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Sweet Taste Receptor Gene Variation and Aspartame Taste in Primates and Other Species

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Abstract

Aspartame is a sweetener added to foods and beverages as a low-calorie sugar replacement. Unlike sugars, which are apparently perceived as sweet and desirable by a range of mammals, the ability to taste aspartame varies, with humans, apes, and Old World monkeys perceiving aspartame as sweet but not other primate species. To investigate whether the ability to perceive the sweetness of aspartame correlates with variations in the DNA sequence of the genes encoding sweet taste receptor proteins, T1R2 and T1R3, we sequenced these genes in 9 aspartame taster and nontaster primate species. We then compared these sequences with sequences of their orthologs in 4 other nontasters species. We identified 9 variant sites in the gene encoding T1R2 and 32 variant sites in the gene encoding T1R3 that distinguish aspartame tasters and nontasters. Molecular docking of aspartame to computer-generated models of the T1R2 + T1R3 receptor dimer suggests that species variation at a secondary, allosteric binding site in the T1R2 protein is the most likely origin of differences in perception of the sweetness of aspartame. These results identified a previously unknown site of aspartame interaction with the sweet receptor and suggest that the ability to taste aspartame might have developed during evolution to exploit a specialized food niche.

Key words: aspartame, modeling, primates, sweet taste, taste receptor, T1R2, T1R3

Introduction

The ability to perceive sweet taste is a common trait in a range of animals that probably reflects the importance of simple carbohydrates as a dietary energy source (Kare and Beauchamp 1984). However, not all compounds that taste sweet are sugars and within the last 2 centuries, researchers have discovered several nonsugar sweeteners, such as saccharin and aspartame. These sweeteners are desirable because they are potentially safer for use among people who metabolize simple sugars poorly, for example, diabetics, and among people who wish to avoid the extra calories of sugar, for example, in sweetening coffee or tea. Some of types of high-potency sweeteners are large proteins found in plants, but many were synthesized, often by accident, in the laboratory (Dubois 2008).

One high-potency sweetener, aspartame, is apparently not sweet to most mammals other than humans and closely related primates (Hellekant et al. 1980, 1981, 1994; Naim et al.

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1982; Sclafani and Abrams 1986; Thomsen et al. 1988; Glaser et al. 1992; De Francisco and Dess 1998; Bachmanov, Tordoff, and Beauchamp 2001; Schilling et al. 2004; Li et al. 2009). We say apparently since we do not know what an animal perceives, but we judge their ability by their behavior when offered aspartame-sweetened water to drink or food to eat. The differences among species in their behavior toward aspartame correspond to differences in afferent sensory responses of the chorda tympani and glossopharyngeal gustatory nerves. For example, aspartame does not elicit gustatory neural responses in the aspartame nontaster species: mice (Inoue et al. 2001), gerbils (Jakinovich 1981), hamsters (Nowlis et al. 1980; Hellekant and Danilova 1996; Danilova, Hellekant, Tinti, and Nofre 1998), rats (Hellekant and Walters 1993), cows, (Hard af Segerstad and Hellekant 1989a, 1989b; Hellekant et al. 2010), pigs (Hellekant and Danilova 1996; Glaser et al. 2000), prosimians and New World simians (Hellekant et al. 1980, 1981, 1993; Glaser et al. 1995; Nofre et al. 1996; Danilova et al. 2002). However, in Old World monkeys and apes, aspartame evokes both behavioral preference and responses in gustatory nerves (Sato et al. 1977; Glaser et al. 1992, 1995, 1996; Hellekant and Danilova 1996; Hellekant et al. 1996). The term aspartame "taster" and "nontaster" is used here to distinguish between species that prefer aspartame-flavored food or water to plain food or plain water or which have a vigorous taste nerve response to aspartame and those that do not. This term is adopted for simplicity but not all members of a species are necessarily the same in their behavioral response to aspartame. For instance, some mice prefer aspartame to water (Meliska et al. 1995; Bachmanov, Tordoff, and Beauchamp 2001). Likewise, there are exceptions to the observation that only humans and closely related primates prefer aspartame, for instance one species related to the raccoon is an aspartame taster (Li et al. 2009), as are fruit flies (Gordesky-Gold et al. 2008). These observations notwithstanding, the main point is that most mammals except for humans and Old World primates are indifferent to aspartame. The explanation for the species difference is unknown.

Recent advancements in our understanding of the molecular mechanisms of taste perception allowed us to form a hypothesis. Differences in taste perception among species, strains, or even among members of a population are often due to variant sites in specific receptor genes (Chandrashekar et al. 2000; Bachmanov, Li, et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Sainz et al. 2001; Jordt and Julius 2002; Kim et al. 2003; Reed et al. 2004; Bufe et al. 2005; Reed et al. 2010). Therefore, to understand species differences in aspartame sensitivity, we focused on the known sweet receptor, a heterodimer of 2 proteins, T1R2 and T1R3 (Max et al. 2001; Nelson et al. 2001, 2002; Li et al. 2002; Ariyasu et al. 2003; Damak et al. 2003; Zhao et al. 2003). From cell-based assays, we have learned that the human receptor (as opposed to the mouse receptor) is required for aspartame responsiveness (Xu et al. 2004). Introduction of the human receptor into a mouse or cell line humanizes its response to aspartame (Jiang et al. 2004; Xu et al. 2004). The preponderance of the evidence indicates that the logical first step toward understanding aspartame sensitivity would be to examine the T1R2 + T1R3 dimer, but we acknowledge that T1R homodimers or unknown heterodimers may comprise an alternative aspartame receptor in some species.

These lines of evidence led us to formulate the hypothesis that disparate aspartame taste responses among primates and other species might be due to species variation in sequences of orthologs of the known sweet receptor genes. To test this hypothesis, we sequenced genes encoding T1R2 and T1R3 in 9 aspartame taster and nontaster primate species. We then compared these sequences with sequences of their orthologs in 4 other nontasters species and determined which of these differences were liable to disrupt the interaction of the sweet receptor to aspartame using computer-assisted modeling. Our goal was to identify the most likely DNA variant sites within the sweet receptor that account for aspartame sensitivity.

Materials and methods

Selection of primate species to sequence

We selected the primate species for sequencing based on the availability of results from previous behavioral and electrophysiological studies that determined whether the species was an aspartame taster or nontaster (Table 1). We included 4 additional nonprimate species because their responses to aspartame were known from previous studies and also because full-length sequences of the sweet taste receptor genes were available. Cats were eliminated from consideration because their aspartame insensitivity extends to all sweeteners tested (Li et al. 2005).

Obtaining DNA and preparing DNA from primates

Genomic DNA samples from 6 primate species were available through 2 commercial sources 1) Coriell Institute for Medical Research: chimpanzee (*Pan troglodytes*, chimpanzee); gorilla (*Gorilla gorilla*, western lowland gorilla); orangutan (*Pongo pygmaeus abelii*, Sumatran orangutan); patas monkey (*Erythrocebus patas*, patas monkey); tamarin (*Saguinus labiatus*, red-bellied tamarin) and 2) Therion International, LLC: rhesus monkey (*Macaca mulatta*, rhesus monkey). The San Diego Frozen Zoo provided genomic DNA from 2 species: squirrel monkey (*Saimiri sciureus*, squirrel monkey) and marmoset (*Cebuella pygmaea*, Pygmy marmoset). The Texas Biomedical Research Institute provided us with baboon DNA (*Papio hamadryas*, baboon). Genomic DNA was measured for concentration and purity using conventional spectrophotometer and diluted to a concentration of 25 ng/μL as a prerequisite for polymerase chain reaction (PCR)-based sequencing.

Table 1 List of species used in this study and their aspartame taster/nontaster status

Species name	Preference	Electrophysiology	References
Humans (*Homo sapiens*)	+	NA	Mojet et al. (2001, 2003, 2004)
Chimpanzee (*Pan troglodytes*)	+	+	Glaser et al. (1992, 1995), Hellekant et al. (1996, 1998); Hellekant, Danilova, and Ninomiya (1997)
Gorilla (*Gorilla gorilla*)	+	NA	Glaser et al. (1992, 1995, 1996)
Orangutan (*Pongo pygmaeus abelii*)	+	NA	Glaser et al. (1992, 1995, 1996)
Patas monkey (*Erythrocebus patas*)	+	NA	Glaser et al. (1992, 1995, 1996)
Baboon (*Papio hamadryas*)	+	NA	Glaser et al. (1992, 1995, 1996)
Rhesus monkey (*Macaca mulatta*)	+	+	Thomsen et al. (1988), Glaser et al. (1992, 1995, 1996), Hellekant, Danilova, and Ninomiya (1997)
Marmoset (*Cebuella pygmaea*)	–	–	Glaser et al. (1992, 1995, 1996), Danilova, Hellekant, Roberts, et al. (1998), Danilova et al. (2002)
Squirrel monkey (*Saimiri sciureus*)	–	NA	Glaser et al. (1992, 1995, 1996)
Tamarin (*Saguinus labiatus*)	–	NA	Glaser et al. (1992, 1995, 1996)
Cow (*Bos taurus*)	–	–	Hard af Segerstad and Hellekant (1989a, 1989b), Hellekant et al. (1994)
Dog (*Canis lupus familiaris*)	–	NA	Glaser (2002)
Rat (*Rattus norvegicus*)	–	–	Sclafani and Abrams (1986), Thomsen et al. (1988), Hellekant and Walters (1992), De Francisco and Dess (1998)
Mouse (*Mus musculus*)	–	–	Bachmanov, Tordoff, and Beauchamp (2001), Inoue et al. (2001)

Preference refers to the case when consumption of aspartame solution is more than 50% of the total fluid intake in a 2-bottle test with aspartame and water. Electrophysiology refers to the case when responses in chorda tympani or glossopharyngeal nerves are observed when stimuli are applied to the oral cavity. +, a species prefers or responds to aspartame; –, a species does not prefer or does not respond to aspartame. NA, data not available.

Selection of genes to sequence

We selected the 2 known sweet receptor genes, *Tas1r2* and *Tas1r3*, for DNA sequencing. All the primate species tested have functional sense of other tastes. Therefore, we thought it was unlikely that variation in genes involved in perception of more than one taste quality (e.g., gustducin, TRPM5) would contribute to the aspartame taster/nontaster species differences. We use *Tas1r2* and *Tas1r3* as the gene symbols (in some cases generically, when referring to multiple species) and T1R2 and T1R3 as the protein symbols, as applicable. Gene or protein symbols with a prefix refer to the specific species, for example, mT1R2 is mouse T1R2 and hT1R3 is human T1R3.

Amplification of *Tas1r2*- and *Tas1r3*-coding regions by PCR

The sequences corresponding to the validated exons of the human *Tas1r2* and *Tas1r3* genes were amplified. Because mouse and human genes have the same intron–exon structure, we assumed that the primate gene structure would be the same as human too. We confirmed this assumption by sequencing the cDNA from one primate species, with the method as follows: we collected circumvallate and foliate papillae from a postmortem tongue tissue sample taken from a female Baboon (*Papio anubis*). We extracted total RNA from the taste papillae by using TRIZOL reagent (Invitrogen), total RNA was transcribed to cDNA using Superscript III kit (Invitrogen), and reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted using intron-spanning primers designed using baboon genomic sequences of *Tas1r2* and *Tas1r3*. Sequencing of RT-PCR products revealed that the exon–intron junctions of T1R2 and T1R3 from baboon are the same as those of humans. Collection of tongue tissue was approved by the animal care and use committee at the University of Pennsylvania and the Monell Chemical Senses Center.

We used a walk-down procedure for designing primers to amplify nonhuman DNA: we first designed primers based on human *Tas1r2* and *Tas1r3* sequences to obtain primate-specific sequences and then used the resulting primate sequence to design additional primate-specific primers. After PCR amplification, the products were purified and sequenced by the DNA sequencing facility at the University of Pennsylvania. Both strands were sequenced and assembled using Sequencher (version 4.0.5, Gene Codes). In cases where primate sequences became available through public sequencing efforts during the data collection phase of this project, we compared our sequencing against that of the published sequence to check for sequencing errors or gaps. In cases where we could fill gaps in our own sequencing with public sequence, we assembled all available sequences and used these data in the alignments.

```
human            MGPRAKTICSLFFLLWVLAEP---AENSDFYLPGDYLLGGLFSLHANMKGIVHLNFLQVP 57
chimpanzee       MGPRAKTICSLFFLLWVLAEP---AENSDFYLPGDYLLGGLFSLHANMKGIVHLNFLQVP 57
gorilla          MGTRATTICSLFFLLWVLAEP---AENSDFYLPGDYLLGGLFSLHANMKGIVHLNFLQVP 57
orangutan        MGPRATTICSLFFLLWVLAEP---AENSDFYLPGDYLLGGLFSLHANMKGIVHLNFLQVP 57
patas_monkey     XXXXXXXXXXXXXLLQVLAEP---AKNSDFYLPGDYLLGGLFTLHANMKGIVHXXXXXXX 57
baboon           MRPRATTICSLFFLLRVLAEP---AKNSDFYLPGDYLLGGLFTLHANMKGIVHLDYLQVP 57
rhesus_monkey    MRPRATTICSLFFLLRVLAEP---AKNSDFYLPGDYLLGGLFTLHANMKGIVHLDYLQVP 57
squirrel_monkey  MEPRVRTVCFLFFLLRVLAEP---AKNSDFYLPGDYLLGGLFTLHANMKGTVHLNFLQVP 57
marmoset         MGPRARTVCFLFFLLWVLAEL---AENSDFHLPGDYLLGGLFTLHANMKGIVHLNFLQVP 57
tamarin          MGPRARTVYFLFFLLRVLAEP---AKNSDFYLPGDYLLGGLFTLHANMKGTVHLNFLQVP 57
cow              MGPQGRAACSLLFLLQALAEP---AENSEFLPGDYLLGGLFTLHANVKGIVHLNYLKVP 57
dog              MGPRAKAVCSLFILLQVLAEP---AENSDFYLPGDYLLGGLFTLHANVKGTVHLSFLQVP 57
rat              MGPQARTLCLLSLLLHVLPKPGKLVENSDFHLAGDYLLGGLFTLHANVKSISHLSYLQVP 60
mouse            MGPQARTLHLLFLLLHALPKPVMLVGNSDFHLAGDYLLGGLFTLHANVKSVSHLSYLQVP 60

human            MCKEYEVKVIGYNLMQAMRFAVEEINNDSSLLPGVLLGYEIVDVCYISNNVQPVLYFLAH 117
chimpanzee       MCKEYEVKVIGYNLMQAMRFAVEEINNDSSLLPGVLLGYEIVDVCYISNNVQPVLYFLAH 117
gorilla          MCKEYEVKVIGYNLMQAMRFAVEEINNDSSLLPGVLLGYEIVDVCYISNNVQPVLYFLAH 117
orangutan        MCKEYEVKVIGYNLMQDMRFSVEEINNDSSLLPGVLLGYEMVDVCYVSNNVQPVLYFLAH 117
patas_monkey     XXXXXEVKVIGYNLMQAMRFAVEEINNDSSLLPDVLLGYEMVDVCYVSNNVQPVLYFLAQ 117
baboon           MCKEYETKVIGYNLMQAMRFAVEEINNDSSLLPDVLLGYEMVDVCYVSNNVQPVLYFLAQ 117
rhesus_monkey    MCKEYETKVIGYNLMQAMRFAVEEINNDSSLLPDVLLGYEMVDVCYVSNNVQPVLYFLAQ 117
squirrel_monkey  MCKEYEVKLSGYNLMQAMRFAVEEINNDSSLLPDVRLGYEMVDVCYVSNNVQPVLYFLAQ 117
marmoset         MCKEYEMKVSGYNLMQAMRFAVEEINNDSSLLPDVLLGYEMVDVCYISNNVQPALYFLAQ 117
tamarin          MCKEYEMKVSGYNLMQAMRFAVEEINNDSSLLPDVLLGYEMVDVCYISNNVQPALYFLAQ 117
cow              KCKEYEMKVLGYNLMQAMRFAVEEVNNDSSLLPNVLLGYEMVDSCYMSNNVQPVLYFLSQ 117
dog              QCKKYEMKVLGYNLMQAMRFAVEEINNRSDLLPGVLLGYEIVDVCYISNNVQPVLYFLAR 117
rat              KCNEFTMKVLGYNLMQAMRFAVEEINNCSSLLPGVLLGYEMVDVCYLSNNIHPGLYFLAQ 120
mouse            KCNEYNMKVLGYNLMQAMRFAVEEINNCSSLLPGVLLGYEMVDVCYLSNNIQPGLYFLSQ 120
                                *

human            EDNLLPIQEDYSNYISRVVAVIGPDNSESVMTVANFLSLFLLPQITYSAISDELRDKVRF 177
chimpanzee       EDNLLPIQEDYSNYISRVVAVIGPDNSESVMTVANFLSLFLLPQITYSAISDELRDKVRF 177
gorilla          EDNLLPIQEDYSNYISRVVAVIGPDNSESVMTVANFLSLFLLPQITYSAISDELQDKVRF 177
orangutan        EDNLLPIQEDYSDYVSRVVAVIGPDNSESVMTVANFLSLFLLPQITYSAISDELRDKVRF 177
patas_monkey     EDDLLPIQENYSNYVPRVVAVIGPDNSDAVXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 177
baboon           EDDLLPIQENYSNYVSRVVAVIGPDNSDAVMTVANFLSLFLLPQITYSAISDELRDKVRF 177
rhesus_monkey    EDDLLPIQENYSNYVPRVVAVIGPDNSDAVMTVANFLSLFLLPQITYSAISDELRDKVRF 177
squirrel_monkey  EDNLLPIQEDYSNYVPRVVAVIGPENSESVTTVANFLSLFLLPQITYSAISDQLRDKQRF 177
marmoset         EDNLLPIQEDYSNYVPRVVAVIGPENSESVMTVAHFLSLFLLPQITYSAISDQLQDKQRF 177
tamarin          EDNLLPIQEDYSNYVPRVVAVIGPENSESVMTVAHFLSLFLLPQITYSAISDELXXXXXX 177
cow              DDYFLPIQEDYSQYVPRVVAIIGPDNSESTKTVANFLSLFLLPQXXXXXXXXXXXXXXXX 177
dog              EDYSLPIQEDYSHYVPRVLAVIGPDNSESTTTVAHFLSLFLLPQITYSAISDDLRDKQHF 177
rat              DDDLLPILKDYSQYMPHVVAVIGPDNSESAITVSNILSHFLIPQITYSAISDKLRDKRHF 180
mouse            IDDFLPILKDYSQYRPQVVAVIGPDNSESAITVSNILSYFLVPQVTYSAITDKLRDKRRF 180
                                                                          *

human            PALLRTTPSADHHVEAMVQLMLHFRWNWIIVLVSSDTYGRDNGQLLGERVA-RRDICIAF 236
chimpanzee       PALLRTTPSADHHVEAMVQLMLHFRWNWIIVLVSSDTYGRDNGQLLGERLA-RRDICIAF 236
gorilla          PALLRTTPSADHHVEAMVQLMLHFRWNWIIVLVSSDTYGRDNGQLLGERLA-RRDICIAF 236
orangutan        PALLRTTPSADHHIEAMVQLMLHFRWNWIIVLVSSDTYGRDNGQLLGERLA-RRDICIAF 236
patas_monkey     XXXXXXXAPSADHHIEAMVQLMLYFHWNWIIVLVSGDTYGRDNGQLLGDRLA-RGDICIAF 236
baboon           PALLRTAPSADHHIEAMVQLMLHFRWNWIIVLVSGDTYGRDNGQLLGDRLA-RGDICIAF 236
rhesus_monkey    PALLRTAPSADHHIEAMVQLMLHFRWNWIIVLVSGDTYGRDNGQLLGDRLA-RGDICIAF 236
squirrel_monkey  PALLRTTPSAKHHIEAMVQLMLHFRWNWISVLVSSDTYGRDNGQLLGDRLA-GGDICIAF 236
marmoset         PALLRTTPSAKHHIEAMVQLMLHFHWNWISVLVSSDTYGRDNGQMLGDRLA-GGDICIAF 236
tamarin          XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 236
cow              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 236
dog              PALLRTVAGADHQIEAMVQLLLHFNWNWIIVLVSSDDYGRYNSQLLNDRLA-TGDICIAF 236
rat              PSMLRTVPSATHHIEAMVQLMVHFQWNWIVVLVSDDDYGRENSHLLSQRLTVTSDICIAF 240
mouse            PAMLRTVPSATHHIEAMVQLMVHFQWNWIVVLVSDDDYGRENSHLLSQRLTVTGDICIAF 240
                                                                        *

human            QETLPTLQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLTLYHFFNEVLRQNFTGA 296
chimpanzee       QETLPALQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLSLYDFFNEVLRQNFTGA 296
gorilla          QETLPTLQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLSLYDFFNEVLRQNFTGA 296
orangutan        QETLPTLQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLTLYDFFNEVLRQNFTGA 296
patas_monkey     QETLPTVQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLTLYNFFNEVLRQNFTGA 296
baboon           QETLPTVQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLTLYNFFNEVLRQNFTGA 296
rhesus_monkey    QETLPTVQPNQNMTSEERQRLVTIVDKLQQSTARVVVVFSPDLTLYNFFNEVLRQNFTGA 296
squirrel_monkey  QETLPTLQPNQDMPEDRQRLVSIVEKLQQSTARVVVVFSPDLTLYDFFREVLRQNFTGA 296
marmoset         QETLPTLQSNQDIMPEDHQRLVSIVEKLQQSTARVVVVFSPDLSLYNFFREVLRQNFTGA 296
tamarin          QETLPTLQSNQDIMPEDHQRLVSIVEKLQQSTARVVVVFSPDLSLYNFFREVLRQNFTGA 296
cow              QETLPTPQPNQTMTPQDQERLEAIVGKLQQSTARVVVVFSPELALHNFFREVLRQNLTGA 296
dog              QETLPMPQPDQVVTEWERQRLEAIVGKLQQSSARVVVLFSPDLILHNFFREVLRQNFTGA 296
rat              QEVLPIPESSQVMRSEEQRQLDNILDKLRRTSARVVVVFSPELSLYSFFHEVLRWNFTGF 300
mouse            QEVLPVPEPNQAVRPEEQDQLDNILDKLRRTSARVVVIFSPELSLHNFFREVLRWNFTGF 300
                            *         *                   *

human            VWIASESWAIDPVLHNLTELGHLGTFLGITIQSVPIPGFSEFREWGPQAGPPPLSRTSQS 356
chimpanzee       VWIASESWAIDPVLHNLTELRHLGTFLGITIQSVPIPGFSEFREWGPQAGPPPLSRTSQS 356
gorilla          VWIASESWAIDPVLHNLTELRHLGTFLGITIQSVPIPGFSEFREWSPQAGPPPLSRTSQS 356
orangutan        VWIASESWAIDPVLHNLTELRHTGTFLGITIQSVPIPGFSEFREDSQAGPPPLGRTSQR 356
patas_monkey     VWIASESWAIDPVLHNLTELRHMGTFLGITIQSVPIPGFSEFRVRDPQAGPPPLSRSSQR 356
baboon           VWIASESWAIDPVLHNLTELRHMGTFLGITIQSVPIPGFSEFRVRDPQAGPPPLSRTSQR 356
rhesus_monkey    VWIASESWAIDPVLHNLTELRHMGTFLGITIQSVPIPGFSEFRVRDPQAGPPPLSRTSQR 356
squirrel_monkey  VWIASESWAIDPVLHNLTGLHRTGTFLGITLQNVPIPGFNEFRVRGPQAGP-----THQR 351
marmoset         VWIASESWAIDPVLHNLTGLHRTGTFLGITIQNVPIPGFSEFRVRGPQAGP-----TNQR 351
tamarin          VWIASESWAIDPVLHNLTGLHRTGTFLGITIQNVPIPGFSEFRVRAPQAGP-----TNQR 351
cow              VWIASESWAIDPVLHNLTELQRAGTFLGITTQNVPIPGFSEFRVRRSQVGRPALNRS--S 354
dog              VWIASESWAIDPVLHNLTELRQTGTFLGVTTQSVPIPGFSEFRIRRTPVRLPEPNRTSLE 356
rat              VWIASESWAIDPVLHNLTELRHTGTFLGVTIQRVSIPGFSQFRVRRDKPGYPVPNTTNLR 360
mouse            VWIASESWAIDPVLHNLTELRHTGTFLGVTIQRVSIPGFSQFRVRHDKPEYPMPNETSLR 360
                                                                  *  *

human            YTCNQECDNCLNATLSFNTILRLSGERVVYSVYSAVYAVAHALHSLLGCDKSTCTKRVVY 416
chimpanzee       YTCNQECDNCLNATLSFNTILRLSGERVVYSVYSAVYAVAHALHSLLGCDNSTCTKRVVY 416
gorilla          YTCNQECDNCLNATLSFNTILRLSGERVVYSVYSAVYAVAHALHSLLGCDHSTCTKRVVY 416
orangutan        STCNQECDNCLNATLSFNTILRLSGERVVYSVYSAVYAVAHALHSLLGCDHSTCTKRVVY 416
patas_monkey     STCNQECDSCLNGTLSFDNVLRLSGERVVYSVYSAVYAVAHALHSLLGCDHGTCTKKEVY 416
baboon           STCNQECDSCLNGTLSFNNVLRLSGERVVYSVYSAVYAVAHALHSLLGCDHGTCTKTEVY 416
rhesus_monkey    STCNQECDSCLNGTLSFNNVLRLSGERVVYSVYSAVYAVAHALHSLLGCDHGTCTKREVY 416
squirrel_monkey  STCNQECDTCLNSTLSFNTILRLSGERVVYSVYSAVYAVAHALHSLLGCDHSACTKRGVY 411
marmoset         STCNQECDTCLNSTLSFNTVLRLSGERIVYSVYSAVYAVAHALHSLLGCDHSTCTKRVVY 411
tamarin          STCNQECDTCLNSTLSFNTILRLSGERIVYSVYSAVYAVAHALHSLLGCDHSTCTKRVVY 411
cow              ANCNQECDTCLNTTESFNSILTLSGERVVYSVYSSVYAVAHALHSLLGCNQSGCRRDVVY 414
dog              ATCNQECDTCQDTTASFNSILMLSGERVVYNVYSAVYAVAHALHSLLGCTQA-CSKEVVY 415
rat              TTCNQDCDACLNTTKSFNNILILSGERVVYSVYSAVYAVAHALHRLLGCNRVRCTKQKVY 420
mouse            TTCNQDCDACMNITESFNNVLMLSGERVVYSVYSAVYAVAHTLHRLLHCNQVRCTKQIVY 420

human            PWQLLEEIWKVNFTLLDHQIFFDPQGDVALHLEIVQWQWDRSQNFFQSVASYYPLQRQLK 476
chimpanzee       PWQLLEEIWKVNFTLLDHQIFFDPQGDVALHLEIVQWQWDRSQNFFQSVASYYPLQRQLK 476
gorilla          PWQLLEEIWKVNFTLLDHQIFFDPQGDVALHLEIVQWQWDRSQNFFQSVASYYPLQRQLK 476
orangutan        PWQLLEEIWKVNFTLLDHQIFFDPQGDMALHLEIVQWQWDRSQNFFQSVASYHPLQRQLK 476
patas_monkey     PWQLLEEIWKVNFTLLXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 476
baboon           PWQLLKEIWKVNFTLLDHQISFDPQGDMALHLEIVQWQWDLSQNFFQSVASYYPLQRQLK 476
rhesus_monkey    PWQLLKEIWKVNFTLLDHEISFDPQGDMALHLEIVQWQGLSQNFFQSVASYYPLQRQLK 476
squirrel_monkey  PWQLLEEIWKVNFSLLDHQIFFDPQGDVALHLEIVQWQWDLSQNFFQSVASY-PLQGHLK 470
marmoset         PWQLVQEIWKVNFTLLDHQIFFDPQGDVALHLEIVQWQWDLSQNFFQSIASYNPLQGRLK 471
tamarin          PWQVLEEIWKVNFTLLDHQIFFDPQGDVALHLEIVQWQWDLSQNFFQSIASYSPLQGRLK 471
cow              PWQLLLEIRKVNFTLLGHQIFFNEQGDLSLNLEIIQWQWDLSQNFFRSVASYQPSKRQLK 474
dog              PWQLLKEIWKVNFTLLGHNVFFGQQGDVLMPMEVIQWQWDLSQNFFQSIASYYPKLRQLK 475
rat              PWQLREIWHVNFTLLGNRLFFDQQGDMPMLLDIIQWQWDLSQNFFQSIASYSPTSKRLT 480
mouse            PWQLREIWHVNFTLLGNQLFFDEQGDMPMLLDIIQWQWGLSQNFFQSIASYSPTETRLT 480

human            NIQDISWHTVNNTIPMSMCSKRCQSGQKKKPVGIHVCCFECIDCLPGTFLNHTEDEYECQ 536
chimpanzee       NIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHVCCFECIDCLPGTFLNHTEDEYECQ 536
gorilla          HIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHVCCFECIDCLPGTFLNHTEDEYECQ 536
orangutan        NIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHVCCFECIDCLPGTFLNHTEDEYECQ 536
patas_monkey     XXXXXXXXXXXXXIPVSMCSKRCQSGQKKKPVGIHICCFECIDCLPGTFLNQTEXXXXXX 536
baboon           TIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHICCFECIDCLPGTFLNQTEDEYECQ 536
rhesus_monkey    KIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHICCFECIDCLPGTFLNQTEDEYECQ 536
squirrel_monkey  DIQDISWHTVNNTIPVSMCSKRCQSGQKKKPVGIHTCCFECIDCPPGTFLNQTANEYDCQ 530
marmoset         HIQDISWHTINNTIPVSMCSKRCQSGQKKKPVGIHTCCFECIDCLPGTFLNQTEDEYDCQ 531
tamarin          HIQDISWHTVNNTIPVSMCSKRCQSGQKKKPVGIHTCCFECIDCLPGTFLNQTEXXXXXX 531
cow              HIHDISWNTPNNTIPVSMCSRSCQPGQKKKPVGLHPCCFECIDCLPGTFLNQTLDEFDCQ 534
dog              AIHNISWHTANNTIPVSMCSKDCHPGQRKKPVGIHSCCFECIDCLPGTFLNRTADEFDCQ 535
rat              YINNVSWYTPNNTVPVSMCSKSCQPGQMKKSVGLHPCCFECLDCMPGTYLNRSADEFNCL 540
mouse            YISNVSWYTPNNTVPISMCSKSCQPGQMKKPIGLHPCCFECVDCPPGTYLNRSVDEFNCL 540
                                                    *

human            ACPNNEWSYQSETSCFKRQLVFLEWHEAPTIAVALLAALGFLSTLAILVIFWRHFQTPIV 596
chimpanzee       ACPNNEWSYQSETSCFKRQLVFLEWHEAPTIAVALLAALGFLSTLAILVIFWRHFQTPIV 596
gorilla          ACPNNEWSYQSETSCFKRQLVFLEWHEAPTIAVALLAALGFLSTLAILVIFWRHFQTPIV 596
orangutan        ACPSNEWSYQSETSCFKRQLAFLEWHEAPTIAVALLAALGFLSTLAILVIFWRHFQTPMV 596
patas_monkey     XXXXXXXXXXXEAYCFKRRLAFLEWHEAPTIVVALLATLGFLSTLAILVIFWRHFQTPMV 594
baboon           ACPSNEWSHQSEASCFKRRLAFLEWHEAPTIVVALLAALGFLSTLAILVIFWRHFQTPMV 596
rhesus_monkey    ACPSNEWSHQSEASCFKRRLAFLEWHEAPTIVVALLAALGFLSTLAILVIFWRHFQTPMV 596
squirrel_monkey  ACPSNEWSHQSETSCFKRRLSFLEWHEAATIAVALLAALGFLSTLAILVIFWRHFETPMV 590
marmoset         ACPSNEWSHQSETSCFKRRLSFLEWHEAATIAVALLAALGFLXXXXXXXXXXXXXXPMV 591
tamarin          XXXXXXXXXXXXXXXXXXRLSFLEWHEAATIVVALLAALGFLSTLAILVIFWRHFQTPMV 591
cow              PCPSSEWSHRKDTSCFKRQLAFLEWDEAPTIVVVMLASLGFLSTLAIVIIFWRHVRTPVV 594
dog              PCPSYEWSHRNDTSCFKRRLAFLEWHEPSTIFVVMLTILGFLSTLAIMVIFWRHLHTPVV 595
rat              SCPGSMWSYKNDITCFQRRPTFLEWHEVPTIVVAILAALGFFSTLAILFIFWRHFQTPMV 600
mouse            SCPGSMWSYKNNIACFKRRLAFLEWHEVPTIVVTILAALGFISTLAILLIFWRHFQTPMV 600

human            RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCLCRQALFPLCFTICISCIAVRSFQIV 656
chimpanzee       RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCLCRQALFPLCFTICISCIAVRSFQIV 656
gorilla          RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCLCRQALFPLCFTICISCIAVRSFQIV 656
orangutan        RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCLCRQALFPLCFTICISCIAVRSFQII 656
patas_monkey     RSDGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCFCRQALFPLCFTICISCIAVRSFQIV 654
baboon           RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCFCRQALFPLCFTICISCIAVRSFQIV 656
rhesus_monkey    RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVSTCFCRQALFPLCFTICISCIAVRSFQIV 656
squirrel_monkey  RSAGGPMCFLMLTLLLVAYMVVPVYVGLPKVSTCLCRQALFPVCFTICISCIAVRSFQIV 650
marmoset         RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVTTCLCRQALFPVCFTICISCITMRSFQIV 651
tamarin          RSAGGPMCFLMLTLLLVAYMVVPVYVGPPKVTTCLCRQALFPICFTICISCITVRSFQIV 651
cow              RSAGGPMCFLMLAPLLMAYTAVPVYVGPPTVWSCIFRQAFFTLCFTICISCITVRSFQIV 654
dog              RSAGGPMCFLMLVPLLLAYAMVPMYIGQPTFFSCLWRQTFFTLCFTICISCITVRSFQIV 655
rat              RSAGGPMCFLMLVPLLLAFGMVPVYVGPPTVFSCFCRQAFFTVCFSICLCITVRSFQIV 660
mouse            RSAGGPMCFLMLVPLLLAFGMVPVYVGPPTVFSCFCRQAFFTVCFSVCLSCITVRSFQIV 660

human            CAFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVIVVIGMLATGLSPTTRTDPDDPKIT 716
chimpanzee       CAFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVIVVIGMLATGLSPTTRTDPDDPKIT 716
gorilla          CAFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVIVVIGMLATGLNPTTRTDPDDPKIM 716
orangutan        CAFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVTVVIGMLATGLNPTTRIDPDDPKIM 714
patas_monkey     CVFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVTVVIGMLATGLNPTTRIDPDDPKIM 716
baboon           CVFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVTVVIGMLATGLNPTTRIDPDDPKIM 716
rhesus_monkey    CVFKMASRFPRAYSYWVRYQGPYVSMAFITVLKMVTVVIGMLATGLNPTTRIDPDDPKIM 716
squirrel_monkey  CVFKMASRFPRAYSYWVRYQGSYVSVAFITALKMVTVVISLLATGLNPTTRTDTDDPKIM 710
marmoset         CVFKMASRFPRAYSYWVRYQGSYVSVAFITALKVVTVVISLLATGLNPTTRADTDDPKIM 711
tamarin          CVFKMASRFPRAYSYWVRYQGSYVSVAFITALKMVTVVISLLATGLNPTTRTDTDDPKIM 711
cow              CVFKMARRLPRAYGCWGRYHGPYVFVACVVMLKLVIVASSVLATTTMPTTRVDPDDPRIM 714
dog              CIFKMARRLPRAYGYWVRCHGPYVFVASFMVLKVVIVAGNVLATTANPTARPDPDDPNIM 715
rat              CVFKMARRLPSAYSFWMRYHGPYVFVAFITAIKVALVVGNMLATTINPIGRTDPDDPNIM 720
mouse            CVFKMARRLPSAYGFWMRYHGPYVFVAFITAVKVALVAGNMLATTINPIGRTDPDDPNII 720
                                                  *

human            IVSCNPNYRNSLLFNTSLDLLLSVVGFSFAYMGKELPTNYNEAKFITLSMTFYFTSSVSL 776
chimpanzee       IVSCNPNYRNSLLFNTSLDLLLSVVGFSFAYMGKELPTNYNEAKFITLSMTFYFTSSVSL 776
gorilla          IVSCNPNYRNSLLFNTSLDLLLSVVGFSFAYMGKELPTNYNEAKFITLSMTFYFTSSVSL 776
orangutan        IVSCNPNYRNSLLFNTSLDLLLSVVGFSFANMGKELPTNYNEAKFITLSMTFYFTSSISL 776
patas_monkey     IVSCNPNYRNSLFFNTSLDLLLSVVGFSFAYMGKELPTNYNKAKFITLSMTFYFTSTVSL 774
baboon           IVSCNPNYRNSLFFNTGLDLLLSVVGFSFAYMGKELPTNYNEAKFITLSMTFYFTSSVSL 776
rhesus_monkey    IVSCNPNYRNSLFFNTGLDLLLSVVGFSFAYMGKELPTNYNEAKFITLSMTFYFTSSVSL 776
squirrel_monkey  IISCNPNYRNSLLFNTSLDLLLSVAGFSFAYMGKELPTNYNEAKFITFSMTFYFTSSVSL 770
marmoset         IISCNPNYRNSLLFNTSLDLLLSVVGFSFAYMGKELPTNYNEAKFITFSMTFYFTSSVSL 771
tamarin          IISCNPNYRNSLLFNTSLDLLLSVVGFSFAYMGKELPTNYNEAKFITFSMTFYFTSSVSL 771
cow              ILSCNPNYHRGLLVNTSLDLLLSLLGFGFAYTGKELPTNYNEAKFITFCMTFYFSSSVFL 774
dog              VLSCN--YRRALLFNTSLDLLLSVAGFSFAYMGKELPTNYNEAKFITLCMTFYFTSSVSL 773
rat              ILSCHPNYRNGLLFNTSMDLLLSVLGFSFAYMGKELPTNYNEAKFITLSMTFSFTSSISL 780
mouse            ILSCHPNYRNGLLFNTSMDLLLSVLGFSFAYVGKELPTNYNEAKFITLSMTFSFTSSISL 780

human            CTFMSAYSGVLVTIVDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 836
chimpanzee       CTFMSAYSGVLVTIVDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 836
gorilla          CTFMSAYSGVLVTIVDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 836
orangutan        CTFMSAYSGVLVTIVDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSVIQGYTM 836
patas_monkey     CTFMSAYNGVLVTIMDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 834
baboon           CTFMSAYNGVLVTIMDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 836
rhesus_monkey    CTFMSAYNGVLVTIMDLLVTVLNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 836
squirrel_monkey  CTFMSVYDGVLVTIVDLLVTVFNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 830
marmoset         CTFMSVYDGVLVTIVDLLVTVFNLLAISLGYFGPKCYMILFYPERNTPAYFNSMIQGYTM 831
tamarin          CTFMSVYDGGLVTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 831
cow              CTFMAVYNGVLVTILDLLVTVLNLLGISLGYFGPKCYMILFYPERNTPAYFNSVIQGYTM 834
dog              CTFMSVYDGVLVTILDLLITVLNLLGISFGYFGPKCYMVLFYPERNTQVYFSSMIQGYTM 833
rat              CTFMSVHDGVLVTIMDLLVTVLNFLAIGLGYFGPKCYMILFYPERNTSAYFNSMIQGYTM 840
mouse            CTFMSVHDGVLVTIMDLLVTVLNFLAIGLGYFGPKCYMILFYPERNTSAYFNSMIQGYTM 840

human            RRD- 839
chimpanzee       RRD- 839
gorilla          RRD- 840
orangutan        TRD- 839
patas_monkey     RRD- 837
baboon           RRD- 839
rhesus_monkey    RRD- 839
squirrel_monkey  RRD- 833
marmoset         RRD- 834
tamarin          XXX- 834
cow              RKD- 837
dog              GKD- 836
rat              RKS- 843
mouse            RKS- 843
```

Sequence analysis

After we obtained DNA sequences of the coding regions by PCR, we translated the DNA sequences into protein sequences based on the exon–intron junctions of human *Tas1r2* and *Tas1r3*. The nucleotide and predicted protein sequences were aligned to detect nucleic acid and amino acid variants among the species using the ClustalW program (Thompson et al. 1994) (version 1.82; see Electronic Resources). The accession numbers for the primate sequences that we have obtained and deposited are as follows: Chimpanzee, DQ386295 (*Tas1r2*) and AF545573 (*Tas1r3*); Gorilla, DQ386296 (*Tas1r2*) and AF545574 (*Tas1r3*); Orangutan, DQ386297 (*Tas1r2*) and DQ381398 (*Tas1r3*); Patas monkey, DQ386299 (*Tas1r2*); Baboon, DQ386300 (*Tas1r2*) and DQ381400 (*Tas1r3*); Rhesus monkey, DQ386298 (*Tas1r2*); Squirrel monkey, DQ386301 (*Tas1r2*) and DQ381399 (*Tas1r3*); Marmoset, DQ386302 (*Tas1r2*) and DQ381401 (*Tas1r3*); and Tamarin, DQ386303 (*Tas1r2*). The sequences for nonprimate *Tas1r3* and *Tas1r2* and for rhesus monkey *Tas1r3* were obtained from public domain. For some species (e.g., patas monkey), full-length DNA sequences could not be obtained after multiple rounds of primer design and PCR amplification. Consistent with the greater nucleotide diversity of *Tas1r3* when compared with *Tas1r2*, the PCR using *Tas1r3*-specific primers were more likely to fail. Therefore, complete or nearly complete sequence for the *Tas1r2* gene was obtained for most of the species studied, but *Tas1r3* sequences for patas monkey and tamarin were not determined. Sequence variants that distinguished taster and nontaster species were identified after sequence alignment. Some areas of the genome of several species were difficult to sequence, and therefore, the data for some species are incomplete. There were no systematic difficulties sequencing particular areas, for example, in no cases was one region of the gene missing DNA sequence in many or all the species tested here. The final list included amino acid locations for which no taster species shared the same amino acids with any nontaster species.

Computer-assisted modeling of taste receptor structures

The 3D structures for the amino terminus domain of the mouse and human T1R2 and T1R3 proteins were generated using homology-based techniques. Structures were constructed for the human (aspartame taster) and mouse (aspartame nontaster) sequences. The experimentally determined structure of the rat metabotropic glutamate receptor subtype 1 (rMGR1) in its glutamate-bound (active) conformation (Protein Data Bank [code 1EWK]) was selected as a template. The taste receptors T1R2 and T1R3 belong to the same class of G protein–coupled receptors (GPCRs) as MGR1 and are expected to share their 3D structure. There are several structures available for MGR1 (Kunishima et al. 2000) in 2 free forms (1EWT at pH 8.5 and 1EWV at pH 7.5), bound to an antagonist (1ISS) and bound to its endogenous agonist glutamate (1EWK). The active conformation of the rMGR1 homodimer shows one chain (chain A) in an active-close conformation, whereas the second chain (chain B) is in an active-open state. The close or open assignment is based on the relative positioning of the lobes of the Venus flytrap (VFT) architecture that forms the active binding site in this class of receptors. Because the relative positioning of the VFT may affect the binding of agonists, we modeled each T1R in both active-close and active-open conformations. Homology modeling was guided by a multiple sequence alignment that included 14 sequences of T1R2 and 11 sequences of T1R3 from different species. The program ClustalX (Thompson et al. 1997) was used to generate the alignments between T1Rs and the template (rMGR1) sequence. The template sequence was aligned to each T1R alignment (Figures 1 and 2), which was kept unchanged during the process. This procedure is referred to as ''profile alignment'' in ClustalX. The inclusion of T1R sequences other than the ones being modeled increases the quality of the alignment and, hence, the accuracy of the modeled structure. The secondary structures for the template (actual) and for the human T1R (predicted) were used to set structure-dependent gap penalties. The T1R2 alignment was adjusted at region H318-G334 (hT1R2). This segment was originally aligned to a region of the template lying on the surface of the receptor and would have caused a beta-strand in the template, which is part of a 5-strand beta sheet, to be absent in the model for hT1R2. Therefore, the sequence alignment was adjusted to have the H318-G334 (hT1R2) segment aligned to the beta-strand instead, under the assumption that secondary structure elements are more conserved than unstructured regions on the surface. Model building began by copying the backbone coordinates of equivalent residues from the template onto the model structure. Loops not found in the template structure were added by fragment selection using a library of fragments from known 3D structures of proteins. Insertions/deletions in the modeled structure were regularized through localized energy minimization. Side chains were added using an iterative

Figure 1 ClustalW (1.82) multiple sequence alignment of T1R2. Deduced T1R2 amino acid sequences of 14 animal species. Amino acids that differ between aspartame tasters and nontasters are shown in underlined bold black text with an asterisk (*) at the end of the aligned residues. Amino acids are numbered for each species and are shown to the right of the alignment. Species above the solid black line are aspartame tasters and those below it are nontasters. "X" denote missing sequence data. The accession numbers for the publicly available sequence are as follows: human, BK000151; cow, NW_930951; dog, AY916758; rat, AF127390; mouse, NM_031873. In the color version of this figure, amino acids are in color according to their chemical properties; red, small hydrophobic including those with an aromatic ring; blue, acidic; pink, basic; green, hydroxyl with an amine or that are basic; gray, other. This figure appears in color in the online version of *Chemical Senses*.

rotamer search approach. The complete modeled structure was then fully energy minimized using the CHARMM99 force fields and charges. The resulting model was checked for overall structure quality and validated using experimental data from the literature. Modeling and structural quality checks were performed using the software packages Yasara (www.yasara.org), Quanta (Accelrys, Inc), and MOE (www.chemcomp.com).

Molecular docking

Two libraries of ligands were constructed and docked to each of the 3D T1R models: 1) a decoy library of 100 ligands not expected to have affinity for the receptors. These included 6 bitter tastants, such as naringin, salicin and phenylthiocarbamide, 26 medicinal drugs known to be perceived by humans as bitter (e.g., carisoprodol, darvon, and isoptin), and 68 aminoquinolines (antimalaria chelating agents), most of which are well known for their very strong bitter taste. 2) A virtual library of 4 common natural sugars (D-fructose, dextrose [β-D-glucopyranose], sucrose [β-D-fructofuranosyl-α-D-glucopyranoside], and lactose [4-O-alpha-D-galactopyranosyl-D-galactose] and 1 artificial sweetener [aspartame]). The binding interactions between the generated models and these ligands were predicted using an in-house software package called "Orunmila." There are 2 molecular docking protocols implemented in Orunmila: "HierVLS" and "ScanHierDock" (Floriano et al. 2004). HierVLS is a computational protocol that performs a series of steps in order to simulate the molecular docking of each ligand in the virtual library into a potential binding site of the target protein. HierVLS uses a hierarchical scheme that reduces the number of protein-ligand conformers being evaluated from thousands to one in multiple levels of accuracy. The ligands are fully flexible (i.e., all rotatable bonds are allowed to change during docking), and a large number (>10 000) of conformers are generated in the least computationally expensive step for each ligand. Subsequent steps reduce the number of docked conformers, whereas increasing the accuracy of the energy functions used to evaluate binding. ScanHierDock extends the molecular docking to include all potential binding sites within the protein and includes an additional step of all-atoms energy minimization for every ligand-protein complex generated, before the binding energies are calculated. Molecular docking of tastants to binding sites other than the assumed active (orthosteric) site allows the identification of putative allosteric sites. For T1R2 and T1R3, we refer as "active" or "orthosteric" to the site corresponding to where glutamate is found to be bound in the crystallographic structure of the closely related MGR1. This is consistent with current use of the term in the literature (Wellendorph et al. 2009). Binding energies were estimated for the predicted complexes using the Dreiding force field (Mayo et al. 1990), Gasteiger (Gasteiger and Marsili 1980) charges for the ligands, and CHARMM22 (MacKerell et al. 1998) charges for the proteins. The final binding energies include solvation energies calculated using a Generalized Born implicit solvent model (Zamanakos 2002). We used ScanHierDock to perform binding site scanning with hierarchical molecular docking in all T1R models (T1R2 and T1R3, each in active-close and active-open conformation, for each human and mouse, to a total of 8 modeled structures). Calculated binding energies for the decoy ligands were used to assess whether values could be compared across binding sites within the same model and to determine threshold values to discriminate binders from nonbinders. These results are presented in Table 2 and Figure 3. Calculated binding energies and ligand-bound complexes for the sweet ligands were examined carefully for significance (Table 3) and consistency with experimental data from literature (in Table 4). The aspartame-bound models deemed to be the best representations of the T1R taste receptors bound to aspartame were analyzed to provide insights for the observed inability of mice to respond to this sweetener.

Results

Sequence comparison for T1R2 and T1R3

The amino acid sequence alignments for T1R2 and T1R3 are displayed, respectively, in Figures 1 and 2. The percent of sequence identity at nucleotide and amino acid levels are listed for T1R2 (Table 5) and T1R3 (Table 6). Results of alignments of T1R2 and T1R3 sequences show that these 2 proteins are conserved among species, and the degree of conservation reflects their known phylogenetic relationship, that is, humans and chimpanzees are much more similar in amino acid sequence than are humans and mice. For nucleotide similarity, the *Tas1r3* gene was almost always less conserved compared with the *Tas1r2* gene, but this relationship was not true for amino acid similarity. For amino acids, both proteins had approximately the same degree of similarity. This finding is illustrated by comparing chimpanzee and mouse: the percent identity between nucleotides (73%) and amino acids (73%) is the same for T1R3, but for T1R2, the percent identity in amino acid sequence (70%) is lower than in nucleotide sequence (78%).

Identification of variant sites associated with aspartame taster and nontaster status

From the alignment, we identified 41 DNA variant sites that distinguished aspartame tasters and nontasters, that is, were associated with aspartame taster/nontaster status (these will be referred to as taster/nontaster variant sites throughout the text; Figures 1 and 2). The source of the variations was usually single amino acid substitutions, except for the T1R2 segment aa 348-352 (Table 7), which carries single amino acid substitutions for some nontasters but is a 5 amino acid deletion for others (squirrel monkey, marmoset, and tamarin). Therefore, the T1R2 segment aa 348-352 was considered a single variant site. Variant sites associations were broadly defined to include any pattern of differences when none of the variant

```
human            --MLGPAVLGLSLWALLHPGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPS 58
chimpanzee       --MLGPAVLGLSLWALLHPGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPS 58
gorilla          --MLGPAVLGLSLWALLQPGAGAPLCLSQQLRMKGDYMLGGLFPLGEAEEAGFRSRTRPS 58
orangutan        --MLGPAVLGLSLWALLHSGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPS 58
baboon           --MLRPAVLGLSLWALLHLGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPS 58
rhesus_monkey    --MLCPAVLGLSLWALLHLGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLGSRTRPS 58
squirrel_monkey  --MLGSGVLGLSLWTLLHLRTGAPSCLSRQLKMKGDYVLGGLFPLGEAGEAALHSRTRPS 58
marmoset         --MLGSAVLGLGLWALLHLRTGAPLCLSRQLKMKGDYMLGGLFPLGEAGESAFHSRTRPS 58
cow              MPPAMLGLTFLGLVAALGIRPGAPLCLSQQLSLPGDYILGGLFPLGSADDTGLGDRTQPN 60
dog              --MAGL--MLLSLMALLGLGAGAPLCLSRQLRMQGDYVLGGLFPLGTAEDTGLSDRTQPN 56
rat              --MPGLAILGLSLAAFLELGMGSSLCLSQQFKAQGDYILGGLFPLGTTEEATLNQRTQPN 58
mouse            --MPALAIMGLSLAAFLELGMGASLCLSQQFKAQGDYILGGLFPLGSTEEATLNQRTQPN 58

human            SPVCTRFSSNGLLWALAMKMAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMFLA 118
chimpanzee       SPVCTRFSSNGLLWALAMKMAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLVFLA 118
gorilla          SPVCTRFSSNGLLWALAMKMAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMFLA 118
orangutan        SPVCTRFSSNGLLWALAMKMAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMFLA 118
baboon           SPVCTRFSSNGLLWALAMKVAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMFLA 118
rhesus_monkey    SPVCTRFSSNGLLWALAMKMAVEEINNRSDLLPGLRLGHDLFDTCSEPVVAMKPSLMFLA 118
squirrel_monkey  SLVCTRFSWNGLLWALAMKMAVEEINNRLDLLPGLRLGYDLFDTCSEPTVTMKPSLMFLA 118
marmoset         SLVCTRFSWNGLLWALAMKMAVEEINNRSDLLPGLRLGYDLFDTCSESVVTMKPSLMFLA 118
cow              ATVCTRLSAPGLLWALAVMMAVEEINNAPTLLPGLRLGYDLFDTCSEPVVAMKPSLVFMA 120
dog              ATVCTRFSSLGLLWALAMKMAVEEVNNRSTLLPGLRLGYDLFDTCSEPVVAMKPSLMFMA 116
rat              GILCTRFSPLGLFLAMAMKMAVEEINNGSALLPGLRLGYDLFDTCSEPVVTMKPSLMFMA 118
mouse            SIPCNRFSPLGLFLAMAMKMAVEEINNGSALLPGLRLGYDLFDTCSEPVVTMKSSLMFLA 118

human            KAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELLSARE 178
chimpanzee       KAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELLSARE 178
gorilla          KAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELLSARE 178
orangutan        KADSRDIAAYCNYTQYQPRVLAVIGPHSSELALVTGKFFSFFLMPQVSYGASMELLSARE 178
baboon           KAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELLSARE 178
rhesus_monkey    KADSRDIXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 178
squirrel_monkey  KANSHDIAAYCNYTQYQPRVLAVIGPHSSELALVTGKFFGFFLMPQVSYGASMDLLSTRE 178
marmoset         KANSHDIAAYCNYTQYQPRVLAVIGPHSSELALVTGKFFGFFLMPQVSYGASMDLLSTRE 178
cow              KAGSRSIGAYCDYTQYQPRVLAVIGPHSSEVALVTGKFFSFFLMPQVSYGATTDRLSNRE 180
dog              KAGSCDIAAYCNYTQYQPRVLAVIGPHSSELALITGKFFSFFLMPQVSYGASTDRLSNRE 176
rat              KVGSQSIAAYCNYTQYQPRVLAVIGPHSSELALITGKFFSFFLMPQVSYSASMDRLSDRE 178
mouse            KVGSQSIAAYCNYTQYQPRVLAVIGPHSSELALITGKFFSFFLMPQVSYSASMDRLSDRE 178

human            TFPSFFRTVPSDRVQLTAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIA 238
chimpanzee       TFPSFFRTVPSDRVQLTAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIA 238
gorilla          TFPSFFRTVPSDRVQLTAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIA 238
orangutan        TFPSFFRTVPSDRVQLTAAADLLQQFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIA 238
baboon           TFPSFFRTVPSDRVQLTAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIA 238
rhesus_monkey    TFPSFFRTVPSDRVQLVAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAASRGICIA 238
squirrel_monkey  TFPSFFRTVPSDRVQLVATVELLQQLGWNWVAALGSDDEYGRQGLSIFSGLAAARGICIA 238
marmoset         TFPSFFRTVPSDRVQLMATVELLQQLGWNWVAALGSDDEYGRQGLSIFSGLAAARGICIA 238
cow              TFPSFFRTVPSDRVQATAMVELLRGLHWNWVAASGSDDEYGRQGLGLFSSLANAKGICIA 240
dog              TFPSFFRTVSSDRVQAVAMVELLQELGWNWVAAVGSDDEYGRQGLSLFSSLANARGICIA 236
rat              TFPSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSIFSGLANSRGICIA 238
mouse            TFPSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSIFSSLANARGICIA 238

human            HEGLVPLPRADDSRLGKVQDVLHQVNQSSVQVVLLFASVHAAHALFNYSISSRLSPKVWV 298
chimpanzee       HEGLVPLPRADDSRLGKVQDVLHQVNQSSVQVVLLFASVHAAHALFNYSISSRLSPKVWV 298
gorilla          HEGLVPLPRADDSRLGKVQDVLHQVNQSSVQVVLLFASVHAAHALFNYSISSRLSPKVWV 298
orangutan        HEGLVPLPRADDLRLGKVQDVLHQVNQSSVQVVLLFASVHAAYALFNYSISSRLSPKVWV 298
baboon           HEGLVPLPRANSPLLGKVQEVLHQVNQSSVQVVLLFASARAAHALFSYSISSKLSRKVWV 298
rhesus_monkey    HEGLVPLPRANSPLLGKVQEVLHQVNQSSVQVVLLFASARAAHALFSYSISSKLSRKVWV 298
squirrel_monkey  HEGLVPLPRANSPWVGKVQELLPQLNQTSIQVVLLFASARAAHTFFSHIISRRLSPKVWV 298
marmoset         HEGLVPLPRADGLWVGKVQELLPQVNQSSIQVVLLFASTHAAHAFFRHSINRRLLPKVWV 298
cow              YEGLMPLPRAGGTRLGSVQSLLHQVNHSSVQVVVVFSSAQATYSLFSYSIRYRLSPKVWV 300
dog              HEGLVPLPHTSSLRLGTVQGLLHQVNQSSVQVVVLFSSTRAARTLFSYSIHCRLSPKVWV 296
rat              HEGLVPQHDTSGQQLGKVVDVLRQVNQSKVQVVVLFASARAVYSLFSYSILHDLSPKVWV 298
mouse            HEGLVPQHDTSGQQLGKVLDVLRQVNQSKVQVVVLFASARAVYSLFSYSIHHGLSPKVWV 298

human            ASEAWLTSDLVMGLPGMAQMGTVLGFLQRGAQLHEFPQYVKTHLALATDPAFCSALGERE 358
chimpanzee       ASEAWLTSDLVMGLPGMAQMGTVLGFLQRGAQLHEFPQYVKTHLALAADPAFCSALGERE 358
gorilla          ASEAWLTSDLVMGLPGMAQMGTVLGFLQRGAQLHEFPQYVKTHLALAADPAFCSALGERE 358
orangutan        ASEAWLTSDLIMGLPGMAQVGTVLGFLQKGAQLHEFSQYVKTHLALAADPAFCAALGERE 358
baboon           ASEAWLTSDLVMGLPGMAQVGTVLGFLQRGAQLHKFSQYVKTRLALAADPVFCATLGERE 358
rhesus_monkey    ASEAWLTSDLVMGLPGMAQVGTVLGFLQRGAQLRKFSQYVKTRLALAADPAFCAALGERE 358
squirrel_monkey  ASEAWLTSDLVMGLPGMAEVGTVLGFLQKGAQLPEFSQYVKTHLALAADPAFCTSLGERE 358
marmoset         ASEAWLTSDLVMGLPGMAEVGTVLGFLQRGAQLPKFPQYVKTHLALAADPAFCASLSERE 358
cow              ASEAWLTSSLVMTLPGMDRVGTVLGFLHQGAEMPEFRSYVQTRLARAAEPAYCASLEAGR 360
dog              ASEAWLTSDLVMTLPGMAEVGTVLGFLQQGAPIPEFPSYVQTCLALAADPAFCASLDAEQ 356
rat              ASESWLTSDLVMTLPNIARVGTVLGFLQRGALLPEFSHYVETRLALAADPTFCASLK-AE 357
mouse            ASESWLTSDLVMTLPNIARVGTVLGFLQRGALLPEFSHYVETHLALAADPAFCASLN-AE 357

human            QGLEEDVVGQRCPQCDCITLQNVSAGLN-------HHQTFSVYAAVYSVAQALHNTLQC 410
chimpanzee       QGLEEDVVGQRCPQCDCITLQNVSAGLN-------HHQTFSVYAAVYSVAQALHNTLQC 410
gorilla          QGLEEDVVGQRCPQCDCITLQNVSAGLN-------HHQTFSVYAAVYSVAQALHNTLQC 410
orangutan        QGLEEDVVGQRCPQCDCITLQNVSAGLN-------HHQMFSVYAAVYSVAQALHNTLQC 410
baboon           QGLEEDVVGRRCPQCDCITLQNVSAGLN-------HHQTFSVYAAVYSVAQALHNTLQC 410
rhesus_monkey    QGLEEDVVGRRCPQCDCITLQNVSAGLN-------HHQTFSVYAAVYSVAQALHNALQC 410
squirrel_monkey  QGLEEHVVGPRCPQCDDITLQNVPARLQ-------HHQTFSVYAAVYSVAQALHNTLGC 410
marmoset         QGLEEHVVGPRCPQCDDVTLQNMSTRLQ-------HHQTFSVYAAVYSVAQALHNTLRC 410
cow              LGLEEHVVGPRCPQCDRASPENVTDGLL-------YHQTFAAYAAVYSVAHALHKAXXX 412
dog              PGLEEHVVGPRCPQCDHVTLEAMSAGLL-------HHQTFAAYAAVYGVAQALHNTLLC 408
rat              LDLEEKVMGPRCSQCDYIMLQNLSSGLMQNLSAGQLHHQIFATYAAVYSVAQALHNTLQC 417
mouse            LDLEEHVMGQRCPRCDDIMLQNLSSGLLQNLSAGQLHHQIFATYAAVYSVAQALHNTLQC 417

human            NASGCPAQDPVKPWQLLENMYNLTFHVGGLPLRFDSSGNVDMEYDLKLWVWQGSVPRLHD 470
chimpanzee       NASGCPAQDPVKPWQLLENMYNLTFHAGGLMLRFDSSGNVDMEYDLKLWVWQGSVPRLHD 470
gorilla          NASGCPAQDPVKPWQLLENMYNLTFHAGGLMLRFDSSGNVDMEYDLKLWVWQGSVPRLHD 470
orangutan        NASGCPAQDPVKPWQLLENMYNLTFHVGGLTLRFNSSGNVDMEYDLKLWVWQGSVPKLHN 470
baboon           NASGCPVQDPVKPWQLLDNMYNLTFHAGGLTPRFNSSGNVDMEYDLKLWVWQGSVPRLHD 470
rhesus_monkey    SASGCPVQDPVKPWQXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 469
squirrel_monkey  NASGCPMQDPVKPWQVLQNMYNMTFHAAGQVLRFDSSGNVDVEYDLKLWVWRGPVPELHN 470
marmoset         NASGCPMQDPVKPWQLLQNMYNMTFHAAGLALRFDSSGNVDVXXXXXXXXXXXXXXXXX 452
cow              XXXXXXXXXXXXXXXLLDNMYNMSFHAYNRTLQFDTSGNVDLAYDLKLWVWRDRMPMLRT 472
dog              NASGCPPREPVRPWQLLENMYNLTFRVRGLALQFDARGNVNMDYDLKLWVWRDLKPELRT 468
rat              NVSHCHTSEPVQPWQLLENMYNMSFRARDLTLQFDAKGSVDMEYDLKMWVWQSPTPVLHT 477
mouse            NVSHCHVSEHVLPWQLLENMYNMSFHARDLTLQFDAEGNVDMEYDLKMWVWQSPTPVLHT 477

human            VGRFNGSLRTERLKIRWHTSDNQKPVSRCSRQCQEGQVRRVKGFHSCCYDCVDCEAGSYR 530
chimpanzee       VGRFNGSLRTERLKIRWHTSDNQKPVSRCSRQCQEGQVRRVKGFHSCCYDCVDCEAGSYR 530
gorilla          VGRFNGSLRTERLKIRWHTSDNQKPVSRCSRQCQEGQVRRVKGFHSCCYDCVDCEAGSYR 530
orangutan        VGGFNGSLWTERLKIRWHTPDNQKPVSQCSRQCQEGQVRRVKGFHSCCYDCVDCKAGSYR 530
baboon           VGRFNGSLRTERLKIRWHTSDNQKPVSRCCQEGQVRRVKGFHSCCYDCVDCEAGSYR 530
rhesus_monkey    XXXXXXSLWIDSPKIRWHTSNNQKPVSQCSRQCQEGQVRRVKGFHSCCYDCVDCKAGSYR 529
squirrel_monkey  VGIFNGSLWPERLKMRWHTPDNQEPVSQCSRQCQEGQVRRVKGFHSCCYDCVDCEAGSYR 530
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              VGSFNGSLELQFSSMIWHTPGNQEPVSQCSRQCREGQVRRVKGFHSCCYDCVDCKAGSYQ 532
dog              VGAFNGRLKVWHSQMSWHTPGNQRPVSQCSRQCGEGQVRRVKGFHSCCYDCVDCKAGTYQ 528
rat              VGTFNGTLQLQHSKMYWP--GNQVPVSQCSRQCKDGQVRRVKGFHSCCYDCVDCKAGSYR 535
mouse            VGTFNGTLQLQQSKMYWP--GNQVPVSQCSRQCKDGQVRRVKGFHSCCYDCVDCKAGSYR 535

human            QNPDDIACTFCGQDEWSPERSTRCFRRRSRFLAWGEPAVLLLLLLLSLALGLVLAALGLF 590
chimpanzee       QNPDDIACTFCGQDEWSPERSTRCFRRRSRFLAWGEPAVLLLLLLLSLALGLVLAALGLF 590
gorilla          QNPDDVTCTSCGQDEWSPERSTRCFHRRSRFLAWGEPAVLLLLLLLSLALGLVLAALGLF 590
orangutan        HSPDDLACTFCRQDEWSPERSTRCFRRRSRFLAWGEPAVLLLLLLLSLALGLVLAALGLF 590
baboon           QNPDDIACPFCGQDEWSPERSTRCFRRRSRFLAWGEPAVLLLLLLLSLALGLVLAALGLF 590
rhesus_monkey    KSPDDLACTFCGQEEWSPERSTRCFRRRLRFLAWGESAVLLLLLLFGLALGLVLAALGLF 589
squirrel_monkey  RNPDDPTCTPCRHDQWSPKRSTRCFHRRPRFLTWGEPAVLLLLLLLGLALGLVLATLGLF 590
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              RHPDDALCSKCDQDQWSPDGSTRCFPRRPRFLAWGEPAVLGLLLLLGIVLGLVLVALGLF 592
dog              RSPDDLLCTQCDQNQWSPDRSTRCFPRRLTFLAWGQPAVLVLLILLALALGLVLVALGLF 588
rat              KHPDDFTCTPCGKDQWSPEKSTTCLPRRPKFLAWGEPAVLSLLLLLCLVLGLTLAALGLF 595
mouse            KHPDDFTCTPCNQDQWSPEKSTACLPRRPKFLAWGEPVVLSLLLLLCLVLGLALAALGLS 595

human            VHHRDSPLVQASGGPLACFGLVCLGLVCLSVLLFPGQPSPARCLAQQPLSHLPLTGCLST 650
chimpanzee       IHHRDSPLVQASGGPLACFGLVCLGLVCLSVLLFPGQPSPARCLAQQPLSHLPLTGCLST 650
gorilla          VHHRDSPLVQASGGPLACFGLVCLGLVCLSVLLFPGQPSPAQCLAQQPLSHLPLTGCLST 650
orangutan        IRHRDSPLVRASGGPLACFGLVCLGLVCLSVLLFPGRPGTARCLAQQPLSHLPLTGCLST 650
baboon           VHHRDSPLVQASGGPLACFGLVCLGLVCLSVLFPGQPSPARCLAQQPLSHLPLTGCLST 650
rhesus_monkey    IRHRDSPLVQASGGPLACFGLVCLGLVCISVLLFPGQPSPARCLAQQPSSHLPLTGCLST 649
squirrel_monkey  IRHRDSPLVQASGGALACFGLVCLGLVCLSVLLFPGQPSPARCLAQQPLSHLPLTGCLST 650
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              TWHRDSPLVQAAGGPRACFGLACLGLVCLSVLLFPGRPSTASCMGQQLLLHLPLTGCLST 652
dog              IRHRDSPLVQASGGPRACFGLACLGLVCLSVLLFPGQPGPASCLAQQPLLHLPLTGCLST 648
rat              VHYWDSPLVQASGGSSLFCFGLICLGLFCLSVLLFPGRPSASCLAQQPMAHLPLTGCLST 655
mouse            VHHWDSPLVQASGGSQFCFGLICLGLFCLSVLLFPGRPSSASCLAQQPMAHLPLTGCLST 655

human            LFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTD 710
chimpanzee       LFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTD 710
gorilla          LFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTD 710
orangutan        LFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTD 710
baboon           LFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTD 710
rhesus_monkey    FILQAAEIFAESELPLSWADRLSGCLRGPWAWLVVLLAMLVEAALCAWYLVAFPPEVVTD 709
squirrel_monkey  LFLQAAETFVESELPPSWADRLWGCLRGPRAWLAVLLAMLVEAALCAWYLLTFPPEVVTD 710
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              LFLLAAEIFVGSELPPSWTDWLHSCLRGPWAWLVVLLAMLAEAALCSWYLAVFPPVVVTN 712
dog              LFLQAAQIFVGSELPSSWADQLRRCLQGPWAWLLVLLALLAEAALCAWYLVAFPPEVVTD 708
rat              LFLQAAEIFVESELPLSWANWLCSYLRGLWAWLVVLLATLVEAALCAWYLMAFPPEVVTD 715
mouse            LFLQAAETFVESELPLSWANWLCSYLRGLWAWLVVLLATFVEAALCAWYLIAFPPEVVTD 715

human            WHMLPTEALVHCRTRSWVSFGLAHATNATLAFLCFLGTFLVRSQPGRYNRARGLTFAMLA 770
chimpanzee       WHMLPTEALVHCRTRSWVSFGLAHATNATLAFLCFLGTFLVRSQPGRYNRARGLTFAMLA 770
gorilla          WHMLPTEALVHCRTRSWVSFGLAHATNATLAFLCFLGTFLVQSRPGRYNRARGLTFAMLA 770
orangutan        WHILPTEALVHCRTRSWVSFGLAHATNATLAFLCFLGTFLVQSRPGRYNRARGLTFAMLA 770
baboon           WHMLPTEALVHCRTRSWVSFGLVHATNATLAFLCFLGTFLVQSRPGRYNRARGLTFAMLA 770
rhesus_monkey    WRMLPTEALVHCRTRSWVSFGLVHATNATLAFLCFLGTXXXXXXXXXXXXXXXXXXXXXX 769
squirrel_monkey  WRVLPTEALVHCRTRSWVSFGLVHTTNAILAFLCFLGTFLVQSQPGRYNRARGLTFAMLA 770
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              WHALPTEALVHCRVSSWIMFGVVHATNAMLAFLCFLGTFLVQSRPGHYNSARGLTFAMLA 772
dog              WWVLPTQVLVHCRMRSWISFGLLHAINAMLAFLCFLGTFLVQSRPGRYNGARGLTFAMLA 768
rat              WQVLPTEVLEHCRMRSWVSLGLVHITNAVLAFLCFLGTFLVQSQPGRYNRARGLTFAMLA 775
mouse            WSVLPTEVLEHCHVRSWVSLGLVHITNAMLAFLCFLGTFLVQSQPGRYNRARGLTFAMLA 775

human            YFITWVSFVPLLANVQVVLRPAVQMGALLLCVLGILAAFHLPRCYLLMRQPGLNTPEFFL 830
chimpanzee       YFITWVSFVPLLANVQVVLRPAVQMGALLLCVLGILAAFHLPRCYLLMWQPGLNTPEFFL 830
gorilla          YFITWVSFVPLLANVQVVLRPAVQMGALLLCVLGILAAFHLPRCYLLIRQPGLNTPEFFL 830
orangutan        YFITWVSFVPLLANVQVVLRPAVQMGALLLCVLGILAAFHLPRCYLLMRQPGLNTPEFFL 830
baboon           YFITWVSFVPLLANVQVVLRPAVQMGALLLCVLGILAAFHLPRCYLLVRQPELNTPEFFL 830
rhesus_monkey    XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 829
squirrel_monkey  YFITWVSFVPLLANVEVALRPAVQMGAFLLCTLGILAAFHLPRCYLLWQPGLNTPEFFL 830
marmoset         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
cow              YFITWVSYIPLFANVHVVSHPPVQMGTILFCVLGILTTFHLPKCYLLLWRPDLNTPEFFL 832
dog              YFITWISFVPLFANVHVAYQPTVQMAAILLCALGILATFHLPKCYLLLQQLELNNPEFFL 828
rat              YFIIWVSFVPLLANVQVAYQPAVQMGAILFCALGILATFHLPKCYVLLWLPELNTQEFFL 835
mouse            YFITWVSFVPLLANVQVAYQPAVQMGAILVCALGILVTFHLPKCYVLLWLPKLNTQEFFL 835

human            GGGPGDAQGQNDGNTGNQGKHE-- 852
chimpanzee       GGGPGDAQGRNDGDTGNQGKHE-- 852
gorilla          GGGPGDAQGRNDGDTGNQGKHE-- 852
orangutan        GGGPGDAQGRNDGDTGNQRKHE-- 852
baboon           GRGPGDARDRNDGDTGNQGKHE-- 852
rhesus_monkey    XXXXXXXXXXXXXXXXXXXXXX-- 852
squirrel_monkey  GGAQIPVGMVVGTEEAQGKNE-- 852
marmoset         XXXXXXXXXXXXXXXXXXXXXX-- 
cow              GGGPSDARGQGGSGHGETQGKNK 856
dog              G---DDARGGGSSGSGGKET---- 845
rat              GRSPKEASDGNSGSSEATRGHSE- 858
mouse            GRNAKKAADENSGGGEAAQGHNE- 858
```

Figure 2 ClustalW (1.82) multiple sequence alignment of T1R3. The accession numbers for the publicly available sequence are as follows: human, BK000152; cow, NW_929081; dog, AY916759; rat, AF456324; mouse, NM_031872. Other details are the same as those given in Figure 1. This figure appears in color in the online version of *Chemical Senses*.

Table 2 Statistical analysis of calculated binding energies (kcal/mol) obtained by molecular docking and force field scoring of 100 decoy ligands to the T1R 3D models

Model	Active site				Allosteric site			
	Mean	Standard error of mean	Standard deviation	Nonbinder/binder threshold	Mean	Standard error of mean	Standard deviation	Nonbinder/binder threshold
Active-close								
hT1R3	−26	1	9	−44	−26	1	6	−38
hT1R2	−27	2	13	−53	−23	1	7	−37
mT1R2	−19	1	5	−29	−28	1	9	−46
Active-open								
hT1R3	−23	1	7	−37	−21	1	8	−37
hT1R2	−17	1	8	−33	−16	1	8	−32
mT1R2	−25	1	7	−39	−24	1	8	−40

These ligands are not expected to have significant binding affinity for the target proteins and, hence, their calculated binding energies represent nonspecific binding interactions. Nonbinder/binder thresholds were calculated as the mean plus 2 times the standard deviation. Calculated binding affinities at or below these thresholds are reasonably expected to represent specific intermolecular interactions. Ligands below these thresholds are expected to bind to the target sites, albeit not necessarily causing biological response.

sites found among taster species were present in any nontaster species. This strategy was adopted because we did not know whether there is a single variant site that determines binding to aspartame or multiple interacting sites in the receptor, and whether acquisition of aspartame sensitivity was a single event in a common ancestor (and thus all sensitive species would have the same variant site), or there were multiple events (and then aspartame sensitivity can be due to different variants at the same site or variants at different sites). We allowed for both possibilities when assessing the amino acid variation in the receptors and therefore did not exclude locations in which one aspartame tasting species differed from another aspartame tasting species, as long as they did not share amino acids in common at that location with nontaster species. Using these criteria, we found 9 variant sites in T1R2 (Table 7) and 32 variant sites in T1R3 (Table 8).

T1R3 is not more variable at the amino acid sequence level than T1R2 among all species assessed here (sequence identities range from 69% to 99% for T1R3 and from 69% to 98% for T1R2), but it makes a lopsided contribution to the variation that parses aspartame tasters from nontasters, with an approximately a 3-fold increase in the variation that separates tasters from nontasters in the T1R3 protein compared with the T1R2 protein. This observation may be an artifact from having only 2 nontaster primate species for the T1R3 protein (squirrel monkey and marmoset), whereas 4 nontaster primate species were used for comparative purposes for the T1R2 protein (patas and tamarin as well as squirrel monkey and marmoset).

Variation in the T1R3 protein is found evenly distributed across its sequence. The most common type of sequence variants associated with aspartame taster status were amino acid substitutions, but a 5 amino acid deletion at human amino acid positions 348–352 of the T1R2 protein was observed in some nontasters. The amino acids were deleted only in the nontasters but not taster primates. The other nontasters, mouse, rat, cow, and dog, did not have the deletion but do instead have amino acid substitutions that distinguish them from tasters at 2 of these 5 positions (349 and 351).

Molecular modeling

Molecular modeling and docking were used to gain insights into the role of the variant sites identified by sequence alignment which are associated with T1R2/T1R3 dimerization and aspartame binding. The goal was to identify structural difference associated to the variant sites that may correlate to taster/nontaster status. We analyzed the distribution of the variant sites throughout the predicted dimer structure (Figure 4), the calculated binding energies for aspartame and natural sugars bound to the various 3D models (Table 3; additional data not shown), and receptor–tastant interactions for the complex structures obtained from molecular docking (Tables 9 and 10; Figure 5, additional data not shown).

Determining nonbinder/binder thresholds

In order to determine threshold values of binding energies for discriminating nonbinding compounds (referred as "nonbinders") from binding compounds (referred as "binders") based on calculated binding energies, we examined the mean values of calculated binding energies for the set of 100 decoy ligands. The rationale is that the calculated binding energies for nonbinder decoys reflect the nonspecific nature of the intermolecular interactions between target receptor and these ligands, whereas binding energies calculated for binders reflect specific protein–ligand interactions. Therefore, it is reasonable to expect that calculated

Table 3 Calculated binding energies[a] (kcal/mol) for aspartame and natural sugars bound to 3D models of the VFT domain of the human T1R2, mouse T1R2, and human T1R3 receptors in the active-close and active-open conformations

Ligand	Active-close[b]		Active-open[b]	
	Active site	Allosteric site	Active site	Allosteric site
hT1R2				
Aspartame	−90	−72	−12	−60
Sucrose	−75	−53	32	−73
Fructose	−67	−38	−26	−63
Dextrose	−74	−49	−43	−46
Lactose	−77	−58	17	−73
mT1R2				
Aspartame	−70	−70	−48	−47
Sucrose		−49		−58
Fructose	−35	−55	−25	−37
Dextrose	−34	−44	−32	−45
Lactose	−50	−50	−39	
hT1R3				
Aspartame	−86	−32	−74	13
Sucrose	−68			−56
Fructose	−55	−40	−44	−56
Dextrose	−61	−41	−33	−40
Lactose	−50		−66	−70

[a]Values below the nonbinder/binder threshold (see Table 2) are underlined. Calculated binding energies above the threshold reflect nonspecific intermolecular interactions and are not expected to be biologically meaningful. Calculated binding energies below the threshold are due to specific intermolecular interactions. Missing binding energies are the result of unfavorable intermolecular interactions (binding energies above 100 kcal/mol) or shallowly bound ligands (buried surfaces below 70%).
[b]Two binding sites are analyzed. The active site is located at the center of the VFT and corresponds to the site where glutamate is found to bind in the experimentally determined structure of rMGR1. The allosteric site is consistent with variant sites identified by DNA analysis and with a mutation site experimentally found to interfere with aspartame response T1R2 (E63K; see Table 4). Based on the calculated energies (more negative is better; positive values indicate unfavorable binding), aspartame binding is more favorable at the allosteric site when the receptor is in an active-open conformation. For an active-close receptor, however, agonist binding is more favorable at the putative active site.

binding energies for binders will have lower values than the mean of binding energies for decoys. Assuming that most (95%) decoy ligands are nonspecific to the target, calculated binding affinities that are better than the decoy mean by 2 standard deviations (Crocker and Algina 1986) can be considered to reflect specific binding. The mean of calculated binding affinities for a set of decoy nonbinders can be used to set threshold values to identify binders. These threshold values (mean plus 2 times the standard deviation) are shown in Table 2. Ligands with calculated binding affinity at or below (more negative) these thresholds present specific binding interactions to the site and are, thus, expected to bind to the target receptor. Supporting this rationale, the calculated binding energies for natural sugars (Table 3), sucrose and dextrose, believed to bind to the VFT domain of hT1R2 and hT1R3 (Xu et al. 2004; Jiang, Cui, Zhao, et al. 2005; Nie et al. 2005; Zhang et al. 2010) are consistently below the nonbinder/binder thresholds for that site. These thresholds were used to assess whether the binding energies for aspartame bound to the putative active and allosteric sites were indicative of specific intermolecular interactions and not the result of nonspecific binding. Values below the threshold for each site/model are underlined in Table 3.

Comparative analysis of calculated binding affinities

Many studies have compared calculated binding energies across multiple protein models representing closely related proteins (Wang and Wade 2001; Murcia et al. 2006; Henrich et al. 2010) or the same protein in different species (Fratev and Benfenati 2008; Tamamis et al. 2010). However, there is no consensus at the present on how one can determine when comparative analysis of calculated binding energies can be performed with reasonable confidence. In our analysis, we compare calculated binding affinities for the same ligand bound with different sites within the same target protein in different structural conformations. It is reasonable to assume that these energies can be compared since they are calculated for equivalent systems (same atoms, different coordinates). Moreover, the means of binding energies for the decoy ligands across multiple binding sites and multiple conformations of the receptors are within standard deviation of each other (Figure 3), consistent with the nonspecific nature of the intermolecular interactions that generated them. The means of binding energies for natural sugars are consistently lower than for decoys, which reflect the higher specificity of these ligands for the receptors (Table 3, Figure 3). These observations increase confidence in the validity of the comparative analysis of binding energies we present below. A caveat, however, is that although force-field-based binding energies can be used to rank ligands according to their affinity for the target protein, their absolute values are not expected to correspond to experimental binding constants (Ferrari et al. 2007; Grigoriev et al. 2007). This is mostly due to intrinsic exclusion of entropic contributions (Gilson and Zhou 2007; Hnizdo et al. 2008; Irudayam and Henchman 2009), which vary with the nature of the ligand.

We used the nonbinder/binder thresholds to infer which values of calculated binding energies should be taken as meaningful (i.e., representing specific intermolecular interactions between ligand and receptor). The analysis of binding energies and bound structures (Table 3 and Figure 5, additional

Table 4 T1R binding sites for aspartame and other sweeteners from *in vitro* studies

Compounds	Interact receptors	Results	References
Aspartame	hT1R2	VTFD of hT1R2 is required for aspartame binding	Xu et al. (2004)
Neotame	hT1R3	S144A and E302A of hT1R2 abolish aspartame sensitivity	
Cyclamate	rT1R2		
	rT1R3		
Aspartame	hT1R2	hT1R2 + hT1R3 but not rT1R2 + rT1R3 responses to aspartame	Li et al. (2002)
Neotame	hT1R3		
Cyclamate	rT1R2		
37 other compounds	rT1R3		
Aspartame	hT1R2	Response to brazzein requires hT1R3 residues 536-545 (cysteine-rich region; hT1R2 + mT1R3 but not mT1R2 + hT1R3 responds to aspartame	Jiang et al. (2004)
Brazzein	mT1R2		
Other compounds	hT1R3		
	mT1R3		
Aspartame	hT1R3	A733 in hT1R3 is required for lactisol sensitivity	Jiang, Cui, Zhao, et al. (2005)
Lactisol	hT1R2, mT1R2, mT1R3		
Cyclamate	hT1R3	TMD of hT1R3 determines responsiveness of cyclamate.	Jiang, Cui, Zhao, Snyder, et al. (2005)
Aspartame	hT1R2	E63K of hT1R2 showed reduced activity for D-Tryp and aspartame.	Jiang, Cui, Ji, et al. (2005)
D-tryptophan, brazzein, sucrose, monellin	hT1R3	D307A and D307N reduce or abolish responses of D-Tryp and aspartame.	Walters et al. (2009)
Trehalose	hT1R3	mT1R3 responses to trehalose	Ariyasu et al. (2003)
Aspartame	mT1R1	rT1R2 + rT1R3 does not respond to aspartame	Nelson et al. (2001)
Other compounds	mT1R2		
	mT1R3		
Aspartame D and L amino acids	mT1R1	hT1R2 + mT1R3 but not mT1R2 + hT1R3 responds to aspartame	Nelson et al. (2002)
	mT1R2		
	mT1R3		
Aspartame	hT1R2	V738A and L735F of the rT1R3 mediate insensitivity to lactisole	Winnig et al. (2005)
Lactisol	hT1R3		
	rT1R2		
	rT1R3		
Aspartame	hT1R2	hT1R2 rescues the mouse's response to aspartame	Zhao et al. (2003)
	mT1R1		
	mT1R2		
	mT1R3		

Table 4 Continued

Compounds	Interact receptors	Results	References
Sucrose	hT1R2	R383A, E302A, D278A, D142A, Y103A, and S40A abolish response; P277A and D307A significantly reduce response; K65A reduces response to sucralose but not to sucrose	Zhang et al. (2010)
Sucralose			

The prefix h, r, or m before the receptor protein designation refers to the human, rat, or mouse receptor sequence, respectively. All compounds listed taste sweet to humans or are sweet-blockers (lactisole). All studies used one of several lines of human embryonic kidney cells. Amino acid changes are denoted by their single letter codes followed by the species-appropriate position. The cysteine-rich region links the VFT domain (VFTD) on the N-terminus to the transmembrane domains (TMDs) and C-terminus of the protein. When receptors are linked by a + sign, this indicates that coexpression and presumably dimerization is required for function.

Table 5 Percent of sequence identity among pairs of species for the T1R2 gene and protein

Species	Human	Chimpanzee	Gorilla	Orangutan	Baboon	Rhesus monkey	Squirrel monkey	Marmoset	Cow	Dog	Rat	Mouse
Human		*98*	*98*	*95*	*92*	*91*	*89*	*86*	*70*	*76*	*71*	*69*
Chimpanzee	**99**		*99*	*96*	*92*	*91*	*88*	*86*	*70*	*76*	*71*	*70*
Gorilla	**99**	**99**		*96*	*92*	*92*	*88*	*87*	*70*	*76*	*71*	*69*
Orangutan	**96**	**96**	**96**		*93*	*92*	*89*	*87*	*71*	*76*	*71*	*70*
Baboon	**94**	**94**	**94**	**94**		*99*	*89*	*87*	*71*	*76*	*72*	*71*
Rhesus monkey	**94**	**94**	**94**	**94**	**99**		*89*	*87*	*71*	*76*	*72*	*71*
Squirrel monkey	**92**	**92**	**92**	**92**	**92**	**92**		*92*	*70*	*75*	*71*	*70*
Marmoset	**90**	**90**	**91**	**90**	**90**	**90**	**94**		70	*74*	*70*	*69*
Cow	**77**	**77**	**77**	**77**	**76**	**76**	**77**	**76**		*72*	*65*	*66*
Dog	**83**	**83**	**83**	**83**	**83**	**83**	**83**	**81**	**79**		*71*	*71*
Rat	**78**	**78**	**78**	**78**	**79**	**79**	**79**	**78**	**72**	**79**		*91*
Mouse	**78**	**78**	**77**	**78**	**78**	**78**	**79**	**77**	**73**	**79**	***91***	

Upper right-hand cells (italics) contain deduced amino acid identity; lower left cells (bold) contain nucleotide identity. Patas monkey and tamarin, for which extended areas of the gene were refractory to sequencing, were excluded from this analysis.

Table 6 Percent of sequence identity among pairs of species for the T1R3 gene and protein

Species	Human	Chimpanzee	Gorilla	Orangutan	Baboon	Squirrel monkey	Marmoset	Cow	Dog	Rat	Mouse
Human		*99*	*98*	*95*	*95*	*84*	*84*	*69*	*75*	*73*	*72*
Chimpanzee	**98**		*98*	*95*	*95*	*84*	*85*	*69*	*75*	*73*	*73*
Gorilla	**98**	**98**		*94*	*94*	*84*	*85*	*69*	*75*	*73*	*73*
Orangutan	**95**	**95**	**95**		*93*	*84*	*84*	*70*	*76*	*73*	*73*
Baboon	**96**	**96**	**95**	**94**		*84*	*84*	*69*	*75*	*73*	*73*
Squirrel monkey	**88**	**88**	**88**	**88**	**88**		*92*	*68*	*74*	*72*	*72*
Marmoset	**88**	**88**	**88**	**87**	**88**	**93**		*65*	*75*	*70*	*71*
Cow	**77**	**77**	**76**	**76**	**76**	**76**	**72**		*75*	*68*	*67*
Dog	**78**	**79**	**79**	**77**	**77**	**78**	**78**	**80**		*73*	*73*
Rat	**75**	**75**	**75**	**75**	**75**	**75**	**73**	**72**	**75**		*92*
Mouse	**73**	**73**	**73**	**73**	**74**	**75**	**74**	**72**	**74**	**93**	

Upper right cells (italics) contain deduced amino acid identity; lower left cells (bold) contain nucleotide identity. See the caption of Table 5 for other details.

Table 7 T1R2 variant sites associated with aspartame taster/nontaster status

Position[a]	Species name													
	Tasters							Nontasters						
	Hu	Ch	Go	Or	Pa	Ba	Re	Sq	Ma	Ta	Co	Do	Ra	Mo
67	I	I	I	I	I	I	I	S	S	S	L	L	L	L
175	V	V	V	V	X	V	V	Q	Q	X	X	Q	R	R
228	R	R	R	R	R	R	R	G	G	G	H	T	T	T
248	N	N	N	N	N	N	N	D	D	D	T	V	V	A
259	T	T	T	T	T	T	T	S	S	S	A	A	N	N
286	N	N	N	N	N	N	N	R	R	R	R	R	H	R
348	P	P	P	P	P	P	P	–	–	–	P	P	P	P
349	P	P	P	P	P	P	P	–	–	–	A	E	V	M
350	L	L	L	L	L	L	L	–	–	–	L	P	P	P
351	S	S	S	G	S	S	S	–	–	–	N	N	N	N
352	R	R	R	K	R	R	R	–	–	–	R	R	T	E
512	V	V	V	V	I	I	I	T	T	T	P	S	P	P
682	M	M	M	M	M	M	M	V	V	V	V	V	V	V

[a]Position based on human sequences. Hu, human; Ch, chimpanzee; Go, gorilla; Or, orangutan; Pa, palas monkey; Ba, baboon; Re, rhesus monkey; Sq, squirrel monkey; Ma, common marmoset; Ta, tamarin; Co, cow; Do, dog; Ra, rat; Mo, mouse. Missing sequence data are shown by "X." Deletions are shown by "–." The aa 348–352 deletion is considered a single variant site. Thus there are 9 variant sites in T1R2.

data not shown) suggests that, for aspartame and for other natural and artificial sweeteners, the most favorable conformation of T1R2 bound to an agonist is active-close, equivalent to the chain A conformation of the rMGR1 homodimer structure. A secondary energetically favorable site exists for agonist binding in the VFT domain of human and mouse T1R2, in both active-close and active-open conformations. This secondary site is equally or more energetically favorable than the putative active site for all bound sweet tastants in the active-open conformation. This secondary site may be important in the transition from active-open to active-close state. Because changes in conformation upon binding are consistent with allosteric regulation, we will refer to this secondary site as "putative allosteric site" throughout the text. As discussed later, this putative allosteric site is consistent with mutation data (Jiang, Cui, Zhao, et al. 2005; Zhang et al. 2010). Aspartame prefers to bind to the site of human T1R3 corresponding to the glutamate-binding site in rMGR1 over all other potential sites identified in the 3D models. Both active-open and active-close conformations of hT1R3 are favorable for aspartame-bound complexes.

Molecular docking to the active site in the active-close conformation of T1R2

Natural sugars (sucrose, fructose, dextrose, and lactose) and aspartame bind more favorably to the active site of the hT1R2 than to any other available pocket in active-close conformation (Table 3 and additional data not shown). There are 22 residues directly (within 4.5 Å from bound ligand) involved in binding in the active site of hT1R2 model. These are: Y103, D142, N143, S144, S165, A166, I167, S168, Y215, R270, V272, V274, F275, S301, E302, S303, A305, T326, R378, L379, S380, and R383 (position numbers relative to human sequence; Table 9). For ligands docked to the mouse T1R2 in the active-close conformation, the active site residues involved in direct binding did not include S144, S168, A305, T326, and L379. All the other residues found in the human aspartame-hT1R2 complex were also found in the mouse complex. These 22 positions are all very well conserved among the species represented here. Tasters have identical amino acids in all these positions, except for S168, which is a G in Chimpanzee. Among nontasters, some conserved variations are observed in positions 142, 165, 168, 274, and 275. Notably, the most variability in the active site is observed at position 378, which is an R in human and other species, M in dog and mouse, I in rat, and T in cow. The high degree of conservation of these 22 positions is consistent with a role in agonist binding. Variations in these positions may explain differences in sensitivity or preference for different natural sugars observed among species. However, it is unlikely to fully account for aspartame taster/nontaster status. Based on the predicted binding mode for aspartame (Figure 5), the positions most likely to alter affinity for this

Table 8 T1R3 variant sites associated with aspartame taster/nontaster status

Position[a]	Species name											
	Tasters						Nontasters					
	Hu	Ch	Go	Or	Ba	Re	Sq	Ma	Co	Do	Ra	Mo
4	P	P	P	P	P	P	S	S	L	L	L	L
60	P	P	P	P	P	P	L	L	T	T	I	I
172	E	E	E	E	E	E	D	D	D	D	D	D
176	A	A	A	A	A	A	T	T	N	N	D	D
197	A	A	A	A	A	A	T	T	M	M	V	V
198	A	A	A	A	A	A	V	V	V	V	V	V
228	A	A	A	A	A	A	G	G	S	S	G	S
290	S	S	S	S	S	S	R	R	Y	C	H	H
317	Q	Q	Q	Q	Q	Q	E	E	R	E	R	R
332	H	H	H	H	H	R	P	P	P	P	P	P
353	A	A	A	A	T	A	S	S	S	S	S	S
364	D	D	D	D	D	D	H	H	H	H	R	H
386	N	N	N	N	N	N	Q	Q	L	L	M	L
438	G	G	G	G	G	X	A	A	Y	R	R	R
464	S	S	S	S	S	–	P	X	R	L	P	P
467	R	R	R	K	R	–	E	–	M	E	V	V
473	R	R	R	G	R	–	I	–	S	A	T	T
480	T	T	T	T	T	I	P	–	L	V	L	L
485	I	I	I	I	I	I	M	–	M	M	M	M
494	K	K	K	K	K	K	E	–	E	R	V	V
540	F	F	S	F	F	F	P	–	K	Q	P	P
545	E	E	E	E	E	E	Q	–	Q	Q	Q	Q
673	S	S	S	S	S	S	W	–	H	R	C	C
713	M	M	M	I	M	M	V	–	A	V	V	V
739	T	T	T	T	T	T	I	–	M	M	V	M
798	L	L	L	L	L	–	F	–	I	I	I	I
818	M	M	I	M	V	–	L	–	L	L	L	L
835	G	G	G	G	G	–	I	–	S	D	K	K
842	D	D	D	D	D	–	V	–	G	S	S	S
844	N	D	D	D	D	–	T	–	G	G	S	G
845	T	T	T	T	T	–	E	–	H	S	S	G
847	N	N	N	N	N	–	A	–	E	G	A	A

[a]Position based on human sequences. See caption for Table 7 for other details.

sweetener are R378 (anchors aspartame's carboxyl group) and E302 (charge–charge interaction to aspartame's amine group). This is consistent with experimental results showing that the mutation of E302 to A abolishes response to aspartame (Xu et al. 2004). S144 was also found to abolish aspartame response experimentally when mutated to A (Xu et al. 2004). Another position of interest is D142, which is the only difference in the putative active site between tasters

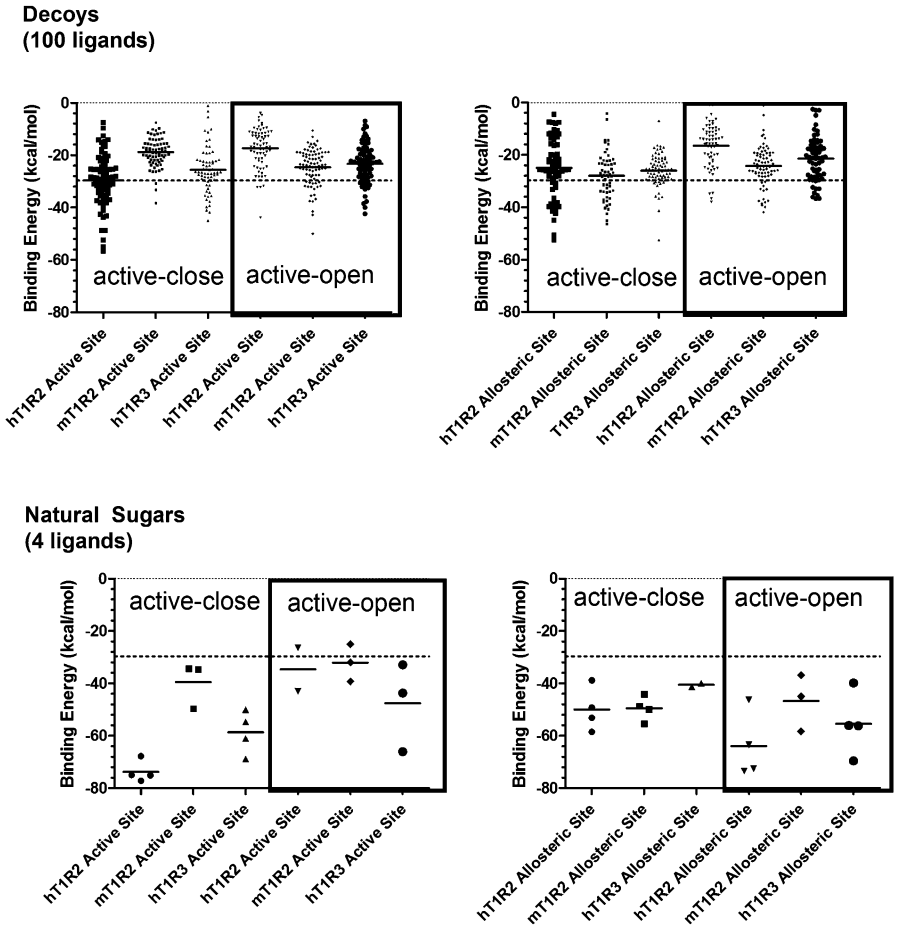

Figure 3 Calculated binding energies (kcal/mol) for 100 decoy ligands and 4 natural sugars docked to the active-close and active-open conformations of the human T1R2, mouse T1R2, and human T1R3 receptors. Decoy ligands are not expected to bind, whereas natural sugars are expected to bind to these receptors. The mean values of binding energies are marked as horizontal lines within the cluster of points corresponding to a particular site/conformer/receptor. Positive values of binding energies are nonfavorable and, hence, were omitted. Ligands with a percentage of buried surface above 70% were eliminated. The lowest mean of the binding energies for the decoy ligands is marked as dashed line in both the decoys and natural sugar graphs, for reference. As expected, the mean binding energies for sugars are consistently lower than for decoys.

and primate nontasters squirrel monkey, marmoset, and tamarin. A more recent study (Zhang et al. 2010) mapping the binding of sweet taste enhancers onto the VFT domain of T1R2 found that mutations Y103A, D142A, E302, and R383 abolish response to sucrose. These positions are all present in our predicted active-close active site for hT1R2, which increases confidence in the docked models.

Aspartame binding to the putative allosteric site in the active-close conformation of T1R2

According to the molecular modeling results, a secondary energetically favorable site exists for aspartame binding in the VFT domain of human and mouse T1R2 in both active-close and active-open conformations. For human, aspartame binding in the putative allosteric site is less favorable than binding to the active site in the active-close conformation. In contrast, this site in mouse T1R2 in the active-close conformation is as favorable for binding as the active site (Table 3). The residues comprising this allosteric site are: F27, L41, H42, K60, E61, Y62, E63, Y69, E340, W341, R352, T353, S354, Q355, and S356 (positions relative to human sequence; Table 10). The same residues are found to be involved in binding in independent analysis of mT1R2-ligand complex structures. The positions comprising the putative allosteric site are well conserved among aspartame tasters, except for E340, which is V for palas monkey, baboon, and rhesus monkey, and W341, which is an R for half of the tasters. Interestingly, sequence variability is observed among nontasters for most of these positions. Mutation of E63 to K in human T1R2 was found experimentally to reduce aspartame response (Jiang, Cui, Ji, et al. 2005), which supports the idea that binding to this putative allosteric site is critical for response. Three key replacements (E63N, R352E, and S356R) between human and mouse T1R2 change the orientation and geometry (binding mode) of aspartame bound to the putative allosteric site in human compared with

Table 9 Residues within 4.5 Å of bound aspartame in the active site of modeled T1R2 (active-close conformation)

Position[a]	Species name													
	Tasters							Non-tasters						
	Hu	Ch	Go	Or	Pa	Ba	Re	Sq	Ma	Ta	Co	Do	Ra	Mo
103	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
142	D	D	D	D	D	D	D	E	E	E	D	D	D	D
143	N	N	N	N	N	N	N	N	N	N	N	N	N	N
144	S	S	S	S	S	S	S	S	S	S	S	S	S	S
165	S	S	S	S	X	S	S	S	S	S	S	S	S	S
166	A	A	A	A	X	A	A	A	A	A	A	A	A	A
167	I	I	I	I	X	I	I	I	I	I	F	I	I	I
168	S	G	S	S	X	S	S	S	S	S	N	S	S	T
215	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
270	R	R	R	R	R	R	R	R	R	R	R	R	R	R
272	V	V	V	V	V	V	V	V	V	V	V	V	V	V
274	V	V	V	V	V	V	V	V	V	V	V	L	V	I
275	F	F	F	F	F	F	F	F	F	F	F	F	F	F
301	S	S	S	S	S	S	S	S	S	S	S	S	S	S
302	E	E	E	E	E	E	E	E	E	E	E	E	E	E
303	S	S	S	S	S	S	S	S	S	S	S	S	S	S
305	A	A	A	A	A	A	A	A	A	A	A	A	A	A
326	T	T	T	T	T	T	T	T	T	T	T	T	T	T
378	R	R	R	R	R	R	R	R	R	R	T	M	I	M
379	L	L	L	L	L	L	L	L	L	L	L	L	L	L
380	S	S	S	S	S	S	S	S	S	S	S	S	S	S
383	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[a]Position numbers based on human sequence. Undetermined residues are labeled "X." Species abbreviations as in Table 7. Amino acids different from human residues at corresponding position are underlined.

mouse (Figure 5). For example, the carboxyl group of aspartame points toward R352 in hT1R2 but flips toward W341 (which is replaced with R) in the mouse T1R2. These differences in binding mode may confer extra stability to aspartame binding in the mouse T1R2 compared with human. This stronger binding to the allosteric site may compete with binding to the active site which would explain, at least in part, mouse nontaster/taster status.

Positioning of the variant sites in the 3D models and with respect to bound aspartame

The mapping of the taster/nontaster variant positions identified by sequence analysis onto the T1R 3D models (Figure 4) suggests that the positions likely to be responsible for species differences in taste response to aspartame are located within segment P348-R352 (hT1R2) (pictured as a green ribbon in Figure 4) and I67 (hT1R2) at the entry of the active site in the VFT. The segment P348-R352 (hT1R2) is a deletion in some aspartame nontasters (squirrel monkey, marmoset, and tamarin), variable among the other nontasters (PMPNE in mouse, PALNR in cow, PEPNR in dog, and PVPNT in rat, compared with PPLSR in human), and well conserved among all primate tasters. This segment is likely involved in allosteric binding, as discussed later. I67 (hT1R2) is replaced by L in nontasters mouse, rat, cow, and dog and by S in all other nontaster in Figure 2. The conserved replacement I-L is not expected to significantly impact the nature of interactions between ligand and receptor, whereas nonpolar polar I for S replacement may change the dynamics of access to the center of the VFT. Positions most likely involved in dimerization are V175 (hT1R2) and A176 (hT1R3) because of their location and orientation at the dimer interface. These 2 amino acids

Table 10 Residues within 4.5 Å of bound aspartame in the putative allosteric site of modeled T1R2 (active-close conformation)

Position[a]	Species name													
	Tasters							Nontasters						
	Hu	Ch	Go	Or	Pa	Ba	Re	Sq	Ma	Ta	Co	Do	Ra	Mo
27	F	F	F	F	F	F	F	F	F	F	F	F	F	F
41	L	L	L	L	L	L	L	L	L	L	L	L	L	L
42	H	H	H	H	H	H	H	H	H	H	H	H	H	H
60	K	K	K	K	X	K	K	K	K	K	K	K	N	N
61	E	E	E	E	X	E	E	E	E	E	E	K	E	E
62	Y	Y	Y	Y	X	Y	Y	Y	Y	Y	Y	Y	F	Y
63	E	E	E	E	E	E	E	E	E	E	E	E	T	N
69	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
340	E	E	E	E	V	V	V	V	V	V	V	I	V	V
341	W	W	W	R	R	R	R	R	R	R	R	R	R	R
352	R	R	R	K	R	R	R	–	–	–	R	R	T	E
353	T	T	T	T	T	T	T	T	T	T	S	T	T	T
354	S	S	S	S	S	S	S	N	N	N	–	S	N	S
355	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	–	L	L	L
356	S	S	S	R	R	R	R	R	R	R	S	E	R	R

[a]Position numbers based on human sequence. Deletion in the sequences are marked as "–." Undetermined residues are labeled "X." Species abbreviations as in Table 7. Amino acids different from human residues at corresponding position are underlined.

are replaced with R and D, respectively, in the mouse receptor, forming a salt bridge between mT1R2 and mT1R3. This replacement may affect dimerization and/or activation after dimerization. Other nontaster species also display complementary replacements, with V175 (hT1R2) replaced by Q and A176 (hT1R3) replaced by T or N. Both Q-T and Q-N pairs may form hydrogen bonds in the dimer. None of the taster/nontaster variant sites were found to be within 4.5 Å of the best (most energetically favorable) active site-bound aspartame in either T1R2 or T1R3 (Table 9 and Figure 4). However, I67 (hT1R2) at the entry of the active site was found to interact with aspartame in some of the higher energy conformations during molecular docking. Overall, the modeling data suggest that the putative allosteric site competes for binding of aspartame with the active site in mouse T1R2 (similar binding affinities) but not in human (much better binding affinity in the active site) (Table 3). The fact that positions E302 in the active site and E63 in the allosteric site predicted in our molecular docking analyses were found to abolish or reduce aspartame response experimentally (Jiang et al. 2004; Xu et al. 2004) supports the idea that both sites play a role in aspartame-induced activation of the receptor. The other taster/nontaster variants are not involved in receptor–ligand or receptor–receptor interactions, and their role in aspartame response, if any, cannot be assessed from the models without performing further calculations to probe structural stability.

Discussion

Nucleotide and amino acid sequence similarity

We found that the nucleotide homology was consistently higher between any 2 pairs of species for the *Tas1r2* gene compared with the *Tas1r3* gene, but these differences in nucleotides did not lead to the same degree of difference in amino acid similarity (Tables 5 and 6). In other words, *Tas1r3* varies more among species at the nucleotide level but these differences are not translated into a more variable T1R3 protein. This observation is consistent with other types of analysis which suggests that the *Tas1r3* gene has been subject to more purifying selection than the *Tas1r2* gene (Shi and Zhang 2006), which should result in *de novo* mutations in the *Tas1r*3 gene to be more often eliminated by natural selection than for the *Tas1r2* gene. T1R3 may be under pressure of purifying selection because it has at least 2 roles: it combines with the T1R1 protein to make the umami or savory receptor, and it combines with the T1R2 protein to make the sweet receptor. It may also combine with other GPCRs to form receptors for minerals (Tordoff et al. 2008). As a consequence, its structure and,

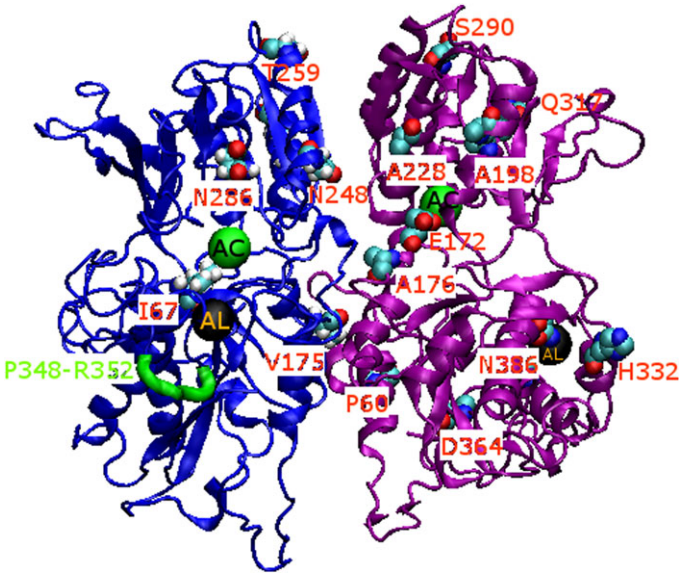

Figure 4 Taster/nontaster variant sites (shown as space-filled representation) and centers of binding regions (shown as green or black spheres) displayed onto the VFT domain of the hT1R2 (active-close)-hT1R3 (active-open) heterodimer. The binding regions at the center of the VFT are referred to as active site (the centers of these regions are shown as green spheres labeled AC), whereas the binding regions near the P348-R352 (hT1R2) segment are referred to as allosteric site (the centers of these regions are shown as black spheres labeled AL). The C-alpha trace for hT1R2 is shown as blue ribbon, whereas hT1R3 is shown in purple. The segment P348-R352 (hT1R2) (shown in green ribbon), which is a deletion in most aspartame nontasters and it is replaced with PMPNE in mouse from PPLSR in human T1R2, is key to the spatial arrangement of the putative allosteric site (its center is shown as a black sphere). Taster/nontaster variant sites V175 (hT1R2) and A176 (hT1R3) are found at the interaction interface between hT1R2 and hT1R3. These amino acids are replaced with R and D, respectively, in the mouse receptors. The introduction of charge interactions and steric effects due to the larger side chains at these positions may affect dimerization and/or activation after dimerization.

therefore, sequence does not tolerate variation, so that it can perform its multiple functions.

Variant sites likely to be responsible for species differences in taste response to aspartame

This study was designed to resolve whether DNA differences in the sweet receptor account for the ability of some species to perceive aspartame as sweet. We identified 41 variant sites that differed among aspartame tasters and nontasters within the 2 sweet receptor genes, any one of which could potentially account for differential response to aspartame. Based upon the modeling work, however, the 5 amino acid segment P348-R352 in T1R2 is the most likely to be responsible for species differences in aspartame taste response. This variation is part of a putative allosteric binding site for aspartame that may compete for binding against the active site in nontaster species that preserve this segment (cow, dog, rat, and mouse). For nontasters with a deletion of this segment, the lack of this allosteric site may prevent the conformational changes which are necessary for dimerization and/or activation of the receptor by aspartame. Our hypothesis is, thus, that binding of aspartame to this allosteric site is a necessary step in receptor activation, but too much affinity for this site will halt the activation process. Other variant sites that may play an important role in aspartame response are I67 (hT1R2) at the entry of the flytrap and the pair V175 (hT1R2)-A176(hT1R3) at the interaction interface of the putative hT1R2/hT1R3 dimer. Their structural positioning suggests that I67 (hT1R2) is involved in the movement of aspartame from the allosteric to the active site, whereas V175 (hT1R2)-A176 (hT1R3) is important for dimer stability.

An alternative binding site for aspartame explains species differences in taste response

The sweet receptor forms a large protein that is characterized by a long N-terminus, thought to form a VFT domain that contains the ligand binding sites for aspartame. Ligand–receptor interactions for aspartame have been studied using 2 methods: computer-assisted modeling, which is used to predict the shape and binding sites for aspartame (Temussi 2002, 2006, 2007; Walters 2002; Jiang, Cui, Ji, et al. 2005; Jiang, Cui, Zhao, et al. 2005; Jiang, Cui, Zhao, Snyder, et al. 2005; Morini et al. 2005; Cui et al. 2006) and cell-based assay systems (Nelson et al. 2001, 2002; Li et al. 2002; Ariyasu et al. 2003; Zhao et al. 2003; Xu et al. 2004; Jiang, Cui, Zhao, et al. 2005; Jiang, Cui, Zhao, Snyder, et al. 2005; Winnig et al. 2005).

Computer methods assume the receptor is similar to the glutamate receptor (chosen because more is known about its exact structure). Previous results using this method are listed in Table 11. None of the variant sites identified in our study map onto key aspartame binding sites suggested by these modeling studies. This observation is also consistent with results of our modeling, where none of the taster/nontaster variant sites was found to be close to the expected active site at the center of the VFT. A possible reason for this lack of agreement is that the response to aspartame is elicited through, or depends upon, a binding site other than that of some natural sugars and sweet proteins.

Cell-based assay results, shown in Table 4, have suggested that T1R2 is more crucial than T1R3 in aspartame sensitivity, and several T1R2 moieties have been identified that are essential for aspartame transduction (S144, E302, E63, and D307). None of the sites identified as essential for aspartame binding in cell-based assays distinguish aspartame tasters and nontasters in the species tested here. All, except E63, are located near the center of the VFT domain, where the closely related MGR1 has the active site for glutamate.

The modeling of the aspartame-T1R2 and aspartame-T1R3 interactions along with the variant sites identified in our work suggests that aspartame may rely on an allosteric site in T1R2 for its activity. According to our 3D models,

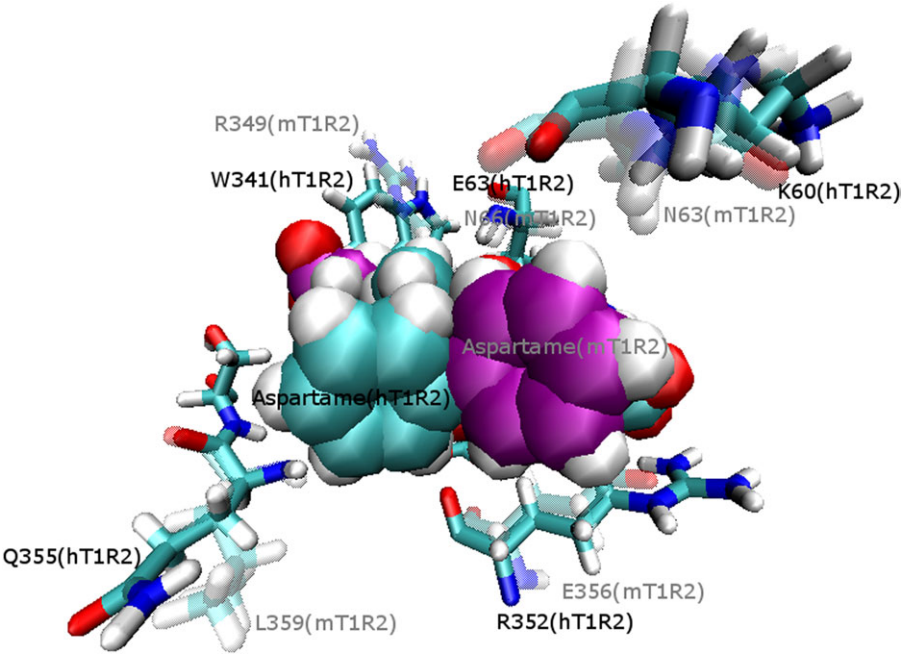

Figure 5 Aspartame (carbon atoms are cyan) bound to the allosteric site of hT1R2 superposed to aspartame (carbon atoms are purple) bound to the allosteric site of mT1R2. Amino acids within 4.5 Å of bound aspartame in hT1R2 are shown in stick representation. The equivalent amino acids in mT1R2 are shown as shadow. Amino acids involved in binding that are identical in mouse and human T1R2 were omitted for clarity. Amino acids E340 and S356 (V and R, respectively, in mouse) are not visible because they are behind the aspartame molecules in this view. Amino acid E63 was found experimentally to reduce aspartame response (see Table 11). E63K (hT1R2) reduces but does not abolish response to aspartame, which is consistent with our proposed model of an allosteric binding site in addition to the active site at the center of the VFT. One of the taster/nontaster variant sites we have identified in hT1R2, R352, is predicted to be directly involved in binding of aspartame into the putative allosteric site. This taster/nontaster variant site corresponds to E356 in mT1R2, which contributes to the difference in geometry and orientation of aspartame into the site, as seen in the picture, and leads to stronger binding of aspartame to the mouse site compared with human.

aspartame binds preferentially and with strong affinity to the active site in human and mouse T1R3. For T1R2, however, a secondary site seems to be energetically favorable for aspartame binding, in addition to the putative active site. For mouse, an aspartame nontaster, the allosteric site is as favorable for binding as the active site in both active-close and active-open conformations. For human T1R2, the allosteric site is preferable in the active-open conformation, whereas the active site is better for binding in the active-close conformation. The fact that many nontasters have a P348-R352 (hT1R2) deletion within this putative allosteric site further supports its involvement in sweet taste response to aspartame. Although there is no evidence for an activation mechanism that combines both sites, we can speculate that aspartame first binds to the allosteric site, inducing conformational changes that make binding to the active site more favorable. The binding of aspartame to the active site then induces activation and response. Whether aspartame migrates from one site to the other or 2 molecules are required for activation are among the questions that require further investigation into this proposed mechanism.

Regardless of the particular mechanism, our variant analysis combined with modeling studies and supported by experimental mutation data suggests that aspartame may act as an allosteric regulator of taste response mediated by T1R2, in addition to the more traditional role of agonist for both T1R2 and T1R3. Allosteric regulation of GPCRs by physiologically relevant ions and small organic molecules has been observed for many GPCRs (May and Christopoulos 2003; Liu et al. 2005; Pin et al. 2005; Winnig et al. 2006; Springael et al. 2007; Conn et al. 2009; Servant et al. 2010).

The curious case of the fruit fly and the lesser panda

One relative of the raccoon, the lesser panda, strongly prefers aspartame (Li et al. 2009) yet does not have the signature sequence of T1R2 of the aspartame tasters species. Similarly, fruit flies have a different types of taste receptors altogether compared with mammals, also strongly prefer aspartame compared with water (Gordesky-Gold et al. 2008). There are at least 2 explanations for these observations: 1) to these species, aspartame may have a pleasant taste which is not sweet, for example, the di-peptide combination may be savory; alternatively, the ability of these species to taste and prefer aspartame may be an example of convergent evolution, in which the phenotype is the same but the mechanism supporting it differs. The motivating force for convergent evolution may be that some foods eaten by these species have compounds with a structural similarity to aspartame.

Table 11 T1R binding sites for aspartame and other sweeteners predicted based on computer modeling

Compounds	Interact receptors	Tools	Predicted binding site	References
Aspartame	hT1R2	SWISS MODEL	Small molecules like aspartame bind to the active site corresponding to the glutamate active site; sweet proteins binds to an active site that is different from the one for glutamate.	Temussi (2002, 2006, 2007)
Brazzein	hT1R3			
Monellin				
Thaumatin				
Aspartame	hT1R2	SWISS MODEL	Four binding sites could be present on heterodimers: 2 sites for small sweet compounds (one in each VFT), one wedge site for sweet proteins, and one site for allosteric modulators in the 7TMD.	Morini et al. (2005)
15 other sweeteners	hT1R3			
Neotame	hT1R3	Quanta program	Neotame, superaspartame and SC-45647 bind to T1R3, interacts with H163, H407, and E318 of this protein.	Walters (2002)
Superaspartame				
SC-45647				
Aspartame	hT1R2	MODELLER	E63 and D307 of hT1R2 interact with brazzein and small molecules like aspartame.	Jiang, Cui, Zhao, et al. (2005)
D-tryptophan	hT1R3			
Brazzein				
Sucrose				
Monellin				
Aspartame	hT1R2	MODELLER	ATD of hT1R2 interacts with aspartame, CRDs of hT1R3 interacts with brazzein, TMD of hT1R3 binds to cyclamate and lactisole.	Cui et al. (2006)
Cyclamate	hT1R3			
Lactisole				

Brazzein, monellin, and thaumatin are large proteins that are sweet to humans. Neotame and superaspartame are structurally similar to aspartame and thought to act at the receptor in the same or similar manner. SC-45647 is a high-potency sweetener. "h" as a prefix before the gene symbol denotes the human form of the receptor. The interactions between aspartame and the specific amino acids of the receptor are shown by the one-letter amino acid code, and the position in the human sequence, for example, H407. VFT domain, the extracellular domain. Seven transmembrane (7TMD) domain, the portion of the receptor that transverses in and out of the taste receptor cell. Wedge site refers the ability of the large proteins to "wedge" open the VFT similar to a large object preventing a door from closing. Allosteric modulators are compounds that bind to the receptor and modulate its activation but do not by themselves cause receptor activation. ATD = amino terminal domain.

Assessing species-related differences in taste response

The assigning of species into aspartame taster and nontaster groups was sometimes done based on behavioral data alone, and in this case, we can know what an animal does but not why. We assume that animals perceive aspartame as sweet when they prefer it to water but they might perceive it as a different but desirable quality, like salty or savory. Therefore, nerve recordings are useful as objective measures because the pattern of firing to aspartame can be compared with sugars like sucrose, but this type of data is not available for all species. Conditioned taste aversion generalization data are another way to try to understand how animals perceive taste quality (Danilova, Hellekant, Tinti, and Nofre 1998). The combination of different types of data would be a more ideal method to chose aspartame taster species because our reliance upon behavioral data alone could be misleading, as it was in the case of the effect of gymnemic acid in the chimpanzee (Hellekant et al. 1996; Hellekant, Ninomiya, and Danilova 1997, 1998).

Evolution of aspartame taste sensitivity

Why humans and closely related primates should find aspartame sweet is puzzling. It is a synthetic di-peptide consisting of aspartic acid and phenylalanine, and it is not known if this small protein exists normally in human food. Thus, it could be by chance that the receptor responds to many

chemically diverse compounds. On the other hand, if aspartame or a structurally similar chemical is found in nature, it is possible that the primate T1R receptor evolved to respond to this compound. Primates that could taste this hypothetical, naturally-occurring aspartame-mimetic compound might have a larger food repertoire and therefore an advantage over nontaster primates. Chemicals like aspartame may exist in edible plants.

Development of novel sweeteners

There is a demand for new high-potency sweeteners with improved characteristics. Many consumers complain about after-taste characteristics or worry about negative health consequences of aspartame consumption, even though recent studies show no association between aspartame intake and cancer risks (Weihrauch and Diehl 2004; Gallus et al. 2007; Bosetti et al. 2009). Finding alternatives to aspartame with improved temporal profiles of taste activation or increased potency (i.e., achieving the same sweetness with a lesser amount) might allay some consumer concerns. Modifications in the structure of aspartame guided by structure–activity relationship studies successfully led to the discovery of neotame, a more potent analog of aspartame (Nofre and Tinti 2000). Findings of this study could guide design of novel sweeteners by modifying the structure of aspartame to take advantage of unsatisfied intermolecular interactions within the binding pocket. We might also use information about the interaction of aspartame with the primate sweet receptor to find naturally occurring plant or animal compounds that have aspartame-like structural characteristics. The discovery and development of a natural source of aspartame might also reduce consumer health concerns.

Concluding remarks/summary

The work undertaken here builds upon the ideas and data of investigators, who demonstrated that humans, Old World monkeys, and apes can perceive aspartame sweetness, whereas New World monkeys and most other mammals cannot (Glaser et al. 1995). Here, we determined that the specific protein variant sites most likely to be responsible for aspartame tasting in mammals are within a putative allosteric binding site. Previous efforts to develop new sweeteners based on aspartames structure have been successful (Nofre and Tinti 2000), and the identification of this allosteric site provides a complementary method for the design of novel sweeteners and sweet taste enhancers.

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