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# Gustatory responses of non-human primates to dipeptide derivatives or analogues, sweet in man

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Dipeptide derivatives or analogues, sweet in man, can be divided into three classes according to their gustatory responses in primates: (i) dipeptides which are sweet to all primates (prosimians, New World simians and Old World simians), such as alitame or L-aspartyl-D-alanine propyl ester (class I); (ii) dipeptides which are sweet to prosimians and Old World simians, but not to New World simians, such as L-aspartyl-(*R*)-α-methylphenethylamine or L-aspartyl-L-(*O-tert*-butyl)serine methyl ester (class II); and, (iii) compounds which are sweet only to Old World simians, but not to prosimians and New World simians, such as aspartame (class III). Analysis of these results by means of the multipoint attachment (MPA) theory of sweetness reception (Nofre & Tinti, 1996) leads to the conclusion that the seven basic recognition sites of the sweetness receptor, as inferred from the MPA theory, are (i) in prosimians: Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Ala-5 or Ser-5, Thr-6, Thr-7; (ii) in New World simians: Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Ala-5 or Ser-5, Ala-6 or Ser-6, Thr-7; and, (iii) in Old World simians: Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Thr-5, Thr-6, Thr-7.


## INTRODUCTION

At the first 'ECRO Sweetness Symposium' in Reims, France, Glaser (1993) reported significant variations in gustatory responses of non-human primates to several natural or non-natural compounds well-known to be sweet in humans, namely, sucrose, neohesperidin dihydrochalcone, stevioside, monellin, thaumatin, D-tryptophan, and aspartame (Fig. 1a), the first known sweet-tasting dipeptide which has a sweetness potency of about 180 times that of sucrose on a weight basis in man. These compounds were divided into four classes according to their responses in non-human primates: (1) compounds which are sweet to all primates; (2) compounds which are sweet to Hominoidea, Cercopithecoidea and Ceboidea, but which induce varied responses in Prosimii; (3) compounds which are sweet to Hominoidea and Cercopithecoidea, but which induce varied responses in Ceboidea and Prosimii; and, (4) compounds which are sweet to Hominoidea and Cercopithecoidea, but which are unsweet to Ceboidea and Prosimii.

The pronounced structural differences existing between the above compounds make it impossible to determine the molecular features responsible for the varied responses observed in primates.

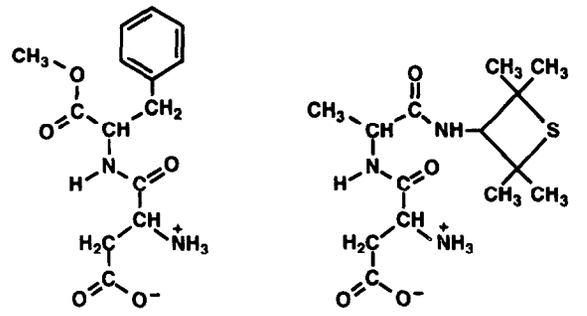
Recently, we observed that alitame (Fig. 1b), another dipeptide derivative (which is about 2000 times sweeter than sucrose in man and which is also based, like aspartame, on L-aspartic acid), is able to induce a sweet taste in *all* primates investigated, i.e. in Old World simians (Catarrhini), like aspartame, but also in New World simians (Platyrrhini) and prosimians (Prosimii) in which aspartame does not taste sweet (Glaser *et al.*, 1995).

In order to try to understand the molecular reasons for the differences observed between aspartame and alitame in primate responses, six representative dipeptide derivatives or analogues, all based on L-aspartic acid and all able to induce a sweet taste in man, were tested on 24 selected non-human primate species or subspecies.

## MATERIALS AND METHODS

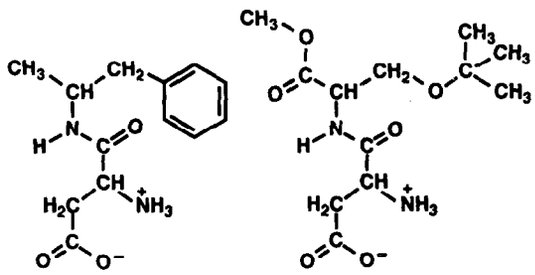
### Chemicals

The six representative dipeptide derivatives or analogues studied in the present work are as follows. *N*-α-L-aspartyl-D-serine propyl ester (ASPE; Ariyoshi *et al.*,



a b

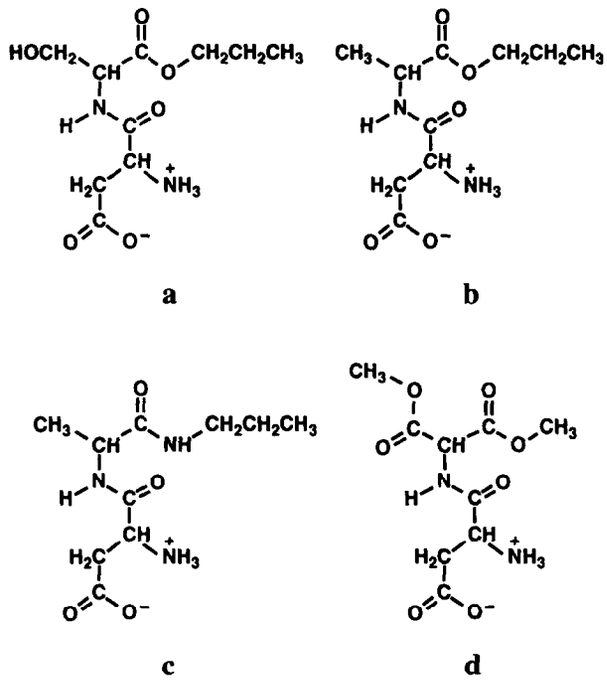
Fig. 1. (a) Aspartame (APM) and (b) alitame (ALT).



a b

Fig. 3. (a) Ampame (AMPA) and (b) ASME.

1974), a dipeptide derivative 320 times sweeter than sucrose in man, was tested in primates at a concentration of 0.4 g litre$^{-1}$ (Fig. 2a). *N*-α-L-aspartyl-D-alanine propyl ester (AAPE; Mazur *et al.*, 1973), a dipeptide derivative 170 times sweeter than sucrose in man, was tested in primates at a concentration of 0.4 g litre$^{-1}$ (Fig. 2b). *N*-α-L-aspartyl-D-alanine propylamide (AAPA; Ariyoshi, 1977), a dipeptide derivative 25 times sweeter than sucrose in man, was tested in primates at a concentration of 5.2 g litre$^{-1}$ (Fig. 2c). *N*-α-L-aspartyl-aminomalonic acid dimethyl diester (AADD), a dipeptide derivative 15 times sweeter than sucrose in man, was tested at a concentration of 8.8 g litre$^{-1}$ and was prepared according to the general procedure described by Fujino *et al.* (1976) (Fig. 2d). *N*-α-L-aspartyl-(*R*)-α-methylphenethylamine (ampame or AMPA; Mazur *et al.*, 1970), a dipeptide analogue 50 times sweeter than sucrose in man, was tested in primates at a concentration of 1.2 g litre$^{-1}$ (Fig. 3a). *N*-α-L-aspartyl-L-(*O*-*tert*-butyl)serine methyl ester (ASME; Dahlmans & Boesten, 1972; Brussel *et al.*, 1975), a dipeptide derivative 140 times sweeter than sucrose in man, was tested in primates at concentrations of between 0.6 and 1.8 g litre$^{-1}$ (Fig. 3b).



a b c d

Fig. 2. (a) ASPE, (b) AAPE, (c) AAPA and (d) AADD.

These compounds were prepared according to the literature cited above. The sweetness potencies indicated are the values reported by the original authors; the sweetness potency of AADD, a compound not yet described, is given relative to a 2% sucrose solution on a weight basis.

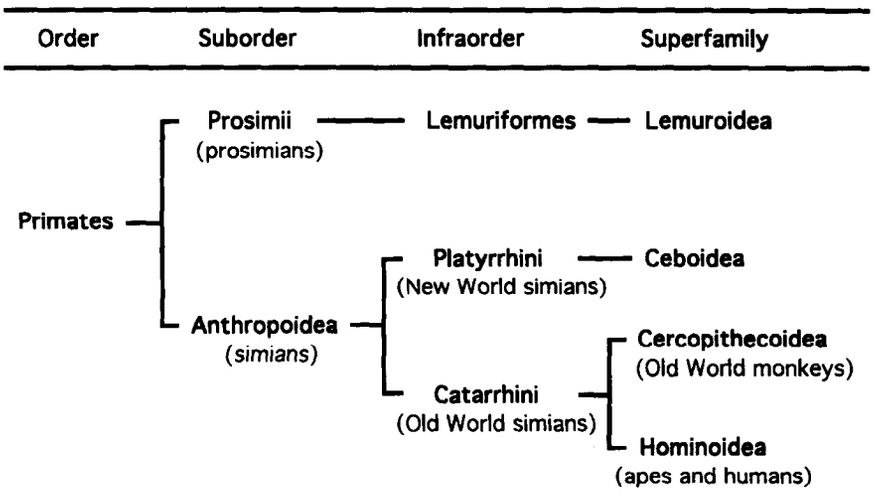



Fig. 4. Simplified classification of the primates used in the present work.

The concentrations previously used for aspartame (APM) were between 0.14 and 2.2 g litre$^{-1}$ (Glaser *et al.*, 1992), and for alitame (ALT), 0.1 g litre$^{-1}$ (Glaser *et al.*, 1995).

### Animals

The primate species or subspecies were selected from four superfamilies: Lemuroidea; Ceboidea; Cercopithecoidea; and Hominoidea (see Fig. 4 for a simplified classification of the primates used in the present study).

In the superfamily Lemuroidea (from Madagascar), we used *Lemur catta* (ring-tailed lemur; one male, five females), *Eulemur mongoz* (mongoose lemur; four males), *Eulemur macaco flavifrons* (Sclater's lemur; three males, two females), *Eulemur fulvus albifrons* (white-fronted brown lemur; one male, one female), *Eulemur coronatus* (crowned lemur; two males, two females), *Eulemur rubriventer* (red-bellied lemur; two males, three females), *Varecia variegata variegata* (black-ruffed lemur; three males, one female) and *Varecia variegata rubra* (red-ruffed lemur; two males, three females).

In the superfamily Ceboidea (South and Central America), we used *Cebuella pygmaea* (pygmy marmoset; one male, one female), *Callithrix jacchus jacchus* (common marmoset; four males, four females), *Leontopithecus rosalia chrysomelas* (golden-headed tamarin; three males, two females), *Callimico goeldii* (Goeldi's monkey; four males, three females), *Aotus trivirgatus* (owl or night monkey; three males, two females), *Cebus apella xanthosternos* (yellow-breasted capuchin monkey; five males) and *Saimiri sciureus* (common squirrel monkey; ten males, four females).

In the superfamily Cercopithecoidea, we used *Macaca nigra* (Celebes black macaque; one male, five females), *Cercopithecus diana roloway* (Roloway guenon; one female), *Cercopithecus preussi* (Preuss's guenon; one female) and *Allenopithecus nigroviridis* (Allen's swamp monkey; one female).

In the superfamily Hominoidea, we tested *Hylobates pileatus* (pileated gibbon; two males, one female), *Hylobates syndactylus* (siamang; two males, one female), *Pongo pygmaeus abelii* (Sumatra orang-utan; two males, seven females), *Gorilla gorilla gorilla* (western lowland gorilla; one male, four females) and *Pan troglodytes troglodytes* (common chimpanzee; two males, two females).

In total, 24 non-human primate species or subspecies were used to test the responses to the six already-mentioned dipeptide derivatives or analogues related to aspartame and alitame.

### Methods

In this study, two complementary behavioural tests were used, the taste-induced hedonic modifications of facial expressions and the two-bottle preference test.

Taste-induced facial expressions clearly show that sweet gustatory stimuli trigger behavioural responses which truly mirror 'hedonic aspects' of gustatory experience (Steiner & Glaser, 1984, 1995): sampling-sipping, lapping or eager drinking, quick swallow, mouth open, lips apart, sucking-smacking, head oriented towards stimulus. These behaviour patterns are clearly differentiable from those triggered by other qualities or simply by tap water (e.g. mouth corners down, spitting, head turn/head shake, gaping, head withdrawal from stimulus). Nearly all primates tested showed these patterns and this behaviour is not species-specific.

The two-bottle preference test, combined with the preceding behavioural observations, was employed to confirm preference for (+), no response to or avoidance of (−) the test solution against tap water. The smaller sized animals were offered the choice of two bottles attached to the cage. The medium-sized animals were provided with two larger drinking bowls, which were placed inside the cages. The Hominoidea (Hylobatidae and Pongidae) were tested with the aid of their usual drinking mugs. So all animals were able to choose between the dipeptide solution and tap water. We randomly changed the side of the dipeptide solution offered. The tests started early in the morning, and the animals had been deprived of fluid intake since the evening before. Each animal was therefore in a thirsty condition. Finally, the intake of the dipeptide solution vs water was measured and compared.

The two complementary behavioural tests were used in all cases, except for a shy and nocturnal animal (*A. trivirgatus*) where only a two-bottle preference test was employed.

All these gustatory studies were made in the Zoological Garden of Zürich (9 species), in the Parc Zoologique et Botanique of Mulhouse (13 species), in the primate facility of Hoffman-LaRoche, Basel (one species) and in the Anthropological Institute of the University of Zürich-Irchel (4 species). In some cases, as a control, experiments were duplicated at two different facilities with four species (*L. catta*, *V. variegata rubra*, *L. rosalia chrysomelas* and *C. goeldii*).

## RESULTS

While all the tested dipeptide derivatives or analogues display a sweet taste in humans, the results reported in Table 1, in which we have included aspartame and alitame, clearly indicate that these compounds can be divided into three different classes according to their gustatory responses in primates.

Class I (alitame-like compounds), comprising alitame (ALT), ASPE, AAPE, AAPA and AADD, corresponds to compounds which, like alitame, elicit a sweet taste in all the primates tested so far, in Lemuroidea, Ceboidea, Cercopithecoidea and Hominoidea.

Class II (ampame-like compounds), consisting of ampame (AMPA) and ASME, corresponds to compounds which induce a sweet taste in Lemuroidea, Cercopithecoidea and Hominoidea, but which do not give a response in Ceboidea.

Class III (aspartame-like compounds) is represented

**Table 1. Compared taste responses to dipeptide derivatives or analogues in primates**

| | Class I | | | | | Class II | | Class III |
|---|---|---|---|---|---|---|---|---|
| | ALT | ASPE | AAPE | AAPA | AADD | AMPA | ASME | APM |
| Prosimii | | | | | | | | |
| Lemuroidea | | | | | | | | |
| *Lemur catta* | + | + | + | + | + | + | + | – |
| *Eulemur mongoz* | + | + | + | + | + | ± | + | – |
| *Eulemur macaco flavifrons* | + | + | + | + | + | + | + | – |
| *Eulemur fulvus albifrons* | + | + | + | + | + | + | + | – |
| *Eulemur coronatus* | + | + | + | + | + | + | + | – |
| *Eulemur rubriventer* | + | + | + | + | + | + | + | – |
| *Varecia variegata variegata* | + | + | + | + | + | + | + | – |
| *Varecia variegata rubra* | + | + | + | + | + | + | + | – |
| Anthropoidea | | | | | | | | |
| Ceboidea | | | | | | | | |
| *Cebuella pygmaea* | + | + | + | + | + | – | – | – |
| *Callithrix jacchus jacchus* | + | + | + | + | + | – | – | – |
| *Leontopithecus rosalia chrysomelas* | + | + | + | + | + | – | – | – |
| *Callimico goeldii* | + | + | + | + | + | – | – | – |
| *Aotus trivirgatus* | + | | | | | – | | – |
| *Cebus apella xanthosternos* | + | + | + | + | + | – | – | – |
| *Saimiri sciureus* | + | + | + | + | + | – | – | – |
| Cercopithecoidea | | | | | | | | |
| *Macaca nigra* | + | + | + | + | + | + | + | + |
| *Cercopithecus diana roloway* | + | + | + | + | + | + | + | + |
| *Cercopithecus preussi* | + | + | + | + | + | + | + | + |
| *Allenopithecus nigroviridis* | + | + | + | + | + | + | + | + |
| Hominoidea | | | | | | | | |
| *Hylobates pileatus* | + | + | + | + | + | + | + | + |
| *Hylobates syndactylus* | + | + | + | + | + | + | + | + |
| *Pongo pygmaeus abelii* | + | + | + | + | + | + | + | + |
| *Gorilla gorilla gorilla* | + | + | + | + | + | + | + | + |
| *Pan troglodytes troglodytes* | + | + | + | + | + | + | + | + |
| *Homo sapiens sapiens* | + | + | + | + | + | + | + | + |

by aspartame (APM), which, like thaumatin (a sweet protein of 207 amino acids), elicits a sweet taste in Cercopithecoidea and Hominoidea, but which does not give a response in Prosimii and Ceboidea.

## DISCUSSION

If we consider only the capability of inducing a sweet taste in man, the family of the L-aspartic acid-based dipeptide derivatives or analogues may seem homogeneous. In fact, if we take into account the responses of these compounds in all the primates experimented on, our results demonstrate the existence of three types of gustatory response, which suggests the presence of three different interaction modes in primates.

What molecular features can be behind these three types of response in primates?

In previous work (Glaser *et al.*, 1995), we explained the differences of gustatory responses between aspartame and alitame in primates as being due to the presence, in the sweetness receptor of Old World simians (Cercopithecoidea and Hominoidea), of two different 'hydrophobic recognition sites', one (designated $G_{APM}$) able to recognize the 'hydrophobic' group of aspartame (i.e. its phenyl group), the other (designated $G_{ALT}$) able to recognize the 'hydrophobic' group (the tetramethylthietanyl group) of alitame; in prosimians and in New World simians (Ceboidea), only the 'hydrophobic recognition site' of alitame ($G_{ALT}$) is present. In order to explain the coexistence in Old World simians of both the recognition sites of alitame and aspartame, it was necessary to assume that alitame and aspartame must interact with the receptor via two different active conformations: alitame in an L-shaped conformation, and aspartame in an extended conformation (Glaser *et al.*, 1995).

The present work shows the existence in prosimians (Lemuroidea) and in Old World simians of an additional 'hydrophobic recognition site' (designated $G_{AMPA}$), which is lacking in New World simians (Ceboidea; see Table 2). This site is able to interact with the 'hydrophobic' group (the phenyl group) of ampame (AMPA) or with that (the *tert*-butyl group) of ASME.

What is the possible nature of these three different 'hydrophobic recognition sites' and their arrangement in the sweetness receptor? It is first necessary to recall some essential topics concerning the multipoint attachment (MPA) theory of sweetness reception as developed by two of us (Nofre & Tinti, 1996).

**Table 2. Distribution of the three identified 'hydrophobic recognition sites' in primate sweetness receptors**

| | $G_{APM}$ | $G_{AMPA}$ | $G_{ALT}$ |
|---|---|---|---|
| Lemuroidea | – | + | + |
| Ceboidea | – | – | + |
| Cercopithecoidea | + | + | + |
| Hominoidea | + | + | + |

According to the MPA theory, which is based on an extensive study of the structure–activity relationships of various sweeteners in man, it appears that the human sweetness receptor, which is probably a seven-pass transmembrane receptor, is formed of seven 'basic recognition sites', which are the seven sites capable of recognizing sucrose. These seven recognition sites (Fig. 5) are, in man, made up of the side-chain of an aspartate (Asp-1) or glutamate (Glu-1) residue (through their $\beta$- or $\gamma$-$CO_2^-$ group); of the side-chain of a lysine residue (Lys-2; through its $\epsilon$-$NH_3^+$ group), of the side-chain of an aspartate (Asp-3) or glutamate (Glu-3) residue (through their $\beta$- or $\gamma$-$CO_2^-$ group); and of the side-chains of four threonine residues (Thr-4, Thr-5, Thr-6 and Thr-7; through the OH and $CH_3$ groups of their $CHOHCH_3$ side-chains). These seven recognition groups (the 1-$CO_2^-$, the 2-$NH_3^+$, the 3-$CO_2^-$, and the four 4-, 5-, 6- and 7-$CHOHCH_3$ groups) are approximately arranged in space according to a *skew heptagon* (the 'sweetness heptagon') with sides of about 0.65 nm in the activated state of the receptor (Fig. 5).

These seven basic recognition sites are each formed of two recognition subsites. These 14 recognition subsites (or recognition points) were named by reference to the names given to the various interaction points of the sweeteners with the receptor; we could thus identify the AH1- and AH2-subsites (corresponding to the bidentate $CO_2^-$ group of Asp-1 or Glu-1), the B1- and B2-subsites (corresponding to the $NH_3^+$ group of Lys-2), the XH1- and XH2-subsites (corresponding to the $CO_2^-$ group of Asp-3 or Glu-3), the E1-, E2-, E3- and E4-subsites (corresponding to the OH group of Thr-4, Thr-5, Thr-6 and Thr-7, respectively), and the G1-, G2-, G3- and G4-subsites (corresponding to the $CH_3$ group of Thr-4, Thr-5, Thr-6 and Thr-7, respectively; Fig. 5).

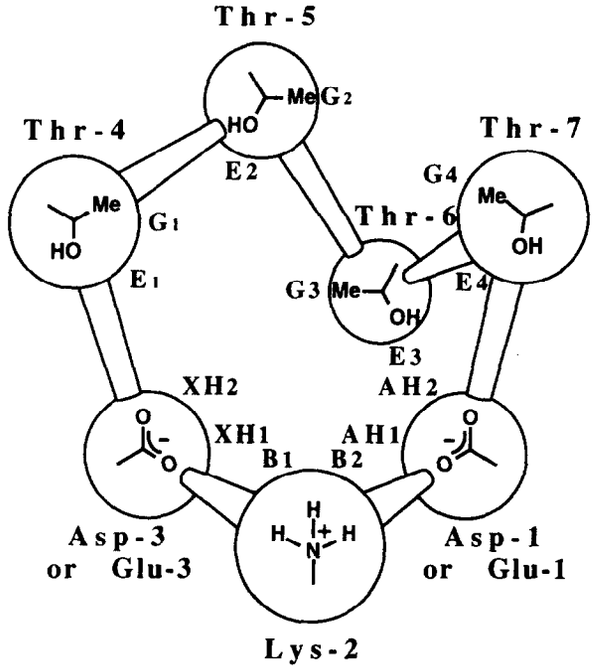



**Fig. 5.** Model of an idealized human sweetness receptor with its seven basic recognition sites (arranged in space according to a skew heptagon) and its 14 recognition subsites (which represent the 14 possible interaction points of a sweetener such as sucrose with the human sweetness receptor) (Nofre & Tinti, 1996).

At the sweetener level, the points of the sweetener molecule that interact with the preceding recognition subsites are, respectively, designated as the B1, B2, AH1, AH2, XH1, XH2, E1, E2, E3, E4, G1, G2, G3 and G4 interaction points of the sweetener; note that the number of interaction points of a sweetener can be equal to or lower than the number of recognition subsites of the receptor. The B1 and B2 interaction points are an anionic group ($CO_2^-$ or $SO_3^-$ for example) or one or two hydrogen-bond acceptor atom(s) (two oxygen atoms for example) of a sweetener; the AH1, AH2, XH1 and XH2 interaction points are hydrogen-bond donor groups ($NH^+$, NH, OH) of a sweetener; the E1, E2, E3 and E4 interaction points are hydrogen-bond acceptor atoms (such as N or O) of a sweetener; finally, the G1, G2, G3 and G4 points are steric interaction points (such as $CH_3$, $CH_2$ or CH) of a sweetener capable of interacting, through van der Waals contacts, with the $CH_3$ groups of the threonine recognition sites. For example, according to the MPA theory, aspartame is considered to be a B1, B2, AH1, XH1, XH2, E1, G1, G2, G4-type sweetener (Fig. 6a), and alitame, a B1, B2, AH1, XH1, XH2, G1, G2, G3, G4-type sweetener (Fig. 6b) (Nofre & Tinti, 1996).

According to the MPA theory, the intermolecular steric interactions between the G-steric interaction points of a sweetener and the G-steric recognition subsites (the threonine $CH_3$ groups) of the receptor are particularly efficient (i) if the interaction gives rise to a very precise *steric fit* of a moiety of the sweetener between at least two threonine methyl groups of the receptor (the sweetener then acting as a wedge leaning on two, or more, opposite $CH_3$ groups of the receptor) and (ii) if the sterically wedging part of the sweetener is *rigid* (such as the phenyl group of aspartame or the 2,2,4,4-tetramethylthietanylamide group of alitame); a flexible group is in fact often an inoperative or a weakly operative group. Note that, in the MPA theory, the *steric interaction concept* (and its corollary, the *steric fit*

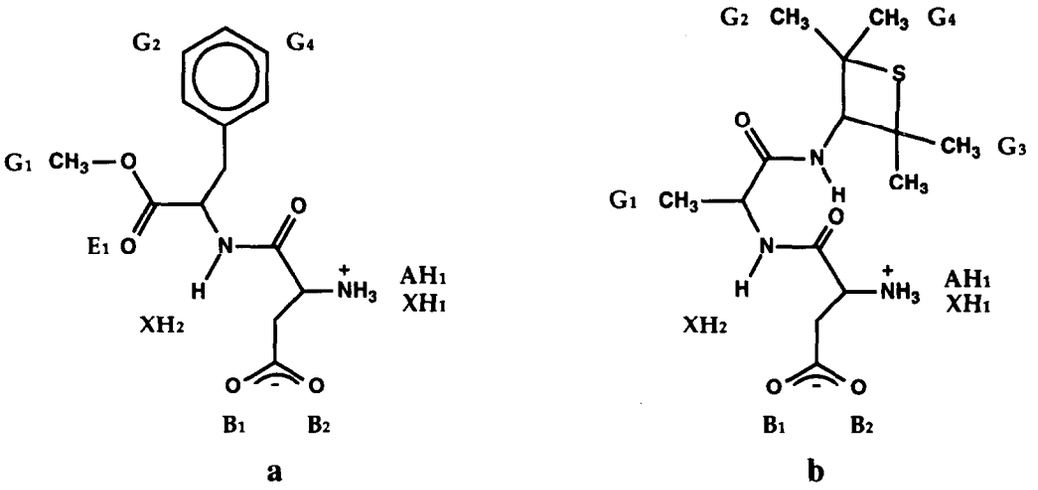



**Fig. 6.** The interaction points of (a) aspartame and (b) alitame with the human sweetness receptor (Nofre & Tinti, 1996).

*concept*) has replaced the former *hydrophobic interaction concept* that we consider as no longer valid (Nofre & Tinti, 1996).

In the light of the MPA theory, which is the specific $G_{ALT}$-steric recognition subsite that is present in the sweetness receptor of all primates tested so far (Glaser *et al.*, 1995)?

If we analyse the putative interactions of the CPK models of the alitame-like sweeteners (class I) with a CPK model of the human sweetness receptor in its activated state (Nofre & Tinti, 1996), it is observed that these sweeteners are able to interact with both the G1- and G4-steric recognition subsites of the receptor (i.e. with the $CH_3$ groups of the Thr-4 and Thr-7 residues), through a G1G4 transversal interaction (Fig. 7); note that alitame (Fig. 6b) furthermore interacts with the G2- and G3-steric recognition subsites. As all the dipeptide derivatives or analogues studied in the present work (the ALT-like compounds, but also the APM-like and AMPA-like compounds as we shall see below) interact

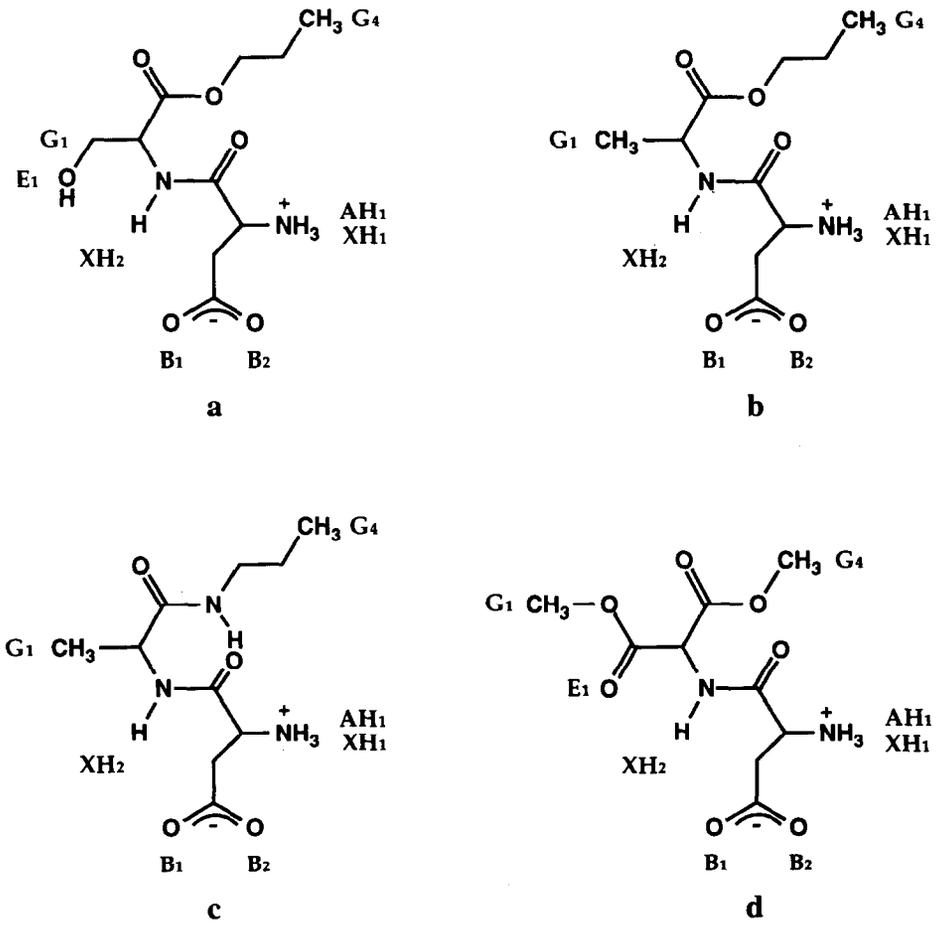



**Fig. 7.** The interaction points of (a) ASPE, (b) AAPE, (c) AAPA and (d) AADD with the human sweetness receptor.

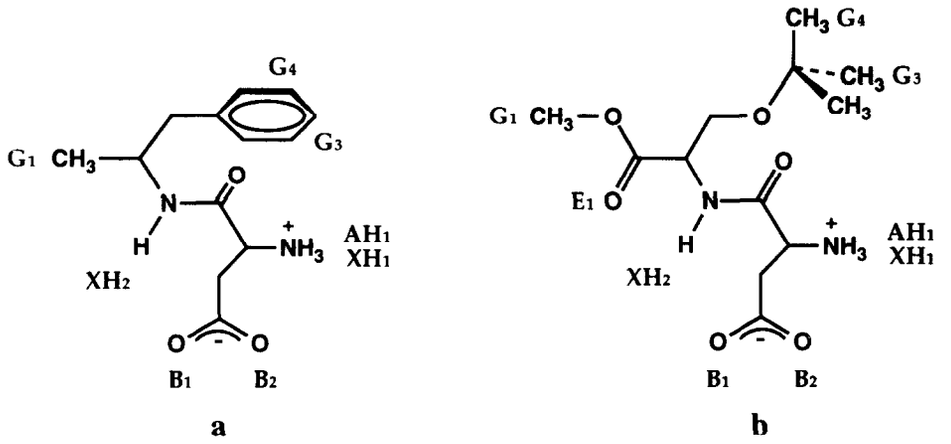



**Fig. 8.** The interaction points of (a) ampame (AMPA) and (b) ASME with the human sweetness receptor.

with the G1-steric recognition subsite, the G4-steric recognition subsite must therefore be the special feature of all the alitame-like compounds (class I sweeteners). Consequently, $G_{ALT}$ must correspond to the G4-steric recognition subsite (the Thr-7 methyl group) of the MPA theory.

Now, if we consider aspartame (APM), we have already said that APM interacts with the receptor through the G1-, G2- and G4-steric recognition subsites of the receptor (Fig. 6a) (Nofre & Tinti, 1996). As the G1-steric recognition subsite is common to the three dipeptide classes (I, II and III) and the G4-steric recognition subsite is specific to the alitame class (I), the G2-steric recognition subsite must be the special feature of the aspartame-like sweeteners, including thaumatin (class III sweeteners); for a discussion on thaumatin, see Glaser *et al.* (1995). $G_{APM}$ must therefore correspond to the G2-steric recognition subsite (the Thr-5 methyl group) of the MPA theory. In fact, the phenylalanine methyl ester moiety of APM is too flexible to exert an efficient G1G4 steric fit in the absence, as in the non-catarrhine primates, of the G2-steric recognition subsite.

Finally, if we consider the putative interactions of the two ampame-like sweeteners (class II) with the CPK model of the activated human sweetness receptor, we observe that these two sweeteners must interact with the G1-, G3- and G4-steric recognition subsites of the receptor (i.e. with the $CH_3$ groups of the Thr-4, Thr-6 and Thr-7 residues) (Fig. 8). As the G1- and G4-steric recognition subsites are common to all the sweetener classes (I, II and III), the G3-steric recognition subsite must therefore be the special feature of the ampame-like sweeteners (class II sweeteners). As a result, $G_{AMPA}$ must correspond to the G3-steric recognition subsite (the Thr-6 methyl group) of the MPA theory.

The distribution of the G1-, G2-, G3- and G4-steric recognition subsites in primate sweetness receptors is given in Table 3.

The steric fits that are able to exert pushing forces between the various steric recognition subsites of the primate sweetness receptors are outlined in Fig. 9 (in order of increasing complexity of the sweetness receptor) for (i) the platyrrhine receptor (New World simians; Fig. 9a), (ii) the prosimian receptor (Lemuroidea; Fig. 9b), and (iii) the catarrhine receptor (Old World simians, including man; Fig. 9c). It must be remarked that the pushing forces between G1 and G2 or between G3 and G4 must be inoperative if they are isolated (i.e. if they are not relayed through a G2G4 or a G1G3 steric fit), which means that one of the main processes for eliciting a sweet response in primates is to move apart the G1 (or the G1/G2) moiety of the receptor from the G4 (or the G3/G4) moiety.

Since, according to the present results, it appears that the mutations observed in the primate sweetness receptors concern only the G2- and G3-steric recognition subsites, what could be then the nature of the recognition sites X-5 and X-6 (Fig. 10) present in Lemuroidea and Ceboidea?

The most probable phylogenetic precursors of the Thr-5 and Thr-6 residues of the human receptor must be alanine (Ala) or serine (Ser), as a result of the small size of their $CH_3$ or $CH_2OH$ side-chains (these chains being, with that of glycine, the only ones sterically compatible with the interaction of sucrose with the receptor), and in accordance with the phylogenetic principle of parsimony,

**Table 3. Distribution of the G1-, G2-, G3- and G4-steric recognition subsites in primate sweetness receptors**

| | G1 | G2 | G3 | G4 |
|---|---|---|---|---|
| Lemuroidea | + | − | + | + |
| Ceboidea | + | − | − | + |
| Cercopithecoidea | + | + | + | + |
| Hominoidea | + | + | + | + |

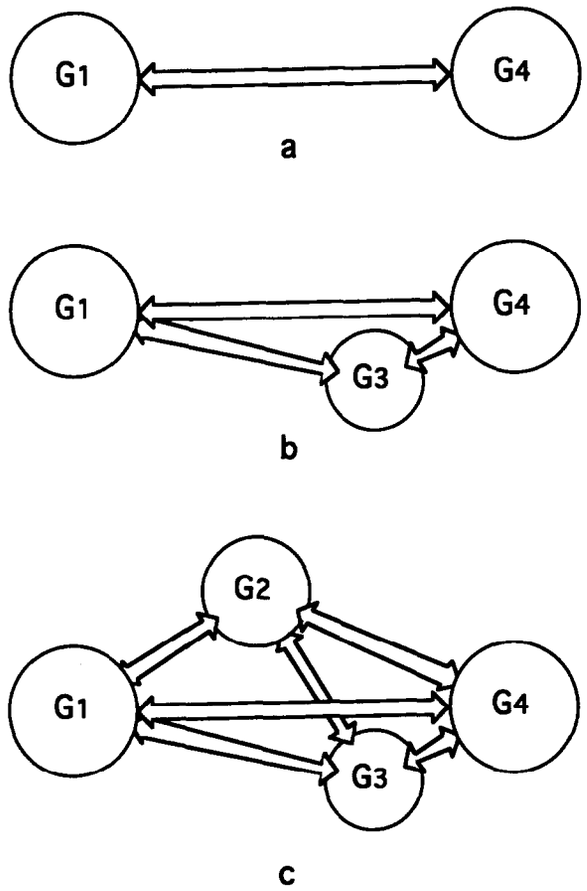



**Fig. 9.** The steric fits able to exert pushing forces between the steric recognition subsites in the sweetness receptors of (a) New World simians, (b) prosimians, and (c) Old World simians.

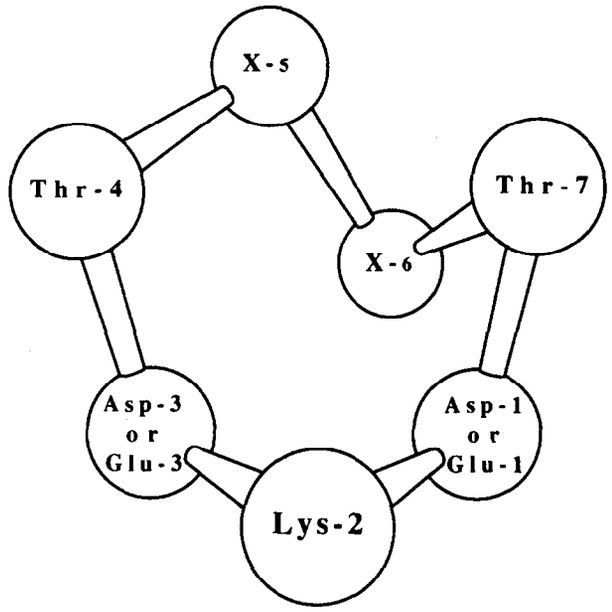



**Fig. 10.** Sweetness receptor model for (i) the New World simians (where X-5 and X-6 are Ala or Ser), (ii) the prosimians (where X-5 is Ala or Ser, and X–6 Thr), and (iii) the Old World simians (where X-5 and X-6 are Thr).

which prefers an Ala → Thr or Ser → Thr mutation (which requires only one nucleotide substitution and is therefore the simplest change) to a Gly → Thr mutation (which requires two nucleotide substitutions).

Consequently, the seven basic recognition sites of the primate sweetness receptors must be in Lemuroidea: Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Ala-5 or Ser-5, Thr-6, Thr-7; in New World simians: Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Ala-5 or Ser-5, Ala-6 or Ser-6, Thr-7; and in Old World simians (including man): Asp-1 or Glu-1, Lys-2, Asp-3 or Glu-3, Thr-4, Thr-5, Thr-6, Thr-7.

Finally, these observations, in relation to the previous results with aspartame, do not confirm that Anthropoidea form a monophyletic clade (Ford, 1994), but confirm that Catarrhini (Cercopithecoidea and Hominoidea) do form a monophyletic lineage (Glaser *et al.*, 1978).

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