Genetic Approach to Characterize Interaction of Sweeteners with Sweet Taste Receptors In Vivo

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Introduction

Genetic analysis of taste responses has played an important role in identification and characterization of the taste receptors. Genetic mapping and positional cloning of the mouse saccharin preference (Sac) locus resulted in the discovery of the Tas1r3 gene encoding the T1R3 receptor (Bachmanov et al., 2001b). The identification of the Sac locus was based on its phenotypical allelic variants corresponding to sequence variants of the Tas1r3 gene and T1R3 protein (Reed et al., 2004). We used these naturally occurring Tas1r3 variants to characterize Tas1r3 functional polymorphisms and to assess T1R3 ligand specificity.

Functional polymorphisms of the Tas1r3 gene

To identify the Tas1r3 sequence variants associated with saccharin preference, we analyzed sequences of the Tas1r3 region in a variety of inbred mouse strains (Reed et al., 2004). We examined genomic sequences including Tas1r3 exons, introns, and upstream and downstream regions, so that polymorphisms affecting amino acid composition or potential regulatory regions could be detected. To minimize possibility of associations due to common origins, we tested mouse strains with unrelated or distant genealogies. To provide adequate statistical power for detection of phenotype-genotype associations, we analyzed 30 mouse strains. Initially, we sequenced ~6.7 kb of the Tas1r3 gene and its flanking regions from six inbred mouse strains with high and low saccharin preference, including the strains in which the Sac alleles were originally described [C57BL/6J, Sacb'; DBA/2J, Sacc (Fuller, 1974)]. Of the 89 sequence variants detected among these six strains, eight polymorphic sites were significantly associated with preferences for 1.6 mM saccharin. Next, each of these eight variant sites was genotyped in 24 additional mouse strains. Analysis of the genotype-phenotype associations in all 30 strains showed the strongest association with saccharin preference at three sites: nucleotide (nt) −791 (3 bp insertion/deletion), nt +135 (Ser45Ser) and nt +179 (Ile60Thr). To examine the role of the polymorphisms that do not change amino acid sequence of the T1R3 protein, we measured Tas1r2 gene expression in the taste tissue of two inbred mouse strains with different Tas1r3 haplotypes and saccharin preferences. The results of these experiments suggest that the polymorphisms associated with saccharin preference do not affect gene expression, change alternative splicing, or interfere with protein translation in the taste tissue. We conclude that the amino acid substitution (Ile60Thr) may influence the ability of the T1R3 protein to form dimers or bind sweeteners.

Ligand specificity of the T1R3 receptor

The mouse T1R2 and T1R3 combination expressed in a heterologous system responded to sucrose, fructose, dulcin, saccharin, acesulfame, guanidinacetic acid sweeteners, glycine and several D-amino acids, but not to several sugars (glucose, maltose, lactose, galactose, palatinose) artificial sweeteners (N-methyl saccharin, cyclamate, aspartame and thaumatin) or L-amino acids (Nelson et al., 2001; Nelson et al., 2002). The rat T1R2 and T1R3 combination expressed in a heterologous system responded to glucose, fructose, maltose, lactose, dulcin, saccharin, acesulfame, sucralose, d-tryptophan and glycine, but not to aspartame, cyclamate, monellin, neotame or thaumatin (Li et al., 2002). The apparently broader range of responses in rats compared with mice may result from differences in expression systems between these studies. The human T1R2 and T1R3 combination responded to several sweeteners that did not activate rodent receptors, namely aspartame, cyclamate, monellin, neotame and thaumatin (Li et al., 2002). Several aspects of these in vitro studies emphasize importance of an in vivo approach. First, discrepancies between results obtained using different expression systems leave open a question of whether responsiveness or unresponsiveness to a particular sweetener reflects differences in in vivo sensitivity of the receptor, or is an artifact of the in vitro system. Second, responses of the heterologously expressed mouse receptors to amino acids were inconsistent with mouse behavioral responses to these stimuli. For example, sweet L-proline and L-threonine did not activate the T1R2 and T1R3 combination, but instead activated the T1R1 and T1R3 combination, which also responded to some umami-tasting and bitter (e.g. L-phenylalanine) compounds (Nelson et al., 2002).

To characterize ligand specificity of the T1R3 receptor, we assessed how Tas1r3 genotype affects behavioral and neural gustatory responses to a variety of chemically diverse sweeteners. These studies analyzed association of sequence variants of the Tas1r3 gene with taste responses to different sweeteners. They were based on an assumption that if a response to a compound is affected by Tas1r3 genotype, then this compound activates a receptor involving T1R3. We have used several genetic approaches: comparisons of multiple inbred mouse strains, genetic analyses of hybrids between high sweetener-prefering C57BL/6ByJ (B6) mice and low sweetener preferring 129P3/J (129) mice, and experiments with 129.B6-Sac congenic mice. We used several experimental populations because they have different genetic composition. The B6 × 129 F2 hybrids and 129.B6-Sac congenic mice vary only at two Tas1r3 alleles (originating from the B6 and 129 parental strains), while number of Tas1r3 alleles in multiple inbred strains may be larger than two. Variation of sweet taste responses in the 129.B6-Sac congenic strain depends only on the Sac/Tas1r3 locus, while in multiple inbred strains B6 × 129 F2 hybrids is also affected by other genetic loci.

In mice from 28 strains with defined candidate functional Tas1r3 polymorphisms (Reed et al., 2004), we tested preferences for saccharin, sucrose, D-phenylalanine and glycine using two-bottle 48 h tests (Bachmanov et al., 2002). There was a strong association between the Tas1r3 alleles and saccharin and sucrose preferences, a weaker association with D-phenylalanine preferences, and no significant association with glycine preferences.
In the F2 hybrids between the B6 and 129 strains, we determined genotypes of markers on chromosome 4 where Tas1r3 resides, measured consumption of taste solutions presented in the two-bottle preference tests, and recorded integrated responses of the chorda tympani gustatory nerve to lingual application of taste stimuli (Inoue et al., 2004). The taste stimuli were selected based on differences between the parental strains, B6 and 129 (Bachmanov et al., 2001a; Inoue et al., 2001). For intakes and preferences, significant linkages to Tas1r3 were found for the sweeteners sucrose, saccharin and D-phenylalanine, but not glycine. For chorda tympani responses, significant linkages to Tas1r3 were found for the sweeteners sucrose, saccharin, D-phenylalanine, D-tryptophan and SC45647, but not glycine, l-proline, L-alanine or L-glutamate. No linkages to distal chromosome 4 were detected for behavioral or neural responses to non-sweet quinine, citric acid, HCl, NaCl, KCl, monosodium glutamate (MSG), inosine 5'-monophosphate (IMP) or ammonium glutamate.

The 129.B6-Sac congenic mouse strain has been produced using serial backcrossing to introgress a Tas1r3-containing donor chromosomal fragment from the B6 strain onto the genetic background of the 129 strain (Bachmanov et al., 2001b; Li et al., 2001). The 129.B6-Sac congenic mice were tested using 48-h two-bottle tests with concentration series of fourteen sweeteners (Theodorides et al., 2003). Congenic mice that had a copy of the Tas1r3 gene from the B6 strain had higher preferences for sucrose, glucose, maltose, fructose, saccharin, acetaldehyde, sucralose, SC45647, erythritol, D-phenylalanine, D-tryptophan and l-proline, compared with their littermates homozygous for the 129 allele of Tas1r3. Thus, allelic variation of the Tas1r3 gene affects behavioral taste responses to these sweeteners, suggesting they are T1R3 ligands. There were no differences between mice of the two Tas1r3 genotypes in preferences for glycine and L-alanine (or any of the control non-sweet solutions: quinine, citric acid, NaCl, IMP or MSG).

These results demonstrate that allelic variation of the Tas1r3 gene affects gustatory neural and behavioral responses to some but not all sweeteners. Allelic variants of Tas1r3 affected taste responses to sugars, a sugar alcohol, amino acids and artificial sweeteners. Allelic variants of Tas1r3 did not affect taste responses to glycine and L-alanine, even though they have sucrose-like taste to mice (Manita et al., 2004). The results are consistent among tests with multiple inbred strains, B6 × 129 F2 hybrids and 129.B6-Sac congenic strains, and between behavioral and neural responses. These data suggest that a wide variety of sweeteners can activate a receptor involving T1R3. Lack of the effect of the Tas1r3 genotype on glycine and L-alanine taste responses can be explained by several mechanisms: (i) binding to the T1R3 receptor at a site that is not affected by the polymorphic variants; (ii) binding to the T1R2 receptor; or (iii) existence of another sweet taste receptor binding them.

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References


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