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Human Bitter Taste Perception

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Introduction

Bitter taste perception is innate and induces aversive reactions. Since numerous harmful compounds, including secondary plant metabolites, synthetic chemicals, inorganic ions and rancid fats, do taste bitter, this basic taste modality may be considered as a defence mechanism against the ingestion of potential poisons. For a complete understanding of this defence mechanism it is obligatory to identify and characterize the chemical detectors of the bitter compounds, which display the remarkable ability to recognize thousands of different chemicals. Screening of the genome data bases ultimately led to the discovery of a novel gene family of ~40 members in mice and ~30 in humans. Some of the genes identified by this approach are located within chromosomal loci associated with tasting various distinct bitter compounds (Adler *et al.*, 2000; Matsunami *et al.*, 2000; Bufe *et al.*, 2002). Therefore, these genes encoding G-protein-coupled receptors, TAS2Rs (previously referred to as T2Rs or TRBs), have been suggested to represent bitter taste receptors. Several lines of independent evidence further support this assumption (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000). First, the expression pattern of these genes on the rodent tongue was consistent with that of bitter taste receptors. Secondly, functional expression studies identified the bitter compound cycloheximide as an agonist for mTAS2R105. Thirdly, mice strains with impaired cycloheximide tasting have variant mTAS2R105 genes encoding receptors that are less responsive to cycloheximide. Fourthly, mTAS2R105 couples *in vitro* to α-gustducin (Chandrashekar *et al.*, 2000), a G-protein α-subunit expressed in taste tissue that has amply been shown before to play a role in bitter taste transduction (Margolskee, 2002). Although this question has not been directly addressed, these investigations led to the impression of a narrow tuning of bitter taste receptors and raised the intriguing problem of how organisms that are equipped with a limited number of TAS2R genes are able to perceive numerous chemicals bitter.

In the present report we address two questions that are important for the understanding of bitter taste in general and human bitter taste in particular. First, given the largely uncharacterized receptor repertoire, are all TAS2Rs true bitter taste receptors and, secondly, can their broad tuning explain how humans equipped with a fairly small number of TAS2R genes are able to perceive thousands of bitter compounds.

Results and discussion

Although it sounds trivial, as an obligatory criterion a true bitter taste receptor must respond to bitter chemicals but not to compounds that lack the bitter taste. To test this property we employ a functional expression system, in which the TAS2Rs are expressed as recombinant receptors at the cell surface of HEK-293 cells. To this end the cDNAs of all 25 identified human hTAS2Rs were elongated with the amino terminal 45 amino acids of rat somatostatin receptor 3 (sst3) and a carboxy-terminal Herpes Simplex Virus (HSV) glycoprotein D-epitope. The sst3 epitope has recently been shown to facilitate cell surface expression of recombinant receptors (Ammon *et al.*, 2002). The HSV epitope allows immunocytochemical detection of chimeric receptors in transfected cell lines and does not impair the receptor's signaling properties (Roosterman *et al.*, 1997). Receptor activation by bath application of appropriate bitter stimuli would result via the stably expressed G-protein Gα16gust44 (Ueda *et al.*, 2003) and phospholipase C activity in the elevation of intracellular calcium concentrations. These can be recorded by means of the calcium sensitive fluorescence dye Fluo-4 using an automated fluorescence plate reader. In fact, cells transfected with hTAS2R10 cDNA responded in the predicted fashion to administration of strychnine, while mock-transfected cells did not respond (Bufe *et al.*, 2002). Similarly, hTAS2R16 expressing cells responded to salicin, while mock-transfected cells or cells transfected with any other hTAS2R construct did not respond. Subsequent structure–activity considerations identified chemical properties that make a compound an agonist for hTAS2R16. These are a gluco- or mannopyranose moiety and an aglycon attached to it in the β-glycosidic configuration. These studies also revealed that aglycons with one or two aromatic rings display stronger agonist properties than a methyl group and that hydrophilic substitutions in the aglycon impair the potency of the agonists. The oxygen atom bridging the sugar ring and the aglycon can be substituted for by a sulfur atom and an additional glucopyranose can be added to carbon atom 6 without significantly impairing the agonist properties of the compounds. hTAS2R38 shows similar properties. By positional cloning the gene for this receptor has recently been suggested to encode a receptor for phenylthiocarbamide (Kim *et al.*, 2003). Functional expression studies carried out in our laboratory now show that hTAS2R38 is activated by phenylthiocarbamide and many other compounds containing an –N=C=S group in a variety of chemicals, including propylthiourea. Thus TAS2R16 and hTAS2R38 represent receptors for chemicals that share certain molecular properties. They do not detect single specific compounds, but are broadly tuned to chemically related substances. hTAS2R14 may even show a broader tuning, as there appears to be no obvious common structural motif shared by its agonists (Behrens *et al.*, 2004). hTAS2R14 was activated *in vitro* by naphthalaldehydic acid, naphthoic acid, nitronaphthalene, picrotin, picrotoxinin, piperonylic acid, sodium benzoate and α-thujone. Although these chemicals have one or more ring systems and at least one electronegative side-chain in common, these parameters are also shared by many compounds that did not activate hTAS2R14.

What evidence do we have that the TAS2Rs represent cognate human bitter taste receptors? First, there is functional evidence. All of the compounds that activated a TAS2R receptor taste bitter. In addition, in the case of bitter β-glucopyranosides and –N=C=S group containing chemicals, the threshold values of activation and the EC_{50} values recorded *in vitro* closely correspond to those determined in human psychophysical experiments (Meilgaard *et al.*, 1999;

Frank *et al.*, 2001; Bufe *et al.*, 2002). Moreover, substances chemically related to above mentioned compounds, such as α-glucopyranosides and β-galactosides or uracil and phenylurea, do neither activate hTAS2R16 or hTAS2R38 *in vitro,* nor taste comparatively bitter to humans.

Secondly, adaptation and cross-adaptation experiments provide additional evidence. Adaptation means that stimuli elicit reduced responses upon prolonged or repeated stimulus presentations (Torre *et al.*, 1995). Repeated stimulation of TAS2R16-expressing cells with phenyl-β-D-glucopyranoside resulted in largely diminished responses to this and the related β-glucopyranosides, salicin and helicin. This cross-desensitization was fully reversible and resembled homologous desensitization of agonist-occupied heptahelical receptors (Ferguson and Caron, 1998; Bockaert and Pin, 1999). We also observed adaptation in human subjects who initially scored phenyl-β-D-glucopyranoside, salicin and helicin as equally intensely bitter. During prolonged stimulation the bitterness of phenyl-β-D glucopyranoside declined and the subjects perceived salicin and helicin also as less bitter. This adaptation was specific for the β-glucopyranosides, as the unrelated bitter substance denatonium benzoate, which cannot activate TAS2R16, did not show diminished responses. In marked contrast, the phenyl-α-D-glucopyranoside failed to cross-adapt with all tested β-D-glucopyranosides, although its own taste response desensitized strongly. This indicates that β-glucopyranosides signal through a common mechanism, most likely involving TAS2R16 as a bitter taste receptor, while the α-isomer uses a separate pathway.

Thirdly, there is genetic evidence. There are five known haplotypes of hTAS2R38. Subjects differ in their sensitivity of tasting the hTAS2R38 agonist phenylthiocarbamide depending on their haplotype (Kim *et al.*, 2003). Moreover, our functional expression studies correlated phenylthiocarbamide tasting to the ability of the receptor variants to elevate calcium concentrations in transfected cell lines.

Fourthly, there is anatomical evidence. *in situ* hybridization revealed the presence of mRNA for hTAS2R16, hTAS2R38 and hTAS2R14 in a subset of taste receptor cells in taste buds of the human circumvallate papillae.

In summary, the present data clearly show that all characterized TAS2Rs responded to bitter stimuli. They apparently are able to detect many compounds instead of only one. This broad tuning of TAS2Rs may therefore, at least in part, solve the problem of how few receptors mediate the perception of numerous bitter compounds. The increasing evidence thus suggests that TAS2Rs may be equated to bitter taste receptors. Whether bitter taste can be equated to TAS2Rs remains to be seen.

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