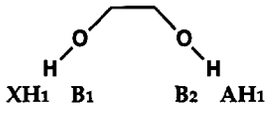
Fig. 8. Interaction of glycine (0.14× sucrose on a molar basis) with the ionic triad leading to a partial activation of the receptor.

We think that it is by splitting this 'sweetness ionic triangle' that 'simple' sweeteners, such as glycine (**4**) (see Fig. 8), 1,2-ethanediol (ethylene glycol) (**5**), erythritol (**6**), L-glucose (**7**) or D-glucose (**8**) (see Fig. 9), must work with the receptor.



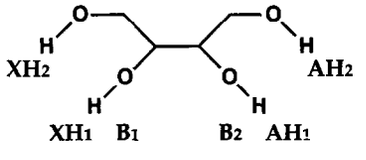
0.65 x

4



0.6 x

5



0.7 x

6

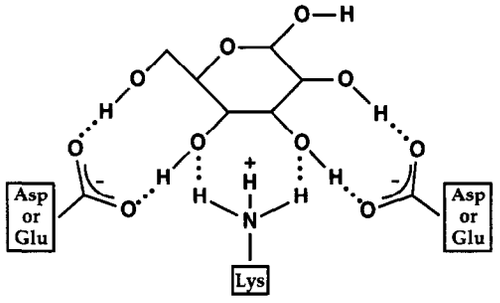
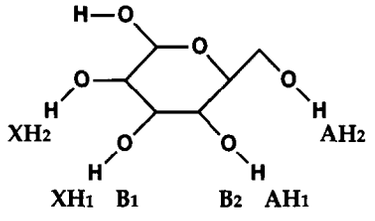
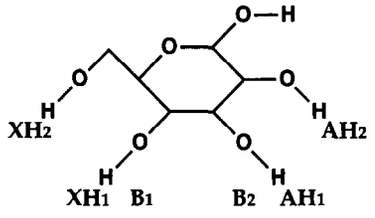


Fig. 9. Interaction of D-glucose (0.25× sucrose on a molar basis) with the ionic triad leading to a partial activation of the receptor.



0.5 x

7



0.5 x

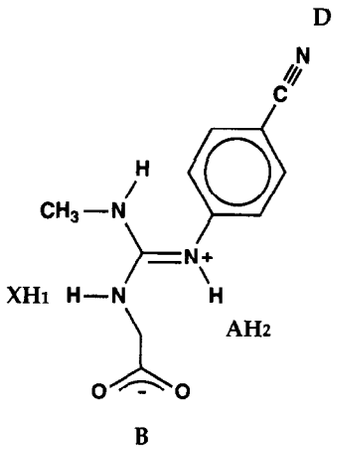
8

The G1E1 region

Compound **9**, which is an *N*-methyl derivative of **3**, is unsweet. Conversely, its superior homologue (**10**) is sweet, with a sweetness potency of about 350× sucrose (Nofre *et al.*, 1990). So the sweetness of this compound, which is the consequence of a simple change from a methyl to an ethyl group, is consistent with the presence of a steric interaction between the ethyl group and the receptor. We will designate this interaction as an E1s-type interaction.

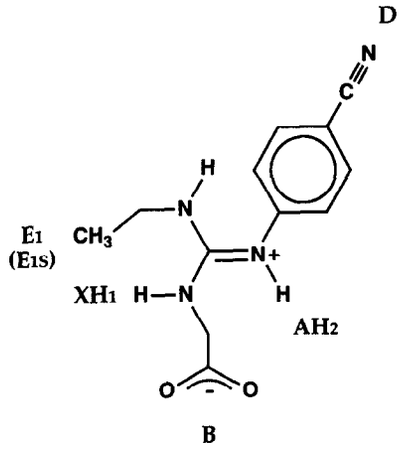
Furthermore, if, in the previous compound, we replace the ethyl group by a cyano group (**11**), a potent H-bond acceptor group, we observe a significant increase in sweetness, the new compound being about 7000× sweeter than sucrose (Nofre *et al.*, 1989). This fact is consistent with the existence, at this level, of an H-bond acceptor subsite which interacts with the receptor through an E1a-type interaction.

Another series of sweeteners (Muller *et al.*, 1991) also enables this G1E1 region to be explored. Compound **12**, for example, which is about 5000× sweeter than sucrose, is much more potent than its parent compound, cyanosuosan (**1**). We attribute this striking enhancement of activity to the presence of an E1s-type interaction.



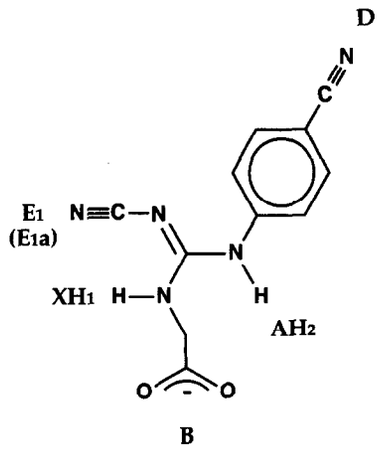
Not sweet

9



350 x

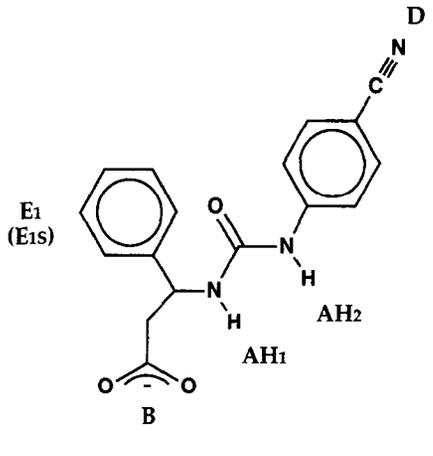
10



7 000 x

11

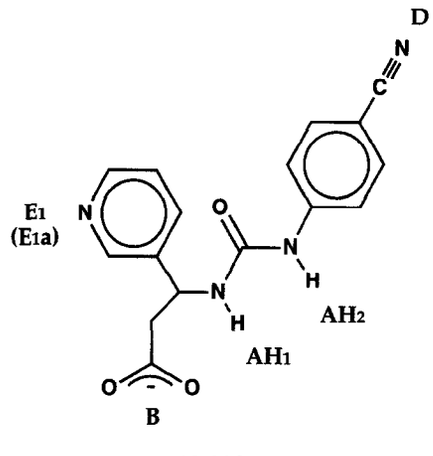
If, in the previous molecule, we replace the phenyl group by a 3-pyridyl group, the resulting compound (**13**) is about 10 000× sweeter than sucrose according to our own assessment (20 000× according to Muller). We explain this very significant rise in activity by the very potent H- bond acceptor role of the heterocyclic nitrogen atom.



5 000 x

12

Consequently, we consider this interaction as being due to an E1 subsite acting through an E1a-type interaction.



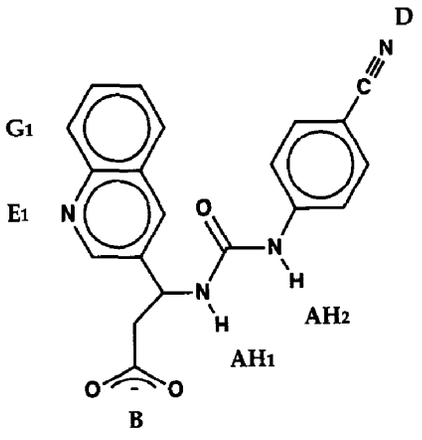
10 000 x

13

If, in the above molecule, we now substitute for the 3-pyridyl group a 3-quinolyl group (**14**), the sweetness potency of this molecule rises from 10 000× to about 15 000× sucrose according to our estimation (20 000× according to Muller). We assume that this marked increase in activity is due to a cooperative effect coming from a G1 subsite, acting on the receptor through a G1s-type interaction.

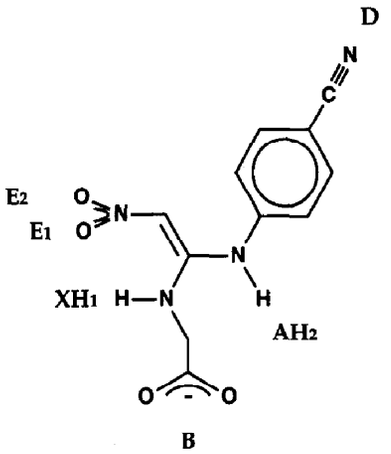
What is the nature of the G1-recognition site which is opposite subsites G1 and E1? We consider that the only compatible site, from a strictly geometric and functional viewpoint, is the 1-hydroxyethyl group of a threonine residue side-chain. This side-chain is probably bound, in the resting state of the receptor, to the adjacent carboxylate group of the XH-recognition site, through an H-bonding interaction (see Fig. 3).

In Fig. 10, the interaction between a threonine side-chain (initially linked to a carboxylate group of an XH-recognition site in the resting state of the receptor) and a moiety of the 3-quinolyl group of compound **14** is given as an example of an interaction between a G1-recognition site and two interaction subsites, G1 (G1s) and E1 (E1a).



15 000 x

14



9 000 x

15

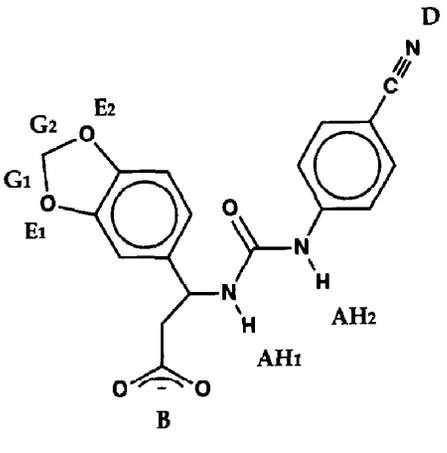
Since we assume that all the GE-type regions are structurally identical in man, we will explore the remaining regions much more rapidly.

The G2E2 region

To explore and map the E2 area in relation to the border G1E1 region, our first example is compound **15**, which is about 9000× sweeter than sucrose (Nofre *et al.*, 1989). We assume that the nitro group (which must here be roughly perpendicular to the plane of the molecule as a result of its intramolecular steric interaction with the neighbouring XH1-amino group) is able to interact with the receptor through an E1a- and an E2a-type interaction. This is consistent with the fact that this compound is much more active than its analogue **11**.

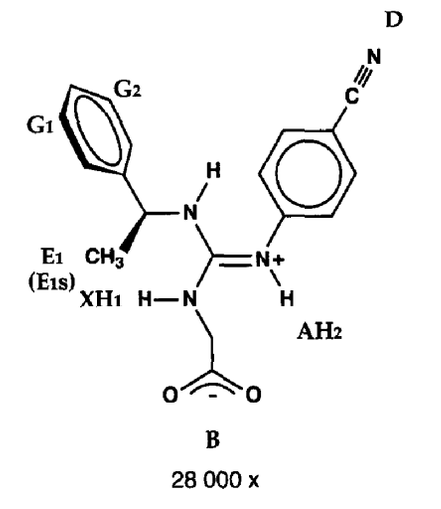
Compound **16** (Muller *et al.*, 1991) has a sweetness potency of about 15000× sucrose according to our assessment (25000× according to Muller). This enhancement of activity with regard to its base compound **12** appears to be due to four supplementary interactions of types E1a, G1s, E2a and G2s.

Compound **17**, a phenyl derivative of compound **10**, has a potency of 28000× (Nofre *et al.*, 1990). This increase in activity, as compared with compound **10**, is attributed to the appearance of two additional steric interactions, the G1s- and G2s-type interactions.



15 000 x

16



28 000 x

17

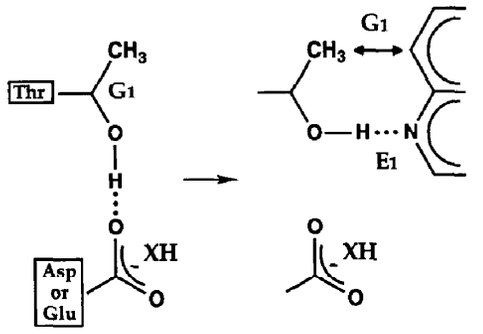
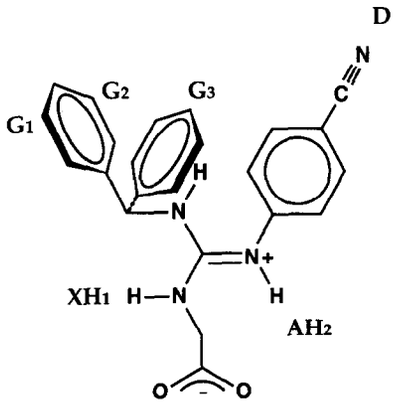


Fig. 10. Interaction between the side-chain of a threonine residue of the receptor (G1-recognition site) and the 3-quinolyl group of compound **14**.

For this G2E2 region, just as for the G1E1 region and exactly for the same reasons, we think that the G2-recognition site is the side-chain of a threonine residue, which, in the resting form of the receptor, must be linked, by means of a hydrogen bond, to the adjacent G1-recognition site (see Fig. 3).

The G3E3 region

If, in compound **17**, we replace the methyl group by a phenyl group, we obtain a compound (**18**), that we have named sucrodiphenate, which is about 150 000× sweeter than sucrose (Nofre & Tinti, 1993*a*). We interpret this result as being due to an additional G3s-type interaction.



B

150 000 x

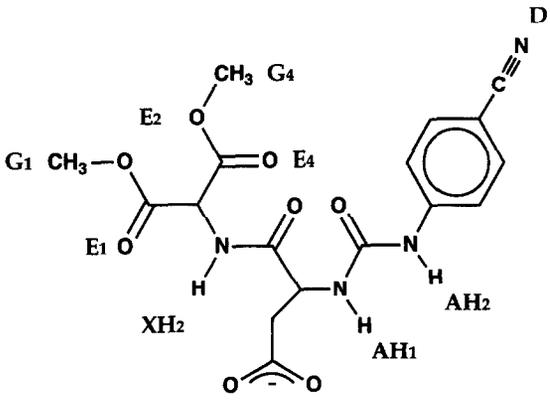
18

We also believe that sucrose possesses a G3 subsite associated with an E3 subsite.

Finally, the G3-recognition site is probably, here too, the side-chain of a threonine residue, which could be attached, in the resting state of the receptor, to the next G4-recognition site, through a hydrogen bond (see Fig. 3).

The G4E4 region

Compound **19**, which has a sweetness potency of about 7000× (Nofre and Tinti, unpublished result), is an example of a molecule possessing both an E4 and a G4 subsite.

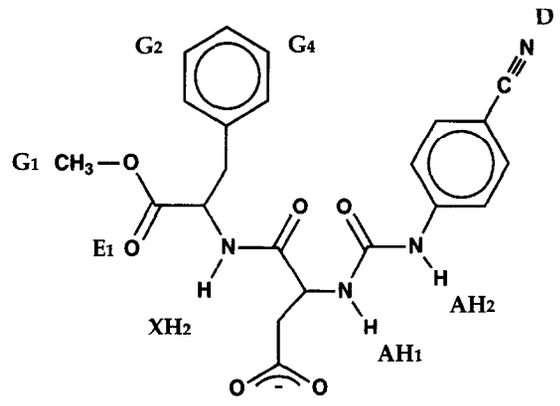


B

7 000 x

19

Superaspartame (**20**), which has a sweetness potency of about 8000× (Nofre & Tinti, 1987*a*), is an example of a sweetener provided with three steric interaction subsites, namely, the G1, G2 and G4 interaction subsites.

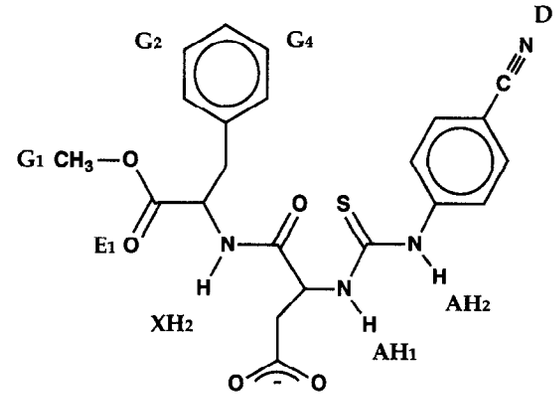


B

8 000 x

20

Note that the replacement of the ureido oxygen atom in superaspartame with a sulphur atom raises the activity by a factor of about 5, the sweetness potency of the resulting compound, thiosuperaspartame (**21**), being about 40 000× sucrose (Nofre & Tinti, 1987*a*). This enhancement of activity is due to an increase in the acidity of the ureido-NH groups under the influence of the sulphur atom, as was already the case for cyanosuosan (**1**) and thiocyanosuosan (**2**).



B

40 000 x

21

Just like the recognition sites of the G1, G2 and G3 interaction sites, and for the same reasons, we assume that the G4-recognition site is a threonine residue side-chain, which, in the resting state of the receptor, is linked to the adjacent carboxylate group of the AH-recognition site, through an H-bonding interaction (see Fig. 3).

So according to the present MPA theory, the G1-, G2-, G3- and G4-recognition sites constitute, through the side-chains of the threonine residues, an amphiphilic tetrad. On this assumption, we think for example that

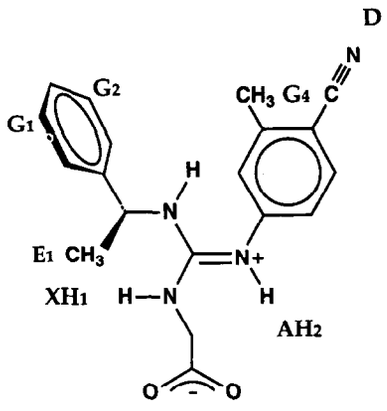
D-fructose (1× sucrose on a weight basis, 0.5× on a molar basis) is an E1(4-OH), E2(3-OH), E3(2-OH), E4(1-OH) sweetener.

We assume that it would be by splitting both the 'sweetness amphiphilic skew quadrilateral' of the amphiphilic tetrad and the 'sweetness ionic triangle' of the ionic triad that certain 'complex' sweeteners, such as sucrose or sweet dipeptides and analogues, would operate with the receptor. For example, aspartame (180× sucrose) is, according to the revised MPA theory, a B1, B2, AH1, XH1, XH2, G1, E1, G2, G4 sweetener in its extended conformation (for a discussion about the active conformation of aspartame, see Glaser *et al.*, 1995); alitame (2000× sucrose), a B1, B2, AH1, XH1, XH2, G1, G2, G3, G4 sweetener in its L-shaped conformation (for a discussion about the active conformation of alitame, see Glaser *et al.*, 1995); and L-aspartyl-DL-aminomalonic acid β-(+)-fenchyl methyl diester (50 000 × sucrose), a B1, B2, AH1, XH1, XH2, G1, E1, G2, E2, G3, G4, E4 sweetener. In the last two sweeteners, note that the steric interaction site (the 'hydrophobic site') of these sweeteners is a G1, G2, G3, G4-steric tetrad which must be easily trapped into the four-methyl crown formed by the G1, G2, G3, G4-steric cavity (or steric pocket) of the receptor. For these sweeteners, the G1, G2, G3, G4-steric cavity of the receptor is in fact a very efficient molecular trap, which we name the steric trap or the steric cage.

The D region

Site D is very often, in our molecules, a CN group attached to an aromatic ring, such as a 4-cyanophenyl group. This site, which is an essential feature of 'sophisticated' high-potency sweeteners, was identified in 1982 (Tinti *et al.*, 1982). Site D being our main reference point for exploring the receptor, this site is obviously of little value for mapping the D region itself. We know only that CN is a potent H-bond acceptor and that it is close to the G4 interaction site. For example, if we introduce a 3-methyl or a 3-chloro substituent on the 4-cyanophenyl group of compound **17**, the sweetness potency of compound **17** rises from 28 000× sucrose to 50 000× (**22**) (Nofre *et al.*, 1990) and 125 000× sucrose (**23**), respectively (Nofre and Tinti, unpublished work). In both these compounds, the methyl and the chloro substituents behave as a G4 interaction site (G4s-type interaction for CH_3 and G4s/E4a-type interaction for Cl), which explains the potency observed with these molecules.

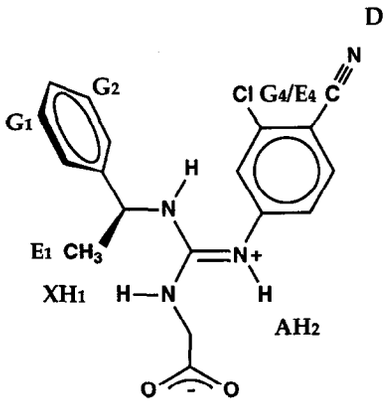
It is interesting to note that, in compound **17** for example, we can replace the 4-cyanophenyl group by a 3,5-dichlorophenyl group (**24**) which has a sweetness potency of about 120 000× sucrose (Nofre *et al.*, 1990). In order to explain the high potency of this molecule, we believe that its 5-chloro substituent could interact with the receptor through an additional steric interaction (via a putative G5-recognition site which could be the phenyl group of a phenylalanine residue close to the D-recognition site).



B

50 000 x

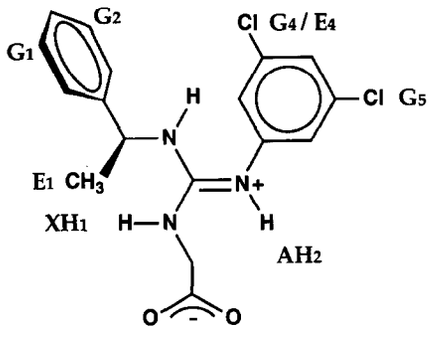
22



B

125 000 x

23

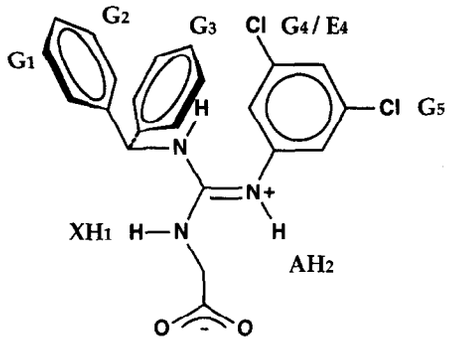


B

120 000 x

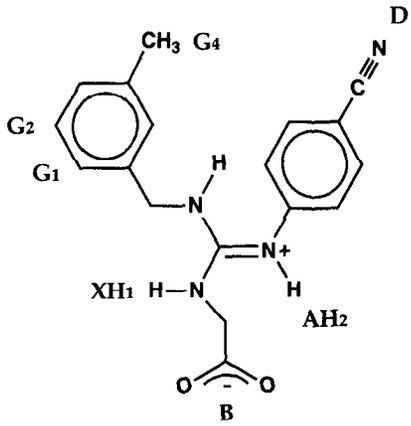
24

In the same way, if we perform the same replacement in compound **18**, we obtain a compound (**25**), that we name carrelame, whose sweetness potency is about 160 000× sucrose on a weight basis (200 000× on a molar basis) (Nofre and Tinti, unpublished work). Carrelame is, on a molar basis, one of the four sweetest compounds invented to date (with compounds **26**, **27** and **28**).



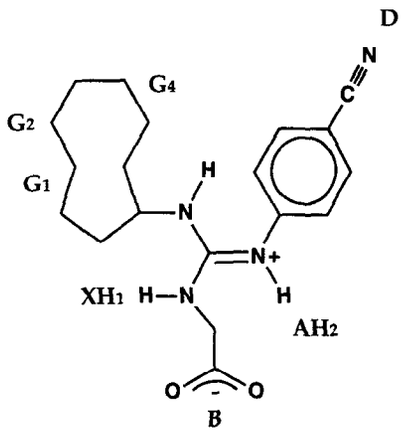
160 000 x

25



200 000 x

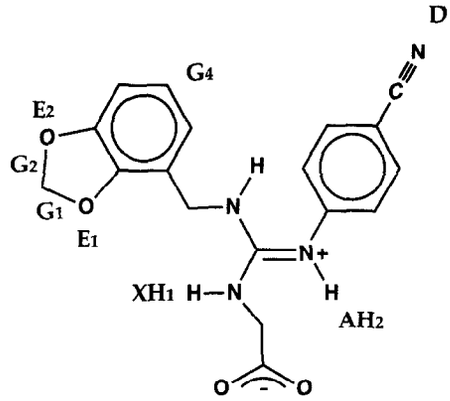
26



200 000 x

27

We assume that the D-recognition site is the side-chain of a serine (or threonine) residue, which could be attached, through a hydrogen bond, to the adjacent G4-recognition site in the resting state of the receptor. Furthermore, we think that there may exist, near the D-recognition site, a G5-recognition site (a phenyl group belonging to a contiguous phenylalanine residue),



225 000 x

28

which could adjust and even reinforce the interaction between the receptor and the D-interaction site of the sweetener through a π-stacking interaction.

COMPREHENSIVE MODEL

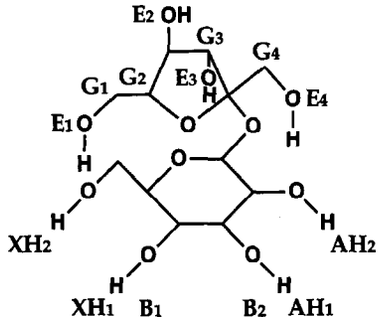
The sweetness receptor model that we have developed is a very comprehensive model since it enables us to explain how all sweeteners, from hyperpotent to low-potency sweeteners, can operate with the receptor.

For example, bernardame (**26**) (Nofre and Tinti, unpublished work) or sucrononate (**27**) (Nofre *et al.*, 1990) have a sweetness potency of 200 000× sucrose on a weight basis (190 000 and 200 000×, respectively on a molar basis). These two compounds are, on a weight basis, among the three most potent sweeteners found to date (with compound **28**). The high potency of these molecules, which are B1, B2, AH2, XH1, G1, G2, G4, D-type sweeteners, is particularly due to the presence of three very operative steric interaction subsites (G1, G2 and G4) which fit perfectly to the related G1, G2, G4-steric triad of the receptor.

Another example of a hyperpotent sweetener is given by lugduname (**28**) (Nofre and Tinti, unpublished work), which is about 225 000× sweeter than sucrose on a weight basis (230 000× on a molar basis). This sweetener is the finest piece of our collection, since it is the sweetest compound described to date. According to our sweetener systemization, this compound is a B1, B2, AH2, XH1, G1, E1, G2, E2, G4, D-type sweetener. The very high potency of this sweetener is explained by its interaction not only with the G1, G2, G4-steric triad of the receptor, but also with the E1- and E2-recognition subsites.

And finally, how does sucrose work? According to the present revised MPA theory, sucrose (**29**), which is the most widely distributed sweet compound in the plant kingdom, is a B1, B2, AH1, AH2, XH1, XH2, G1, E1, G2, E2, G3, E3, G4, E4-type sweetener. In other words, it interacts with the receptor through 14 elementary interactions. This multiplicity of interactions explains

the high selectivity of the sweetness receptor towards sucrose with regard to many other natural compounds. Its low potency is due to the absence of D and ionic interaction sites, to the low affinity of its polar interaction subsites (hydroxyl groups) as hydrogen-bond acceptor or donor groups, and to the low efficiency of its steric interaction subsites.



1 x

29

Trihalo- or tetrahalodeoxysucroses, such as sucralose (4,1′,6′-trichlorogalactosucrose) (650× sucrose) or 4,1′,4′,6′-tetrabromogalactosucrose (7500× sucrose) (for reviews see, for example, Jenner, 1991 or Hough & Khan, 1993), which are B, AH1, AH2, XH2, G1, E1, G2, E2, G3, E3, G4, E4-type sweeteners, are examples of sucrose derivatives whose potency enhancement is essentially explained by an improvement in the B (C-4) interaction and in their G1, G4 (C-6′, C-1′) or G1, G2, G4 (C-6′, C-4′, C-1′) steric fit on to the receptor.

Finally, the present sweetness receptor model strongly argues in favour of the existence of only one type of sweetness receptor in man.

ACKNOWLEDGEMENTS

The authors wish to thank The NutraSweet Company, Deerfield, Illinois, for financial support, and Anne-Marie Peytavi, Edith Juvin, Pascale Georjon and Odile Sarcelle, for their contributions to this work.

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