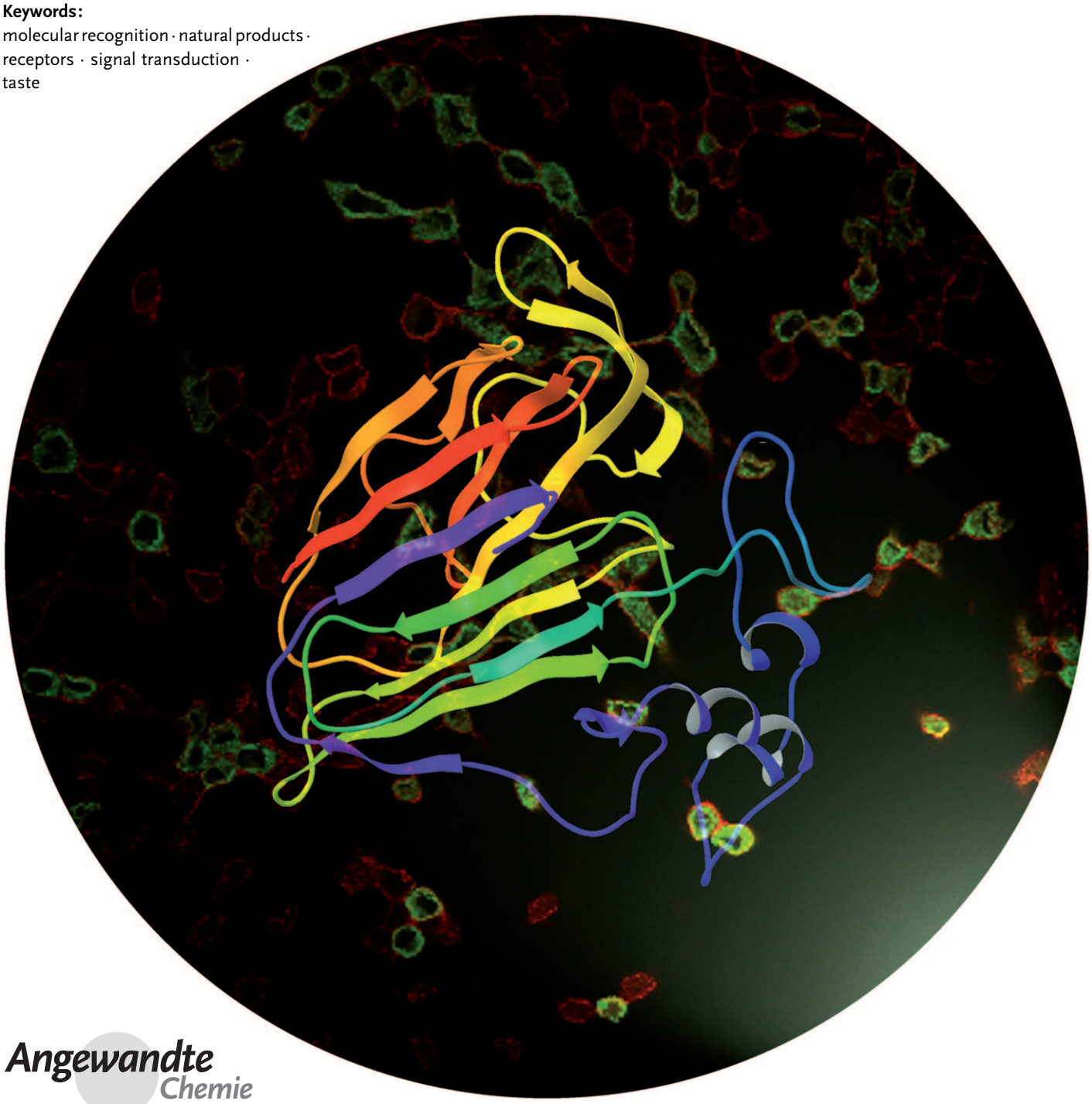
DOI: 10.1002/anie.201002094

Chemistry of Taste

Sweet and Umami Taste: Natural Products, Their Chemosensory Targets, and Beyond

*Maik Behrens, Wolfgang Meyerhof, Caroline Hellfritsch, and Thomas Hofmann**

Keywords:
molecular recognition · natural products · receptors · signal transduction · taste



Much of our appreciation of food is due to the excitement of the perception of "sweet" and "umami" taste. With a special focus on natural products, this Review gives a summary of compounds that elicit and modulate "sweet" or "umami" taste responses. It will be discussed how the interaction of these molecules with the oral sweet and umami taste receptors stimulates receptor cells to secrete neurotransmitters to induce neural activity that is conveyed to the cerebral cortex to represent sweet and umami taste, respectively. Recent data also show that a sweet taste is metabolically relevant for fuel homeostasis and linked to appetitive ingestive behavior.

From the Contents

1. Introduction

All five of our sensory systems are involved in determining palatability, thus promoting the selection, intake, absorption, and digestion of foods. While most of our senses, including sight, hearing, touch, thermoception, and proprioception, are tuned to the detection of physical events, the perception of smell and taste is induced by volatile and nonvolatile molecules activating specific chemoreceptor cells in the nose and the oral cavity, respectively. Psychophysicists have long suggested the existence of the four basic taste qualities sweet, bitter, sour, and salty. Although first reported in 1908 by K. Ikeda (Tokyo Imperial University) after the discovery of L-glutamate (the key taste stimulus in seaweed broth), the so-called umami taste—popularly referred to as savory—has only recently been recognized as the fifth basic taste since the cloning of a specific amino acid taste receptor in 2002.[1] All five tastes are mediated by the activation of taste receptor cells, which are assembled into taste buds that are distributed across different papillae on the tongue and palate epithelium.

The attraction to sweet taste is innate, so newborns already like sweet-tasting stimuli. The perception of a sweet taste might have helped our early ancestors to identify carbohydrate-rich food and to ensure their intake of energy. For centuries honey has been the main source of sweetness in many parts of the world. Later, the desirable taste profile of sucrose combined with its wide availability and its low-cost extraction from sugar cane (*Saccharum officinale* L.) and sugar beet (*Beta vulgaris* L.) provided the main sources of "sugar" since they contain high amounts of sucrose.[2] On the other hand, during the last century a number of undesirable health effects, such as dental caries[3] as well as cardiovascular diseases and its risk factors,[4] have been associated with the consumption of sucrose as a sweetener. Risk factors such as obesity and type-2 diabetes increased the demand for low-calorie sweeteners worldwide. Artificial sweeteners such as saccharin, aspartame, acesulfame K, sucralose, and neotame are approved and consumed in the USA[5] as well as in the European Union,[6] but again and again debates concerning the safety of some of these artificial high-potency sweeteners arise.[7]

Facial-expression studies performed with neonatal human infants in response to stimulation with different tastes revealed that umami-tasting stimuli such as monosodium glutamate (MSG) solutions induce eager sucking/smacking and licking movements and trigger facial expressions very similar to those induced by the sweet taste.[8] These results suggest that L-glutamate is already a palatable taste stimulus for human infants; by virtue of its presence in breast milk (>50% of the total free amino acid content),[9] its taste signals the presence of dietary proteins and might conceivably contribute to the acceptability of this liquid. The umami taste of L-glutamate is known to be drastically potentiated by 5′-ribonucleotides such as inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP), and this synergy is a hallmark of this taste quality.[10] This palatability enhancement has resulted in L-glutamate-rich foods having been used for centuries in many civilizations. Starting with the use of fermented fish sauce in ancient Greece and Rome, it is today common practice in Asia for soy sauce to be used to make savory dishes more delicious, and in Japan, seaweed and bonito are added to soups to make them more tasty. In France, meat (or fish) is combined with vegetables to produce more flavorful stocks, and in Italy, parmesan cheese, anchovies, and fully ripened tomatoes are cooked with seafood to produce a much tastier dish. The combination of these ingredients certainly bring L-glutamate and 5′-ribonucleotides

[*] C. Hellfritsch, Prof. Dr. T. Hofmann
Chair of Food Chemistry and Molecular Sensory Science
Technische Universität München
Lise-Meitnerstrasse 34, 85354 Freising-Weihenstephan (Germany)
Fax: (+49) 8161/71-2949
E-mail: thomas.hofmann@wzw.tum.de
Dr. M. Behrens, Prof. Dr. W. Meyerhof
Department of Molecular Genetics
German Institute of Human Nutrition Potsdam-Rehbruecke
Arthur-Scheunert-Allee 114–116, 14558 Nuthetal (Germany)
Fax: (+49) 3320088-384

together in sufficient amounts to impart a greatly enhanced umami taste to the foods prepared. This fact is of great relevance for the food industry in which mixtures of MSG, IMP, and GMP are used to enhance the flavor of culinary products, snacks, sauces, soups, and seasonings. However, some health concerns have been raised regarding MSG, as it is accused of causing the "Chinese restaurant syndrome" with symptoms such as acute headache, flushing, and sweating. Although there is no clear scientific evidence for a link between MSG consumption and this syndrome,[11] this concern as well as the progress made in molecular-biological techniques have spurred new research strategies—similar to drug identification strategies—to understand ligand-initiated activation of the umami receptor and to discover alternative umami-tasting substances and umami-enhancing molecules.

As a consequence of the increasing interest of the food industry in naturally occurring sweet and umami-modulating compounds as well as the recent molecular-biological findings on the molecular nature of the underlying taste receptors, this Review will give a state-of-the-art survey on natural products that elicite and modulate sweet or umami taste responses. Moreover, recent insight will be discussed of how the interaction of these molecules with the oral sweet and umami taste receptors stimulates receptor cells to secrete neurotransmitters to induce neural activity that is conveyed to the cerebral cortex to represent sweet taste and linked to appetitive ingestive behavior.

2. High-Potency Sweeteners from Plants

Following the increasing demand of consumers for natural food ingredients, many efforts have been made in recent years to discover natural low-calorie sweeteners. Different approaches have been undertaken to successfully locate, isolate, purify, and identify a vast number of intensely sweet tasting molecules in the plant kingdom. For example, botanists have used terms such as *dulcis* (sweet) or *saccharum* (sugar) when describing sweet-tasting plants for the first time, and, therefore, the published botanical literature provides a useful pool of plants, in which potential highly sweet compounds are expected to be found.[2,12] In addition, distinct plants, or specific parts of them, have been used by native people for a long time, thus giving important hints to natural product chemists where to look for sweet-tasting molecules in natural sources. Moreover, the systematic screening for candidate plants through human organoleptic detection during field work also led to the discovery of intensely sweet tasting molecules.[2]

Natural products need to satisfy certain requirements to be used as a potential sweetener. Besides having a sucrose-like taste profile, they should be nontoxic, noncariogenic, readily water soluble, and stable under acidic pH conditions as well as at higher temperatures. With a sweetness potency of at least 50–100 times that of sucrose, these compounds are also called "high-potency" or "low-calorie" sweeteners.[13]

Most of the highly sweet natural products known today belong to the chemical classes of either terpenoids or steroids and, in many cases, occur as glycosides. The following sections

Thomas Hofmann studied food chemistry at the University of Erlangen-Nürnberg and completed his PhD (1995) and habilitation (1998) both with Prof. P. Schieberle at the Chemistry Department of the TU München (TUM). From 1999 to 2002 he was deputy director of the German Research Center for Food Chemistry at the Leibnitz Society. In 2002 he took over the Chair of Food Chemistry of the University of Münster, and since 2007 he has held the Chair of Food Chemistry and Molecular Sensory Science at TUM. He is currently also vice president of the TUM.

Maik Behrens studied biology at the University of Hamburg. He received his PhD at the University of Hamburg, Institute for Cell Biochemistry and Clinical Neurobiology, with Prof. H. Schmale in 1997. After postdoctoral research at the Department of Anatomy and Neurobiology, University of Maryland, Baltimore, he joined the Department of Molecular Genetics at the German Institute of Human Nutrition Potsdam-Rehbruecke.

Wolfgang Meyerhof studied Biochemistry at the Free University of Berlin, where he received his PhD with Prof. W. Knochel in 1984. He was awarded the venia legendi in cell biochemistry in 1993 at the University-Hospital Eppendorf, Hamburg. In 1994 he became Professor for Molecular Genetics, University of Potsdam, and Head of the Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbruecke, an institute of the Leibniz Society. His current research interests are the molecular neurobiology of taste and the regulation of hunger and satiety.

Caroline Hellfritsch received her diploma in chemistry in 2006 from the Friedrich Schiller University of Jena where she worked under the guidance of Prof. W. Plass. In 2007 she began her PhD studies at the Technical University of Munich under the supervision of Prof. T. Hofmann.

aim to provide an overview of several sweet-tasting candidates with a mono-, sesqui-, di-, and triterpene backbone as well as of intensely sweet steroidal saponins, followed by sweet-tasting phenolics and proteins originating from various botanicals. The highlighted sweet molecules, their plants of origin, as well as their sweetening potency, relative to sucrose on a weight basis (sucrose = 1), are summarized in Table 1. The sweetness potency is determined by diluting an aqueous solution of the test compound until the perceived sweetness is equivalent to that of, for example, a 2% solution of sucrose (wt/vol). A sweetness potency of 100 on a weight basis means that a 100 times lower concentration of the test compound is needed to achieve the same sweetness intensity as the sucrose reference.[14] It is important to note that the sweetness potency of a molecule varies with concentration and depends on the organoleptic method used for its determination.

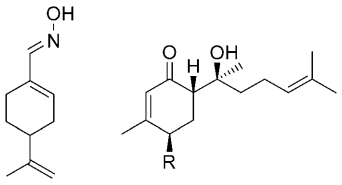
Table 1: Selected high-potency sweeteners from plants.

Compound class	Compound name	Sweetness potency[a]
monoterpenes:		
	perillartine[b] (**1**)	370
sesquiterpenes:		
	(+)-hernandulcin (**2**)	1000–1500
	(+)-4β-hydroxy-hernandulcin (**3**)	N.S.[c]
diterpenes:		
ent-kaurene glycosides:	dulcoside A (**4**)	30
	rebaudioside A (**5**)	242
	rebaudioside B (**6**)	150
	rebaudioside C (**7**)	30
	rebaudioside D (**8**)	221
	rebaudioside E (**9**)	174
	rebaudioside F (**10**)	N.S.[c]
	rubusoside (**11**)	115
	steviolbioside (**12**)	90
	stevioside (**13**)	210
labdane glycosides:	baiyunoside (**14**)	500
	phlomisoside I (**15**)	N.S.[c]
triterpenes:		
cucurbitane glycosides:	mogroside IV (**16**)	233–392
	mogroside V (**17**)	250–425
	siamensoside I (**18**)	563
dammarane glycosides:	cyclocarioside A (**19**)	200
	cyclocarioside I (**20**)	250
oleanane glycosides	albiziasaponin A (**21**)	5
	albiziasaponin B (**22**)	600
	glycyrrhizin (**23**)	93–170
steroidal saponins:		
	osladin (**24**)	500
	polypodoside A (**25**)	600
	polypodoside B (**26**)	N.S.[c]
phenolics:		
	glycyphyllin (**27**)	N.S.[c]
	naringin dihydrochalcone[b] (**28**)	300
	neohesperidin dihydrochalcone[b] (**29**)	1000
	phyllodulcin (**30**)	400
proteins:		
	thaumatin (**31**)	1600
	brazzein (**32**)	2000
	pentadin (**33**)	500
	monellin (**34**)	3000
	mabinlin (**35**)	375[d]
	curculin (neoculin) (**36**)	550

[a] Sweetness potency is given relative to sucrose on a weight comparison and was reviewed in Ref. [7]. [b] Semisynthetic sweetener. [c] Sweetness potency not given. [d] Sweetness potency for mabinlin II is given on a molar basis, see text.

2.1. Sweet Terpenoids

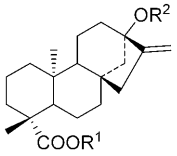
The observation that the naturally occurring terpenoid perillaldehyde, isolated from the volatile oil of *Perilla frutescens* (L.) Britton (Labiatae), has a slightly sweetish taste led to the discovery of the corresponding α-*syn*-oxime, perillartine (**1**), which was found to be about 370 times sweeter than sucrose.[2] Although the poor water solubility as well as the bitter and menthol-liquorice off-taste of this semisynthetic compound limit its more widespread use, **1** is commercially used in Japan for the sweetening of tobacco products.[2,15]



1 **2** R=H
3 R=OH

Hernandulcin (**2**), a sesquiterpene of the bisabolane class, is the sweet principle present in leaves and flowers of *Lippia dul*cis Trev. (Verbenaceae). This sweet natural product was named hernandulcin in honor of the 16th century Spanish physician Francisco Hernández, who reported on the sweet plant already known to the Aztecs. Naturally occurring (+)-hernandulcin (**2**) was described to be about 1000 times sweeter than sucrose (on a molar basis), but exhibits a rather unpleasant bitter aftertaste.[16,17] Another sweet derivative, (+)-4β-hydroxyhernandulcin (**3**), was also isolated from *Lippia dulcis*. As these sesquiterpenes are rather hydrophobic, the 4β-OH group of **2** might provide a potential linkage for the introduction of more polar groups to increase the water solubility of this class of sweeteners.[14]

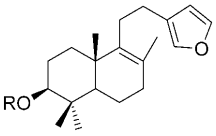
The leaves of *Stevia Rebaudiana* (Bert.) Bertoni, a plant originating from Paraguay, have been used for centuries by the native population to sweeten and to overcome the bitter taste of herbal teas. This member of the compositae family is also known as "sweet herb" and was found to contain a series of nine sweet *ent*-kaurene glycosides (**4**–**10**, **12**, **13**), commonly named as steviol glycosides. Depending on the cultivar and growing conditions, the content of these glycosides varies between 4 and 20% based on the dry weight.[18] Stevioside (**13**) and rebaudioside A (**5**) are the most abundant among the nine known sweet diterpenic glycosides, which contain steviol (13-hydroxykaur-16-en-18-oic acid) as the common aglycone.[19] The values for the relative sweetness of rebaudioside A–F (**5**–**10**), stevioside (**13**), and steviolbioside (**12**) according to Kinghorn and Soejarto are listed in Table 1.[2] Rebaudioside A is believed to have the best organoleptic sweetening properties and to exhibit the least bitter off-taste. Therefore, increased levels of rebaudioside A in plants and plant extracts are the aim of breeding work and the development of extraction and purification processes.[20] A series of toxicological studies have been performed and recently reviewed.[21] On the basis of this, stevioside-containing extracts of *S. Rebaudiana* are permitted as food additives in Japan, Brazil, South Korea, Argentina, and Paraguay, and are used as a dietary supplement in the United States.[17] Steviol glycosides have not yet been approved as a sweetener in the European Union, but purified rebaudioside A gained GRAS (Generally Recognized as Safe) status in the USA in 2008, and in the same year the Joint FAO/WHO Expert Committee on Food Additives suggested a temporary admissible daily intake (ADI) of 0–4 mg kg^{-1} body weight, expressed as steviol.[22]



	R^1:	R^2:		R^1:	R^2:
4	β-glc	β-glc^2-α-rha	**9**	β-glc^2-β-glc	β-glc^2-β-glc
5	β-glc	β-glc^2-β-glc (β-glc^3)	**10**	β-glc	β-glc^2-β-xyl (β-glc^3)
6	H	β-glc^2-β-glc (β-glc^3)	**11**	β-glc	β-glc
7	β-glc	β-glc^2-α-rha (β-glc^3)	**12**	H	β-glc^2-β-glc
8	β-glc^2-β-glc	β-glc^2-β-glc (β-glc^3)	**13**	β-glc	β-glc^2-β-glc

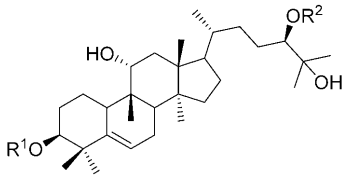
Besides *Stevia Rebaudiana* (Bert.) Bertoni, the leaves of the Chinese herb *Rubus suavissimus* S. Lee (Rosaceae) also contain a sweet-tasting *ent*-kaurene glycoside with a β-D-glucopyranosyl moiety bound to the hydroxy group at position 13 and a carboxy group at position 19.[23] This molecule, coined rubusoside (**11**), has been rated as 114 times sweeter than sucrose, but also exhibits a rather lingering bitter aftertaste.[2]

Baiyunoside (**14**), a labdane-type diterpene glycoside with (+)-baiyunol as the aglycon, was isolated in 1983 from *Phlomis betonicoides* Diels, a plant used in traditional Chinese medicine. While baiyunoside was found to be 500 times sweeter than sucrose, its lingering aftertaste is perceived for longer than one hour, thus limiting its application as a sweetener in food. Phlomisoside I (**15**), which differs from labdane glycoside **14** only by the presence of an α-rhamnose instead of a β-xylose in the glycoside moiety, was also isolated from *P. betonicoides*, but any data on the sweetness potency of this molecule are still lacking.[2,24]



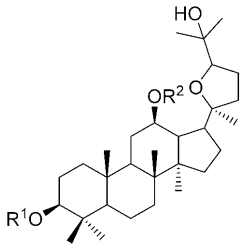
14 R= β-glc^2-β-xyl
15 R= β-glc^2-α-rha

In the group of triterpene glycosides, the so-called mogrosides belong to the most intense sweet-tasting candidates. Mogroside IV (**16**) and V (**17**) are the main sweet triterpene glycosides. They are isolated from dried fruits of the cucurbitaceae "lo-han-kuo" (*Siraitia grosvenorii* (Swingle) C. Jeffrey), extracts of which have been used in China as a medicinal remedy for the treatment of colds and sore throats.[25] The sweetness of mogroside V, which is obtained in yields of about 1% (wt/wt), was rated as 250–450 times more intense than sucrose, depending on the concentration. Even more potent than mogroside V is siamenoside I (**18**), another triterpene glycoside identified in *S. grosvenorii* as well as in other species of the same genus (*S. siamensis*), the sweetness intensity of which was rated as 563 times more intense than sucrose.[26] Today, "lo-han-kuo" extracts containing mogroside V are used to sweeten foods and beverages in Japan[17,27] and are classified with the GRAS status in the United States.[28]



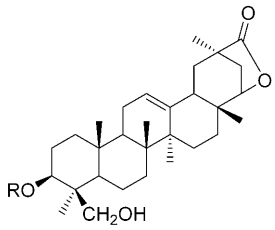
	R^1:	R^2:
16	β-glc^6-β-glc	β-glc^2-β-glc
17	β-glc^6-β-glc	β-glc^2-β-glc (β-glc^6)
18	β-glc	β-glc^2-β-glc (β-glc^6)

Other triterpene glycosides, the cyclocariosides, which feature a dammarane-type aglycone, were found in the leaves of *Cyclocarya paliurus*, a plant that has been used as a treatment for diabetes in China. Cyclocarioside A (**19**) and cyclocarioside I (**20**) were purified and their sweetness potency was found to be 200 and 250 times that of sucrose, respectively.[7,29]

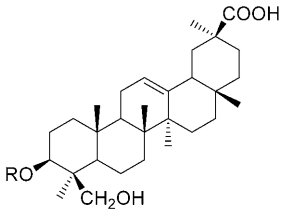


	R^1:	R^2:
19	α-ara-5-Ac	α-rha
20	α-rha	β-qui

Several oleanane-type triterpene saponins were isolated from sweet-tasting extracts prepared from stems of *Albizia myriophylla*, which are used in traditional medicine in Thailand and Vietnam as a substitute to licorice (*Glycyrrhiza glabara* L.). Of these, the saponins albiziasaponin A (**21**) and B (**22**) were identified as the key compounds, and their sweetness was investigated by human sensory analysis. The sweetness was highly dependent on their chemical structure, with the more abundant albiziasaponin B (**22**), which has a free carboxylic group, found to be 600 times sweeter than sucrose, whereas albiziasaponin A, with a lactone group, showed a sweetness potency of only 5.[30]

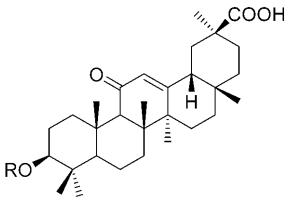


21 R = β-glcA^2-β-glcA^2-α-rha



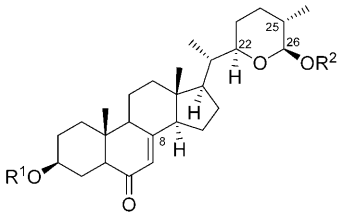
22 R = β-glcA^2-β-glcA^2-α-rha

Glycyrrhizin or glycyrrhizic acid (**23**), the diglucuronide of the aglycone glycyrrhetinic acid, is the well-known sweet principle of the roots of licorice (*Glycyrrhiza glabara* L.) and other species of the same genus. Glycyrrhizic acid can be extracted either from roots, which contain 6–14% of its calcium and potassium salt,[15] or from rhizomes, which contain **23** in amounts of 3–23%.[19] Depending on its concentration, glycyrrhizin was rated to be 93–170 times sweeter than sucrose[7] and found to exhibit a low sweetness onset and a long-lasting licorice aftertaste, thus limiting its use as a sugar substitute.[17] Some purified root extracts of *G. glabara* are used to sweeten food, cosmetics, and medicine in Japan.[31] Whereas the ammonium salt of glycyrrhizic acid (50 times sweeter than sucrose) has GRAS status for use as a flavoring compound in the USA,[32] the Scientific Committee on Food of the EU suggests that not more than 100 mg of glycyrrhizic acid should be consumed per day, as there are concerns that the consumption of glycyrrhizic acid or one of its salts may lead to pseudoaldosteronism, which is reflected, for example, in increased blood pressure. Pseudoaldosteronism can be caused by inhibition of the enzyme 11-β-hydroxysteroid dehydrogenase-2 by glycyrrhetic acid, which is the product of the hydrolysis of glycyrrhizinic acid. This causes a cortisol-dependent increased potassium excretion as well as increased sodium and water retention.[33]



23 R = β-glcA^2-β-glcA

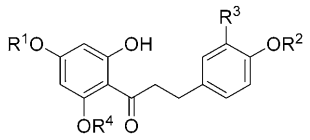
More than 40 years ago a sweet steroidal saponin was isolated from the European fern *Polypodium vulgare* L. (Poliypodiaceae) by Jizba and Herout, and named osladin (**24**) after the Czech name of the fern ("osladic").[34] After the compound, assigned as osladin, was synthesized by means of independent organic synthesis and found not to induce any sweetness sensation, the stereochemistry of osladin had to be reassigned.[35] Single-crystal X-ray diffraction studies of natural osladin revealed that the correct structure is the 22*R*,25*S*,26*R* isomer and not the 22*S*,25*R*,26*S* isomer suggested earlier. In addition, the sweetness potency relative to sucrose was revised from 3000 to 500.[36] Two additional steroidal saponins polypodoside A (**25**) and polypodoside B (**26**) were isolated from the rhizomes of the North American fern *Polypodium glycyrrhiza* DC. Eaton (Polypodiaceae), a plant also known as "licorice fern". The aglycone of these two glycosides was identified as the $\Delta^{7,8}$ derivative of the aglycone of osladin and named polypodogenin. The configuration of polypodoside A also had to be revised from the 22*S*,25*R*,26*S* to the 22*R*,25*S*,26*R* stereoisomer.[36] Although the sweetness potency was rated as 600 times sweeter than a 6% solution of sucrose,[37] the potential of its commercial application seems to be limited by a licorice-like aftertaste, its low water solubility, as well as the poor availability of *P. glycyrrhiza* rhizomes.[38]



	R^1:	R^2:	other:
24	β-glc^2-α-rha	α-rha	7,8-dihydro
25	β-glc^2-α-rha	α-rha	-
26	β-glc	α-rha	-

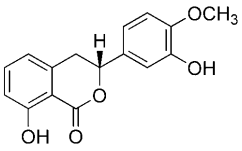
2.2. Sweet Phenolics

Besides the terpenoids, several phenolics have been identified in the past as sweet-tasting phytochemicals. For example, the dihydrochalcone glycyphyllin (**27**) has been found to be the sweet principal of the Australian climbing plant *Smilax glycyphylla* Sm., which belongs to the family of Smilacaceae. It was isolated by Rennie in 1886 and identified as a rhamnoside of phloretin. Several glycosidic forms of phloretin are presumed to contribute to the sweet taste of apples.[15] The taste of glycyphyllin is described as liquorice-like and it is reported as being readily soluble in hot water.[39] Treatment with alkali and hydrogenation of the bitter-tasting flavanone glycosides naringin isolated from the peel of grapefruits (*C. paradisi* Macfad.) and neohesperidin isolated from the peel of the Seville oranges (*Citrus aurantium* L.) is used in industry to produce the intensely sweet tasting compounds naringin dihydrochalcone (**28**) and neohesperidin dihydrochalcone (**29**), respectively.[40] Dihydrochalcone **29**, which is 600–1500 times sweeter than sucrose, was approved as a sweetener by the EU in 1994 and is used as a flavor enhancer (E 959) in a wide range of foods and beverages.[41]



	R^1:	R^2:	R^3:	R^4:
27	H	H	H	α-rha
28	β-glc^2-α-rha	H	H	H
29	β-glc^2-α-rha	CH_3	OH	H

The dihydroisocumarin phyllodulcin (**30**) was identified as the sweet molecule liberated from its glycoside upon crushing and/or fermentation of leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae). In Japan, this sweetener, called "Amacha", is typically used to prepare the tea brew during the Hanamatsuri celebration. Although phyllodulcin has been rated as 400 times sweeter than a 3% (wt/vol) solution of sucrose, its wider use as a low-caloric sweetener is restricted by a late onset of sweetness, a lingering aftertaste, and insufficient solubility in water.[17]



30

2.3. Sweet Proteins

2.3.1. Thaumatin

In 1968 Inglett and May reported that the covering of the seeds of the West African plant *Thaumatococcus daniellii* (Bennett) Benth. (Marantaceae) has an intense sweet taste. Later, in 1972, van der Wel and Loeve identified the two proteins thaumatin I (**31a**) and II (**31b**) as the key molecules that induce the sweet taste impression, and reported their sweetness potency to be 1600 and 100000 times that of sucrose on a weight basis or on a molar basis, respectively.[42] Both thaumatins consist of 207 amino acids, which are arranged in a single polypeptide chain to form a protein with a molecular weight of 22209 Da (thaumatin I) and 22293 Da (thaumatin II).[43] These proteins show only a rather low thermostability because of the presence of disulfide bonds.[15] X-ray studies revealed that the structure of these proteins consists of three domains, an 11-stranded β sandwich, two β stands that form a β ribbon, and an α helix (see Figure 1).[44,45] The aluminum salt of several thaumatins is commercially available as a sweetener (Talin protein). Following its approval as a food additive in Japan in 1979, thaumatin is today an approved sweetener in some European countries and Australia, and has GRAS status as a flavor enhancer for certain food products in the USA.[46]

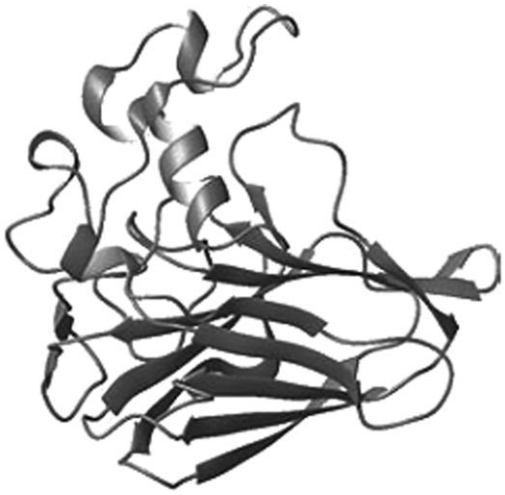


Figure 1. Crystal structure of thaumatin (**31**). Reprinted from Ref. [45] with the permission of Springer (2006).

2.3.2. Brazzein and Pentadin

Although thaumatin is not thermostable, brazzein (**32**), a sweet protein isolated by Ming and Hellekant from the African plant *Pentadiplandra brazzeana* Baillon (Pentadiplandraceae), was found to maintain its sucrose-like sweet taste up to a temperature of 80 °C.[47] The protein consists of a single polypeptide chain with 54 amino acids (6473 Da) and is reported to be 2000 times sweeter than a 2% solution of sucrose.[47] A second protein, named pentadin (**33**), was later isolated from the same plant and found to have a molecular weight of about 12000 Da and to be 500 times sweeter than sucrose.[48]

2.3.3. Monellin

The sweet-tasting protein monellin (**34**), named after the Monell Chemical Senses Center in the USA where it was discovered in 1969,[49] was isolated from the fruit of the West

African shrub *Dioscoreophyllum cumminsii* (Stapf) Diels., also know as "serendipity berry". Bohak and Li showed that monellin consists of two subunits, A and B, which contain 42 and 50 amino acids, respectively.[50] Interestingly, none of these subunits alone exhibited a sweet taste, but the undissociated dimer was determined to be 3000 times sweeter than sucrose on a weight basis.[51] As the extraction and purification of monellin from its plant of origin is rather costly, several approaches have been undertaken to produce the recombinant protein biotechnologically in yeast and *E. coli*.[52] However, monellin is currently not used as a commercial sweetener, because of its low thermostability as well as its lingering aftertaste.[38]

2.3.4. Mabinlin

The seeds of *Capparis masakai* Lévl (Capparidaceae) contain a number of sweet-tasting proteins called mabinlins (**35**).[53] The first proteins, named mabinlin I and II, were isolated in 1983 by Hu and He.[54] The plant—also known as "mabinlang"—has been used in traditional Chinese medicine and is native to the subtropical regions of the Chinese province Yunnan. Chewing the seeds results in the mabinlins inducing a long-lasting sweet-taste impression accompanied by a bitter and astringent oral sensation. Mabinlin II, which consists of two amino acid subunits made up from 33 and 72 amino acids shows by far the highest thermostability of this protein group.[55] The B chain of the protein was identified as the essential structural element that interacts with the sweet receptor.[56] Human sensory studies revealed mabinlin II to be 375 times sweeter than sucrose on a molar basis.[57]

2.3.5. Neoculin (Curculin)

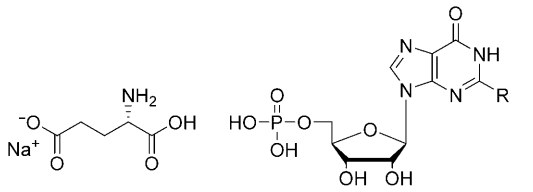
The fruit of *Curculigo latifolia* Dryand. (Hypoxidaceae) has long been known to elicit a sweet taste response—as well as a taste-modifying effect for converting sourness into sweetness. Native people in Malaysia eat the dried fruits to impart a sweet taste to sour food or to sweeten black tea. The taste-modulating component was found to be a protein, named curculin, and was initially regarded as a homodimer of two identical subunits. The sweetness of curculin was described to be 550 times that of sucrose on a weight basis.[58] After the recombinant protein was found not to taste sweet, the chemical structure of the protein isolated from *Curculigo latifolia* Dryand. was re-investigated by means of X-ray crystallographic analysis. These studies demonstrated that the protein was a heterodimer consisting of 13 kDa and 11 kDa subunits connected by disulfide bonds, and was renamed neoculin (**36**). Sensory analyses revealed that the sweetness of aqueous solutions of neoculin was intense when tested at low pH values, while it elicits only a weak sweet taste at neutral pH. Possible pH effects on the structure of neoculin have been discussed as being responsible for its taste-modifying effect and sweet-taste perception.[59]

3. Natural Umami Compounds

At the beginning of the 20th century, the Japanese Professor K. Ikeda noticed that an unidentified taste quality, disctinct from the four basic taste qualities sweet, bitter, sour, and salty was present in highly palatable foods. He detected this savory taste quality most clearly in "dashi", a soup stock prepared from the seaweed *Laminaria japonica* and traditionally used in Japanese cooking, and identified L-glutamic acid as the key component. Ikeda named this taste "umami", after the Japanese word *umai* ("delicious"), and claimed it as the fifth basic taste sensation.[60] Based on Ikeda's findings, it might be hypothesized that the sensitive oral detection of the proteinogenic L-glutamic acid or its salt monosodium L-glutamate (MSG), which exhibit a low umami taste recognition threshold of 3.0 or 1.5 mmol kg^{-1}, respectively, might have led our early ancestors to protein-rich foods. For a long time Ikeda's description of the umami taste, published in Japanese, remained unappreciated by the western scientific community. Although the discovery of the umami taste receptor in 2002 firmly established the umami taste as the fifth basic taste,[1,61,62] from a culinary perspective, the taste of umami is not new. Fermented fish sauces and vegetable extracts as well as meat extracts have been valued in world cuisines for more that 2000 years, for example, Roman *Garum*, Thai *Num Pla*, Vietnamese *Nuoc Mum Tom Cha*, Indonesian *Terasi*, as well as British Beef Tea and their concentrated flavors.

Besides L-glutamic acid and its corresponding monosodium salt (MSG, **37**), aqueous solutions of L-aspartic acid as well as C_4-dicarboxylic acids such as succinic acid and tartaric acid were reported to exhibit some MSG-like taste qualities[63] and, moreover, L-lactic acid was shown to contribute to the umami and savory taste of beef bouillon and meat juice.[64] Furthermore, purine 5′-ribonucleotides such as guanosine 5′-monophosphate (GMP, **38**), inosine 5′-monophosphate (IMP, **39**), and adenosine 5′-monophosphate (AMP) were found to induce an umami taste sensation, with recognition thresholds of 30 and 5 mmol kg^{-1} for GMP and IMP, respectively.[65] In addition, both ribonucleotides were demonstrated to synergistically enhance the umami taste of MSG (**37**) and contribute to the attractive savory taste of meat, fish, seafood, mushrooms, as well as food products containing yeast autolysates.[10] The palatable umami taste in prepared Asian foods is imparted by the combinatorial use of taste-active ingredients such as, for example, dried bonito flakes rich in IMP, shiitake mushrooms rich in GMP, seaweed rich in L-glutamate, as well as fermented products such as soy sauce and fish sauces with high levels of L-glutamate (Table 2).[66] The western world appreciates the flavor enhancement ability of umami when L-glutamate is present as the free amino acid, such as in a bouillon or an extract made from beef meat, aged cheeses, cured ham, and tomatoes (Table 2).

Besides L-glutamate and ribonucleotides, additional umami-tasting molecules have been identified in various natural sources. Analysis of the peptides in vegetable protein hydrolysates revealed pyroglutamyl peptides such as *p*Glu-Pro-Ser, *p*Glu-Pro, *p*Glu-Pro-Glu, and *p*Glu-Pro-Gln as important umami-like-tasting molecules in these liquid sea-



37

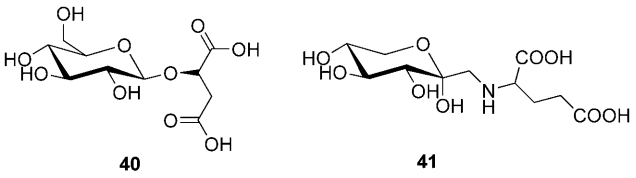
38 R=NH_2
39 R=H
44 R=$NHCOCH_3$
45 R=$NHCOCH(OH)CH_3$

Table 2: Concentration of umami-tasting L-glutamate, inosine 5′-monophosphate (IMP), guanosine 5′-monophosphate (GMP), and adenosine 5′-monophosphate (AMP) in food (n.d.: not detectable).[66]

Food item	L-Glutamate [mg/100 g]	IMP [mg/100 g]	GMP [mg/100 g]	AMP [mg/100 g]
parmesan cheese	1680	n.d	n.d	n.d
emmentaler cheese	308	n.d.	n.d.	n.d.
cheddar cheese	182	n.d.	n.d.	n.d.
seaweed	1608	n.d.	n.d.	n.d.
tomato	256	n.d.	n.d.	21
shiitake mushroom	71	n.d.	150	n.d.
fermented fish sauce (Japan)	1383	n.d.	n.d.	n.d.
fermented soy sauce (Japan)	782	n.d.	n.d.	n.d.
scallop	140	n.d.	n.d.	172
snow crab	19	5	4	32
tuna (bonito)		268	n.d.	n.d.
beef meat	10	70	4	8
pork meat	9	200	2	9

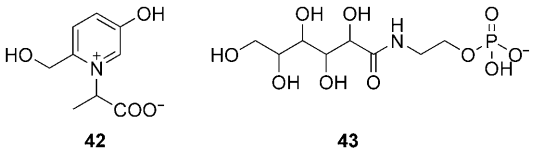
sonings.[67,68] Moreover, enzymatic hydrolysis of fish proteins was reported to lead to savory products which are rich in acidic peptides such as Asp-Glu-Ser, Glu-Asp-Glu, Thr-Glu, and Ser-Glu-Glu.[69] Such acidic di- and tripeptides were found to express weak umami activity by themselves but have been demonstrated to be significantly enhanced in the presence of IMP.[70] More recently, Kaneko et al. investigated the key compounds imparting the umami taste sensation induced by the consumption of so-called "mat-cha", a Japanese green tea, and identified—besides L-glutamate and succinic acid—(1*R*,2*R*,3*R*,5*S*)-5-carboxy-2,3,5-trihydroxycyclohexyl-3,4,5-trihydroxybenzoate (known as theogallin) as well as *N*-ethylglutamine (known as L-theanine) as important umami taste enhancing molecules.[71]

Thermal processing including drying has long been known to enhance umami and savory tastes of food products such as, for example, sun-dried tomatoes and mushrooms, respectively. A so-called "sensomics" approach was developed to enable the targeted discovery of taste compounds and taste-enhancing molecules in complex processed foods. This approach combines techniques of advanced natural product analysis and analytical psychophysical tools, and was used to systematically and comprehensively identify, catalog, and quantify the taste-active key metabolites produced upon food processing.[72] Application of this sensomics approach revealed that the attractive savory taste of air-dried morel mushrooms is due to the enhancement of the umami taste of L-glutamate by the previously not reported (*S*)-malic acid 1-O-β-D-glucopyranoside (**40**).[73] This so-called (*S*)-morelid (**40**) is generated by the non-enzymatic glucosylation of L-



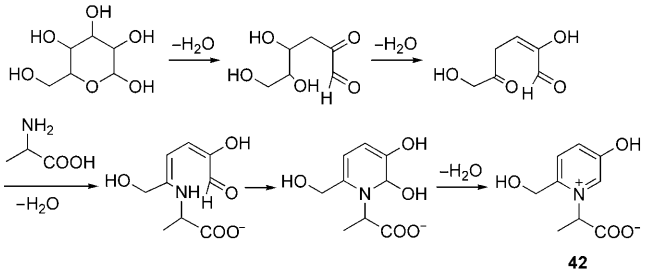
40 41

malic acid upon drying the mushrooms. Furthermore, *N*-(1-deoxy-D-fructos-1-yl)-L-glutamic acid (**41**) was identified in sun-dried tomatos in yields of up to 1.5% (on a weight basis), and confirmed to be generated by the Maillard reaction of L-glutamic acid and glucose upon the drying of foods.[74] After independent synthesis, this Amadori product was found to exhibit an umami-like taste quality with a recognition threshold of 1.8 mmol L^{-1}.[75] Moreover, the previously not reported *N*-(1-carboxymethyl)-6-hydroxymethylpyridinium-3-ol inner salt, named alapyridaine (**42**), was discovered as a taste enhancer in beef bouillon by means of the sensomics



42 43

approach.[76] Model experiments demonstrated that this pyridinium salt is generated as a racemic mixture by the Maillard reaction of L-alanine and glucose (Scheme 1).[77] Although being tasteless on its own, the enantiomer (+)-(*S*)-alapyridaine was confirmed to significantly decrease the



Scheme 1. Reaction sequence leading to the formation of alapyridaine (**42**) from the Maillard reaction of alanine and glucose.[68]

human recognition threshold of umami as well as sweet stimuli, whereas (−)-(*R*)-alapyridaine was physiologically not active.[78] Additional structure–activity relationship studies revealed that substitution of the alanine moiety in **42** by a glycine moiety converted the sweetness-enhancing alapyridaine into a bitter taste inhibitor.[79]

Besides the identification of such natural umami enhancers in raw food materials as well as processed foods by means of a sensomics approach, other discovery strategies include the thermal reaction of known isolated food ingredients under food-related model conditions and the screening of the reaction products formed for taste enhancement activity by human sensory testing, followed by the verification of their natural occurance in foods by means of HPLC-MS/MS. For example, *N*-gluconylethanolamine phosphate (**43**) was obtained from the reaction of 2-aminoethyl dihydrogen phosphate and δ-gluconolactone, and found to exhibit umami-enhancing activity.[68] *N*-Gluconylethanolamine, reported to have a natural, sugar-type flavor character, was identified in "Beerenauslese" wines.[80]

Thermal treatment of guanosine 5′-monophosphate (**38**) in the presence of acetic acid or lactic acid was reported to generate *N*-acetyl- (**44**) and *N*-lactoylguanosine 5′-monophosphate (**45**), respectively, which exhibit a characteristic long-lasting umami taste profile.[81,82] The detection of **45** in dried and fermented skipjack, known as "bonito" in Japanese cuisine, by HPLC-MS analysis verified its natural occurance.[81]

Besides such umami compounds, some molecules that enhance mouthfulness, thickness, and complexity as well as increasing the continuity of the perception of food taste were found in foods and were coined "kokumi" compounds about 10 years ago by the Japanese. The first kokumi compounds were isolated from water extracts of garlic and onion and were identified as sulfur-containing amino acid and peptide derivatives such as, for example, *S*-allyl-L-cysteine sulfoxide and γ-glutamyl-*trans*-*S*-propenyl-L-cysteine sulfoxide.[83] A series of kokumi-active γ-glutamyl di- and tripeptides were identified in common beans as well as matured Gouda cheese by application of a sensomics approach.[84] Although being entirely tasteless on their own up to levels of about 10 mmol L^{-1}, in excess of the threshold amounts these γ-glutamyl peptides were found to increase mouthfulness, complexity, and palate length of savory solutions containing sodium chloride and L-glutamic acid, as well as of an artifical cheese base. Very recently, various kokumi-active γ-glutamyl peptides including γ-glutamylvaline and γ-glutamylcysteinylglycine (glutathione) were demonstrated to activate the extracellular calcium-sensing receptor (CaSR), a close relative of the class C G-protein-coupled sweet and umami receptors.[85] However, a distinct function of the CaSR in human kokumi perception still needs to be confirmed.

4. Structure and Function of the Sweet and the Umami Taste Receptors

4.1. Isolation and Identification of the Sweet and Umami Taste Receptors

As demonstrated above, sweetness and umami taste are mediated by a vast number of compounds that differ greatly in their molecular structure. This chemical diversity challenged investigations on the possible molecular nature of sweet taste receptors. In the past few decades, structure–activity relationship studies have been performed with various sweeteners to find a model that allows the receptor active sites to be predicted. The original AH-B model (A = acid, B = base) of Shallenberger and Acree assumed the presence of a hydrogen-bond donor and acceptor spaced at a distance of 0.3–0.4 nm.[86] This model has been refined several times on the basis of newly appearing sweet chemicals with rigid structures.[87] However, all the models fell short in explaining the sweetness of either molecules having flexible structures or the large sweet-tasting proteins.[88] This led to the assumption of the existence of several sweet taste receptors that respond to the different classes of sweeteners. However, molecular-biological studies only recently succeeded in identifying the molecular nature of the sweet taste receptor.

Today it is known that the products of three genes are responsible for the detection of appetitive sapid stimuli, that is, sweet and umami compounds. *T1R1* and *T1R2* DNA sequences were isolated in 1999 by using a sophisticated molecular-biological approach that relied on the analysis of a subtracted cDNA library obtained from rat taste tissue.[89] Both genes appeared to be selectively expressed in taste cells, but with different expression patterns. Whereas *T1R1* mRNA was frequently found on the anterior tongue, *T1R2* was more abundant on the posterior tongue. Sequence analysis showed that the genes appeared to encode G protein-coupled receptors (GPCR) of class C related to a class of pheromone receptors, the Ca^{2+}-sensing receptor, metabotropic glutamate receptors, and γ-aminobutyric acid type B receptors. Both the T1R1 and T1R2 receptors were considered to be real taste receptors, even though a specific taste has not been associated with them. The third gene was found two years later by a combined approach of human genome database mining and physical genetic mapping. Several research groups simultaneously searched the *sac* locus on the distal arm of mouse chromosome 4 for putative receptor genes.[90–93] It was previously known that *sac* determines sweet taste sensitivity in mice.[94] Different inbred strains differ in their sensitivity to saccharin and other sweeteners. The dominant taster allele *sac* is linked to a higher sweet taste sensitivity than the nontaster allele. These attempts identified the *T1R3* gene, a close relative of *T1R1* and *T1R2*, in the chromosomal segment spanning *sac*.[90–93] The identity of *T1R3* with *sac* was proven by recovery of the sweet taste deficits of a nontaster strain of mice through transgenic expression of *T1R3* isolated from a taster strain.[93] When the expression profile of *T1R3* was analyzed in taste tissue it was observed that *T1R3* mRNA either colocalized with *T1R1* mRNA or *T1R2* mRNA in the same subsets of taste cells, while a small proportion of cells expressed *T1R3* alone.[92,93] This observation together with the fact that other class C GPCRs function as dimers led researchers to express combinations of T1Rs in engineered heterologous cell lines. The results of these expression studies were very clear and demonstrated that T1R1 and T1R3 assemble into a functional heterodimeric receptor for L-amino acids, if the subunits were from mice, or for L-glutamate if they were of human origin.[1,62] A selective L-glutamate response is a hallmark of umami taste. Moreover, the T1R1-T1R3 receptor also shows another signature of umami taste, namely the ability to give enhanced responses in

the presence of 5′-ribonucleotides, including GMP and IMP. Therefore, the T1R1-T1R3 dimer is also referred to as the umami receptor. On the other hand, T1R3 combines with T1R2 to form a functional general receptor for sweet taste.[62,93] All available data from in vitro expression of the receptor dimer[1,62,93] and from knockout mice[95–97] suggest T1R2-T1R3 to be the universal sweet taste receptor (see Sections 4.2 and 4.3), even though some data suggest that the T1R3 homodimer may function as a low-affinity receptor for mono- and disacchrides.[96] Thus, three polypeptides combine into at least two functional receptors, each having a common and a specific subunit.

4.2. Response Profile of the Sweet Taste Receptor

Functional expression of the sweet receptor dimer T1R2-T1R3 has been achieved in human kidney cell lines engineered to express promiscuous signal-transducing guanine nucleotide binding (G) proteins, $G\alpha_{15}$ or $G\alpha_{16}$.[62,93,98,99] These G proteins couple numerous receptors to the release of calcium from intracellular stores. In some cases, chimeric G proteins have been employed, constructed such that their carboxy termini carry sequences of the "gustatory" G-protein subunit, α-gustducin, Gαz, or of $G\alpha_{i3}$, thereby resulting in improved receptor-coupling properties while the amino terminus activates the cellular effector proteins.[93,99,100] Under these conditions the activated sweet receptor couples through the α G protein subunit to phospholipase C activity, liberates the second messenger inositol trisphosphate (IP_3) from membrane lipids which in turn causes release of calcium ions from internal stores. The intracellular calcium signal is detected by fluorescence changes of a calcium-binding dye and read out as light units in appropriate devices that convert these values into quantitative numerical data and graphs (Figure 2). Numerous sweet-tasting molecules have been demonstrated to stimulate the sweet taste receptor in the in vitro assays. The compounds reflect a broad chemical diversity ranging from mono- and disaccharides such as sucrose, fructose, lactose, maltose, galactose, and glucose,[1,62,93,99,100] the amino acids D-Ala, D-Asn, D-Gln, D-His, D-Phe, D-Trp, Gly,[1,62,99] the proteins monellin, thaumatin, brazzein, neoculin, and lysozyme,[62,99,101,102] plant secondary metabolites including stevioside,[93,100] to various synthetic chemicals, for example, sucralose, acesulfame K, saccharin, dulcin, aspartame, neotame, cyclamate, neohesperidin dihydrochalcone (NHDC),[1,62,93,99,100,102,103] and others such as guanidinoacetic acids (GA-1, GA-2).[93] To the best of our knowledge all the proven sweet-tasting compounds that have been used in these assays were capable of activating the receptor dimer, and is thus consistent with its universal role as the only human sweet taste receptor. In rodents, however, a T1R-independent taste may exist for certain sugar polymers such as polycose.[104] The establishment of the sweet receptor assays is necessary, as they are indispensable for setting up biotechnological high-throughput screens of compound libraries for the discovery of new sweeteners or sweet-taste modulators similar to the drug-identification strategies used for many years by the pharmaceutical industry. Although

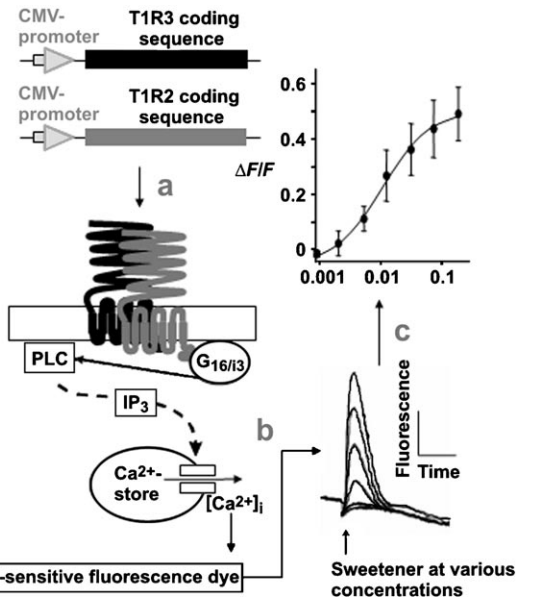


Figure 2. A functional in vitro expression assay for the sweet taste receptor T1R2-T1R3. a) Expression constructs of T1R3 and T1R2 are co-transfected into mammalian cell lines. The cells produce both T1R subunits, which initiate, after stimulation with sweet compounds, a signal transduction cascade involving an engineered, chimeric G protein subunit (G16/i3) and phospholipase C (PLC) that results in the generation of the secondary messenger inositol trisphosphate (IP_3). IP3 triggers, via its receptor, the transient release of calcium ions from intracellular stores into the cytosol. In cells loaded with the calcium-sensitive dye, a shift in the intracellular calcium concentrations results in changes in fluorescence. b) The changes in fluorescence of stimulated sweet receptor expressing cells are monitored with a fluorometric imaging plate reader (FLIPR). This device plots the fluorescence observed after excitation with monochromatic light over time to give the resulting traces. The fluorescence signal observed is dependent on the concentration of the sweet compound used for stimulation. c) The data derived from calcium-imaging experiments can be used to plot dose-response relationships (*y*-axis = changes in fluorescence ($\Delta F/F$); *x*-axis = log(agonist concentration)) to determine the activation thresholds, substance concentrations leading to half-maximal stimulation of the receptor-expressing cells (EC_{50} values), and the amplitude of the signal.

being artificial compounds discovered by the receptor-assay-based screening of chemical drug libraries, the first sweet-taste modulators have just been identified in this way and will become available soon.[105,106]

It is also of interest to note the differences of sweet taste receptors between species. Various compounds including the sweet protein thaumatin (**31**), neohesperidin dihydrochalcone (**29**), as well as the synthetic high-potency sweetener alitame are not perceived by rodents and stimulate only the human but not the murine receptor.[62,96,98,103,107,108] Perhaps the most striking example for variations in sweet taste receptors between species is observed for the family *Felidae* (cats), which are indifferent to sweet stimuli because they lack a functional T1R2 gene as a consequence of pseudogenization.[109] Thus, the biochemical properties of the receptor proteins are responsible for the abilities of mammals to detect such substances or not. In contrast to the extensive differences between species, individual differences in sweet taste sensi-

tivity have not been observed consistently in the human species.[108] However, a recent report demonstrated that two single nucleotide polymorphisms in the gene promoter region of T1R3 are associated with sensitivity to tasting sucrose.[110] Thus, altered T1R3 mRNA and polypeptide levels, but not altered properties of the receptor itself likely account for observed differences in the sensitivity to sweet taste.

4.3. Structure–Function Relationships of the Sweet Taste Receptor

To date, the structure of the sweet taste receptor has still not been resolved at atomic resolution. Nevertheless, combinations of different strategies including molecular modeling, mutational analysis of receptor function by heterologous expression in cell lines, and even by using biochemical and biophysical measurements have increased the detailed knowledge about the structure and function of this receptor molecule and the way it interacts with its various agonists.[62,98–100,103,107,111–113] As class C GPCRs, the sweet taste receptor subunits both possess a very large amino-terminal ectodomain (ATD). The ATD includes a venus-flytrap binding domain (VFTD) that likely contains the orthosteric binding site for various activators of the receptor. The VFTD shares sequence homologies to various known binding proteins of eukaryotes and bacteria. A cysteine-rich domain (CRD) connects the ATD to the heptahelical domain (HD), which is common to all GPCRs.[88,91,108,114,115] Models of the sweet receptor are based on sequence homologies with other receptor polypeptides which have been crystallized and their structures resolved at atomic resolution.[114] Thus, the ATD structure has been modeled on the basis of the crystal structure of the metabotropic glutamate receptor 1 ATDs.[116] The CRD is based on a template from the corresponding region of the tumor necrosis factor α receptor, whereas the model of the HD segments derives from the crystal structure of bovine rhodopsin.[117]

The observed interspecies differences of the sweet taste receptors to respond or not to certain sweeteners have proved to be very fruitful for the identification of the binding sites of the sweet receptors. First, researchers used interspecies hybrid receptors and then chimeric receptors to delineate the binding sites for the "sweet" agonists to either the ATDs or HDs of T1R2 or T1R3 (Figure 3).[98,99,103,107,112] Once the region was identified, single-point mutations were introduced in the T1R sequences, guided by comparisons of rat and human DNA sequences. By using this strategy or similar approaches, the interaction sites of several sweet compounds with the receptors have been mapped (Figure 4). Functional expression data do not allow effects of ligand binding to be distinguished from effects of receptor activation. Therefore, researchers combined these data with predictions from modeling studies to attribute the effects caused by the mutations introduced into the receptor subunits more precisely to ligand binding (Figure 5). Collectively, various such attempts suggest that the sweet receptor possesses multiple binding sites (Figure 4). Orthosteric binding occurs in both of the ATDs, while allosteric binding of activators as well as the

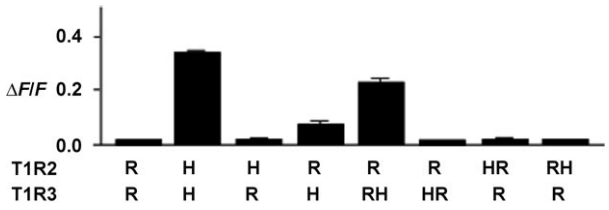


Figure 3. Calcium responses expressed as changes in the fluorescence ($\Delta F/F$) elicited by neohesperidin dihydrochalcone (**29**) in cells expressing various rat or human T1R2 and T1R3 subunits or chimeras thereof. R: subunit stems from rat; H: subunit is from man; RH: rat ATD attached to human HD; HR: human ATD attached to rat HD. The data reveal that the human receptor dimer, but not the rat counterpart, responds to application of the compound. The response appears to be mediated by the human T1R3, as the dimer is activated only when this subunit is of human origin. The dimer containing the rat T1R3 does not respond. The sweet receptor is also responsive if it contains a chimeric rat–human T1R3 containing the human HD region. This finding indicates clearly that sweetener **29** interacts with the HD region of human T1R3 to stimulate the sweet receptor. The data originate from Winnig et al.[103]

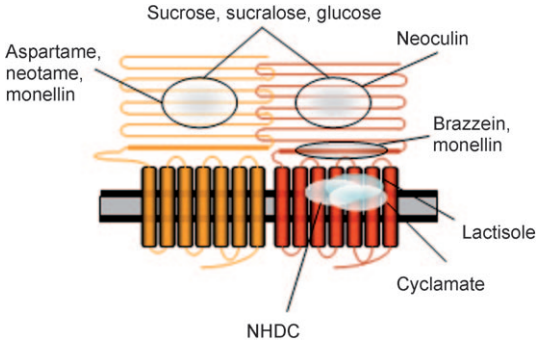


Figure 4. Binding sites of the sweet taste receptor T1R2-T1R3. The CRD is indicated by bold lines connecting the ATDs and HDs. The data were collected from various references.[98,99,101,103,107,111,112]

known sweet taste inhibitor lactisole occurs in the HD region of T1R3 (Figure 4). The binding of sugars to both ATD regions was verified by biophysical measurements.[111] When the ATDs of the murine T1R2 and T1R3 were expressed in and purified from bacteria without their associated HDs, they both bound sucrose, glucose, and sucralose, with reasonable affinities ranging from 2.5 to 15 mM.[111] The binding affinities of all three sweeteners for the T1R3 ATD decreased when the *sac* mutation was introduced, thus confirming the interaction of sugar molecules with the ATD.[111]

The molecular pharmacological properties of the sweet taste receptor determined in vitro were valuable for forming a mechanistic basis for the sensory perception of human subjects. The so-called "sweet-water" taste is observed in experiments when subjects sipped high concentrations of saccharin.[118,119] Subjects having highly concentrated solutions of saccharin in their mouth are known to hardly perceive a sweet taste, such as they do when tasting low saccharin concentrations. Under such conditions a strong bitter taste is noted which is mediated through the activation of two bitter

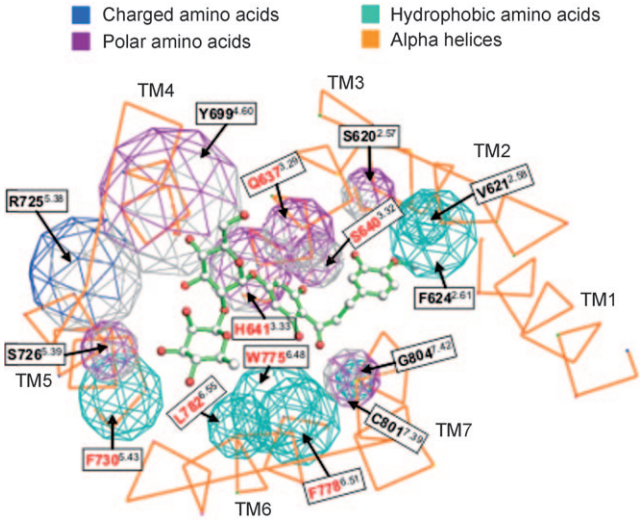


Figure 5. View of neohesperidin dihydrochalcone (**29**; stick model; white C, red O) docked into the T1R3 HD binding pocket. Intra- and extracellular loops have been removed for clarity. Transmembrane helices are shown by the orange zig-zag lines. Important residues are labeled with the Ballesteros–Weinstein nomenclature in addition to the amino acid one letter code. This nomenclature indicates first the number of the TM domain (TM = transmembrane) and separated by a dot for the position within the TM, with 50 being the most conserved residue in that TM. Residues located in the C terminal direction from this reference position are counted upwards, and those towards the N terminus downwards. Amino acids that influence the activation of T1r2-T1R3 through **29** are shown as spheres, the size of which correspond to the size of the amino acid side chains. The model suggests that the OH group in C3 of **29** is important for receptor–ligand interactions and the C3′-position should be in proximity to $H641^{3.33}$. **29** also interacts through the hydroxy group at C3 with $S640^{3.32}$ and the hydroxy groups at C6′ and C2′ of **29** could potentially interact with polar amino acids located in TM3 and TM7, such as $H641^{3.33}$, $Q637^{3.29}$, and $C801^{7.39}$. Favorable hydrophobic interactions between the B phenyl ring of **29** could be envisioned with $V621^{2.58}$ and $F624^{2.61}$, and between the linker of the A and B phenyl rings with $L800^{7.38}$ (not shown) and $G804^{7.42}$. The model predicts interactions between the C3-OH group of **29** with $S620^{2.57}$ in TM2. Moreover, the polar sugar moieties of **29** could interact with polar amino acids of TM4 and TM5, for example, $Y699^{4.60}$, $T724^{5.37}$ (not shown), $R725^{5.38}$, and $S726^{5.39}$. The data originate from Ref. [103].

taste receptors. If, however, the subjects expectorate the highly concentrated saccharin solution and rinse their mouth with pure water, an intense sweet perception becomes evident, namely, the "sweet water" taste.[119] These phenomena are explained by the presence of two binding sites for saccharin on the sweet receptor. One is a high-affinity orthosteric site that is occupied at low saccharin concentrations and activates the receptor, thus mediating sweet-taste perception. At higher saccharin concentrations a second allosteric site also becomes occupied and inhibits the sweet taste receptor again. Finally, washing out the compound from the mouth with table water first releases the sweetener from the inhibitory low-affinity site, thereby leaving the orthosteric site occupied. This results in a strong and long-lasting activation of the sweet receptor, thus mediating the erroneous impression that the water is intensely sweet.[119]

4.4. Structure–Function Relationships of the Umami Taste Receptor

Although T1R1-T1R3 is now widely accepted as the primary receptor for umami stimuli, the task in establishing its role was complicated by a number of observations. Firstly, sensory experiments and electrophysiological nerve recordings revealed contrasting evidence about the perception of L-amino acids as the fifth basic taste quality. Secondly, a number of alternative umami receptors, such as a truncated variant of the neural metabotropic glutamate receptor 4 (taste-mGluR4) have been proposed[61] and may be responsible for the partial persistence of L-glutamate taste in T1R3-deficient knock-out models,[97] although independently generated knock-out mouse models for T1R1 and T1R3 did not show residual responses.[96] The expression of taste-mGluR4 in type II taste receptor cells, as well as its reduced sensitivity to L-glutamate when compared to the neuronal full-length mGluR4, argue for a role in the perception of the umami taste.[61] However, its heterologous expression in mammalian cell lines failed to reproduce the most characteristic feature of umami taste: the well-known synergy between L-glutamate and 5′-ribonucleotides such as IMP. Therefore, the following discussion of the structure and function of the umami receptor will be focused on the heterodimeric receptor T1R1-T1R3.

Relatively little is known about the structure–function relationship of the umami heteromer compared to the sweet taste receptor heteromer T1R2-T1R3. However, as a consequence of the similar composition of both receptors, one can infer that the principle mechanism of the umami receptor heteromer should not be too different from what has been observed for the sweet taste receptor. The T1R1 subunit, which is unique to the umami receptor, was shown to interact with the L-glutamate molecule, as heterologous functional expression of chimeric receptors consisting of human T1R1 and rodent T1R3 was highly selective for L-glutamate and consistent with psychophysical evidence.[1] The proposed binding site for L-glutamate in T1R1 is located in the VFTD of the extracellular domain (Figure 6).[62] Mechanistically, it is proposed that binding of L-glutamate to the hinge region of the VFTD causes a closure of the domain. Interestingly, inosine-5′-monophosphate (IMP, **39**) also interacts at a different position of the VFTD and is presumed to stabilize the closed, active conformation.[120] A strikingly similar mechanism was recently discovered for the activation of the human sweet taste receptor,[105] spawned by the discovery of the first

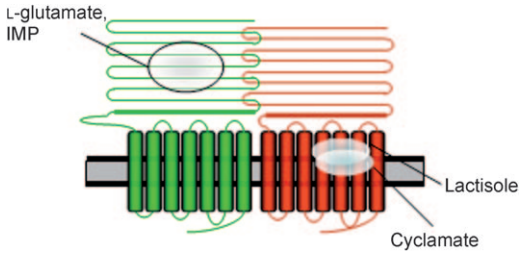


Figure 6. Binding sites of the umami taste receptors. The CRD is indicated by bold lines connecting the ATDs and HDs. The data are from Refs. [62, 98, 120].

positive allosteric modulators.[106] Again, the agonist—in this case sucrose or the artificial sweetener sucralose—was shown to interact with several amino acid residues within the VFTD. The allosteric modulator interacts with a different set of amino acid residues close to the orthosteric agonist. Molecular modeling studies even indicated that direct contacts are established between the agonist and positive modulators.

In an elegant set of experiments using chimeric T1Rs, in which extracellular domains of either human or rodent subunits were fused to the corresponding heterospecific transmembrane domains, the T1R3 transmembrane domain was shown to mediate responses to some agonists and modulators of the sweet taste receptor heteromer.[98] Whereas binding of the artificial sweetener cyclamate activates the receptor, the compound lactisole is a general inhibitor of T1R2-T1R3. As the T1R3 subunit is shared among the sweet and umami receptors, the assumption was made that cyclamate and lactisole might also modify the response of the umami receptor. Indeed, the authors demonstrated a modulatory role of both substances on heterologously expressed human T1R1-T1R3. While the sweet taste receptor blocker lactisole was shown to also inhibit responses of the umami receptor, cyclamate failed to activate T1R1-T1R3, but significantly enhanced L-glutamate responses.[98] The observation that the transmembrane domains of T1R1 and T1R2 in contrast to T1R3 exhibit constitutive activity and lead to activation of heterotrimeric G proteins in biochemical experiments suggests that G protein coupling is facilitated through the umami- and sweet-specific subunits.[121]

A number of studies have been devoted to answer the question as to whether single nucleotide polymorphisms (SNPs) occur in human T1R1 and T1R3 genes and if these SNPs may cause the reported individual differences in human umami taste perception.[122] The majority of nonsynomymous SNPs were identified in the umami-specific subunit T1R1. Out of the 18 different variant positions,[123–126] two were found to be located in the large extracellular amino terminal domain. For the human T1R3 subunit, 11 nonsynonymous SNPs were identified by the same studies.[123–126] In contrast to T1R1, the observed SNPs are more evenly distributed between the extracellular (7 nsSNPs) and transmembrane domains (4 nsSNPs).

In the study by Shigemura et al., the nonsynonymous polymorphism at amino acid position 372 of the T1R1 corresponded to significant differences in the perception of monosodium L-glutamate alone as well as to mixtures of monosodium L-glutamate and inosine-5′-monophoshate (**39**). Functional characterization of the variants T1R1-372A and T1R1-372T by heterologous expression revealed an about twofold difference in sensitivity, with the T1R1-372T variant being more sensitive. In the same set of experiments, the variant amino acid position 757 of T1R3 was analyzed. In this case, the variant T1R3-757R was more sensitive than T1R3-757C for monosodium L-glutamate alone, as well as for the combination of monosodium L-glutamate and inosine-5′-monophosphate. The in vitro data on position 757 of T1R3 also corresponded with human psychophysical experiments.

In an independent study, 142 French subjects were genotyped and analyzed for their taste sensitivity to L-glutamate. Although a correlation of nonsynonymous positions in T1R1 (A110V, A372T) and T1R3 (R757C) with individual differences in L-glutamate perception was evident, the observed variance between individuals could only be partially explained.[125] It should be noted that two of the three positions identified in this study are identical to those characterized by Shigemura et al.[126] The finding that T1R1-372T was more frequent in "tasters" and T1R3-757C was associated with the "nontaster" phenotype is in perfect agreement with the data presented by Shigemura et al.[126]

However, it should be noted that a similar analysis performed by Chen et al. on 242 subjects reported that the less-frequent alleles of three nonsynonymous T1R3 SNPs (T1R3-5T, T1R3-247H, and T1R3-757C) all result in higher umami ratings for certain concentrations of monopotassium L-glutamate.[124] With respect to position 757, this is in contradiction to the previously reported studies[125,126] and requires unequivocal clarification.

5. Functional Anatomy of the Taste System

5.1. Papillae and Taste Buds

Similar to stimuli of the other basic tastes, sweet and umami molecules interact with the apical membrane of specific sensor cells, namely, the taste receptor cells (TRCs) in the oral cavity. Unlike the sensor cells for odorants in the nasal cavity, TRCs are not of neuronal origin, but are specialized epithelial cells.[127,128] They are innervated by afferent nerves (transmitting towards the control nervous system) to transduce gustatory signals to the brain.[128] TRCs are found together with other cells in small aggregates called taste buds which are the proximate sensory organs of taste. These onion-shaped structures contain about 50–100 cells that are embedded in the nonsensory epithelium.[127] Taste buds are found in the three types of chemosensory papillae on the tongue: the fungiform papillae of the anterior tongue, the foliate papillae of the posterior tongue edges, and the circumvallate papillae of the posterior tongue.[127] Figure 7 illustrates the appearance of a fungiform and circumvallate human papilla and their taste buds. The number of papillae and taste buds vary among individuals, but estimates show that humans possess some 300 fungiform papillae with approximately 1100 taste buds. On average, 9 circumvallate papillae contain about 2200 taste buds and the two foliate papillae with their approximately 5 fissures harbor about 1300 taste buds.[127,129] Taste buds also occur numerously in the soft palate and are present in the pharynx, larynx, and epiglottis. Extralingual taste buds, however, are not organized in papillae.[130] Minor salivary glands connect with their ducts to the trenches and the fissures of the circumvallate and foliate papillae. Thus, secretions of the minor salivary glands surround the taste buds of the papillae of the posterior tongue and form a perireceptor milieu that could affect the detection of taste molecules.[131]

Taste bud cells fall into distinct classes. Historically, the various classes were defined on the basis of their morphological characteristics seen in light and electron micro-

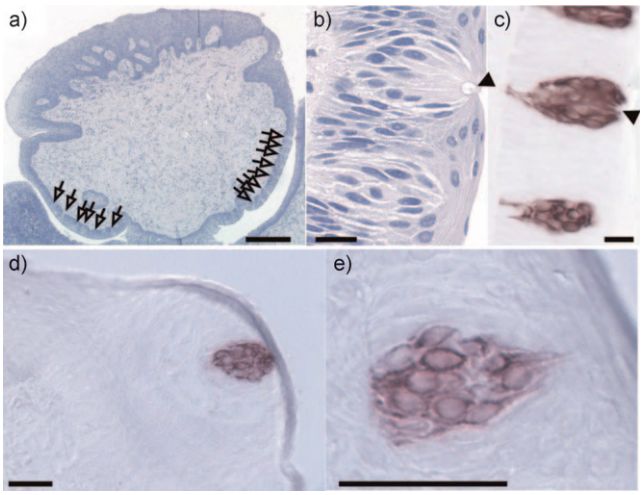


Figure 7. Human taste papillae and taste buds. a) Micrograph of a hemalum-eosin-stained cross-section of a circumvallate papilla. Taste buds are only hardly visible as small light dots (identified by arrows with open heads) in the otherwise blue-stained epithelium lining the trenches. b) Higher magnification of two circumvallate taste buds shown in (a). c) Circumvallate taste buds visualized by indirect immunohistochemistry with an antiserum against cytokeratin 20. Single taste bud cells are easily seen. The arrow heads in (b,c) point to taste pores. d) Fungiform papilla visualized as described in (c). e) Human fungiform taste bud visualized as described in (b). Scale bars: 200 μm (a), 20 μm (b–e).

graphs.[127,128] Today, we are coming closer to a functional classification that is based on the expression of specific taste polypeptides, functional imaging studies, and the characterization of gene-targeted mice which lack specific taste proteins.[132,133] The organization of the taste buds and their connection to the nervous system (Figure 8) has mostly been investigated in animal (mostly rodent) models. However, evidence suggests that the human system closely resembles that of other mammals, so that the observations in animals should apply to the human system as well. Roughly, five different cell types can be distinguished.[127,128]

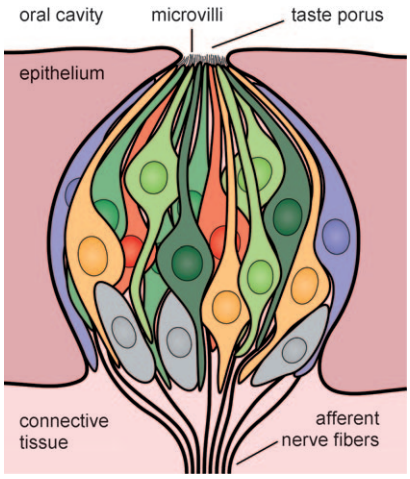


Figure 8. A taste bud. Yellow: type I cells, green: type II or receptor cells (subpopulations dedicated to sweet, umami, or bitter stimuli are shown in different green tones), orange: type III or presynaptic cells, gray: type IV or basal cells, blue: perigemmal or type V cells.

5.2. Type I cells

The electron-dense type I cells appear dark in electron microscopy images (in older literature type I cells are also called "dark cells"). They have an elongated shape and are characterized by numerous cytoplasmic processes that wrap around other cells, which suggest that they have glial-like functions. Type I cells also contain apical slender microvilli that extend into the taste pore, a depression in the epithelium where taste-active molecules contact their receptors. We emphasize that taste molecules only have access to the microvillous apical aspects of taste bud cells; tight junctions shield the basolateral cellular parts from the oral cavity.[127,128] Type I cells express the ecto-ATPase NTPDase 2 and a transporter protein GLAST for the excitatory neurotransmitter L-glutamate, a protein that transports L-glutamate back into the cytosol.[134] Both proteins terminate the actions of neurotransmitters, which is compatible with the proposed glial-like function of these cells. Recent evidence suggested that a subset of type I cells express functional epithelial sodium channel (ENaC), which is thought to be involved in the transduction of salty taste, thus resulting in this cell type having an active role in taste perception.[135]

5.3. Type II Cells and Sweet Receptor Signal Transduction

Type II cells are electron transparent, which causes them to appear light in electron microscopy images (type II cells are also known as "light cells") and they exhibit an elongated shape.[127,128] They are sometimes found in the upper part of a taste bud and do not always extend to its basal part. They possess several blunt microvilli that extend into the taste pore. These cells are also referred to as receptor cells. Numerous studies have shown that they express, in a mutually exclusive fashion, the taste receptors for sweet, umami, or bitter stimuli, thus forming three functional subsets. All three types of receptor cells express the same intracellular signaling proteins, including the G-protein subunits α-gustducin, β_1 or β_3, and γ_{13}, phospholipase C-β2 (PLC-β2), inositol trisphosphate receptor type III (IP_3R3), and transient receptor potential channel M5 (TRPM5), pannexin 1, and various connexins—the last two are proteins that form plasma membrane hemichannels.[132,133,136,137] Knockout mice lacking PLC-β2, α-gustducin, or IP_3R3 showed strongly diminished responses to sapid stimuli.[95,138] The identity of type II cells has further been confirmed by functional imaging experiments on isolated taste cells, taste buds, and lingual slice preparations.[139] The experiments showed that type II cells respond well to the administration of taste stimuli, in accordance with their role as receptor cells.

Type II cells appear to be narrowly tuned to stimuli of only one taste quality, that is, sweet, bitter, or umami.[140] The finding that type II receptor cells are not tuned across modalities is perfectly matched by the expression patterns

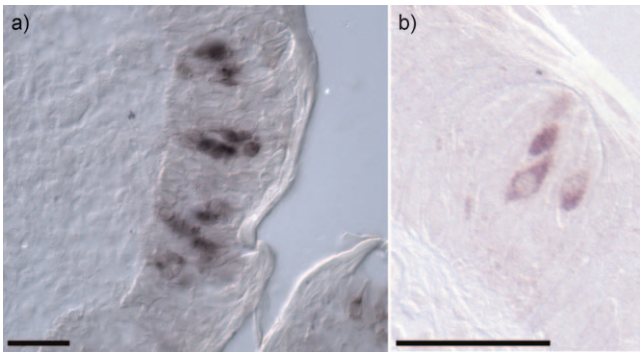


Figure 9. Nonspecific sweet and umami taste receptor subunit T1R3 in human circumvallate taste cells. a) In situ hybridization with a specific antisense probe identified T1R3 mRNA in circumvallate taste buds. b) Indirect immunohistochemistry using a specific antiserum against T1R3 detected in a subset of taste receptor cells. Scale bar: 20 μm (a).

of the taste receptors (Figure 9), which are mutually exclusive.[95,136,141] The so called taste 2 bitter receptors (TAS2Rs or T2Rs), which form their own class of GPCRs, are not coexpressed with T1R receptors in the same cells.[89,93,96,142] Cells expressing T1R1—the umami receptor-specific T1R subunit—differ from cells expressing T1R2—the sweet receptor-specific T1R subunit.[89,93,96,142] These data not only identify the particular receptor cell type, but, together with other findings from biochemical experiments or functional expression studies in heterologous cells, also indicate that sweet, umami, and bitter taste receptors signal through the same intracellular proteins that eventually lead to the release of the neurotransmitter adenosine-5′-triphosphate (ATP, Figure 10).[95,137,143] This transmitter is not released through classical synaptic mechanisms; instead it is discharged through pannexin and/or connexin hemichannels.[137] The release of nonsynaptic transmitters is in accordance with the observation that type II cells lack proteins of the synaptic release machinery as well as voltage-gated calcium channels.[139,144] The extruded ATP molecule binds to ionotropic purinergic $P2X_2/P2X_3$ receptors and thereby stimulates the afferent nerves to fire action potentials and to transduce the taste nerve response to the central nervous system.[143] Afferent nerve endings are present in the vicinity of type II cells but do not engage in synapses. Double knockout mice for $P2X_2/P2X_3$ receptors lost their taste responses to stimuli of all taste qualities, thus highlighting the crucial role of ATP for transmitting gustatory information.[143] ATP also excites type II cells in a positive feedback loop through purinergic $P2Y_4$ receptors, probably to further stimulate its own release.[145]

5.4. Type III Cells and Sour Taste

Type III cells visualized by electron microscopy display, compared to type I and type II cells, intermediate electron density and have a single long slender microvillus that contacts the taste pore. They express various neural proteins such as the neural cell adhesion molecule NCAM.[127,128]

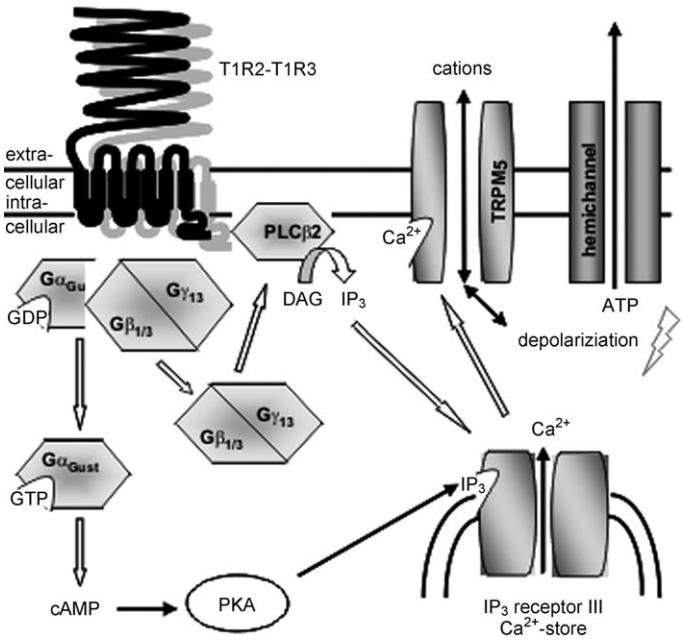


Figure 10. Signal transduction cascade of sweet receptors. Activation of the receptor by sweet compounds results in activation of a heterotrimeric G protein consisting of $G\gamma_{13}$ and $G\beta_1$ or $G\beta_3$. The α subunit could be α-gustducin (α_{Gust}) or a few other candidates that are under discussion. Following exchange of guanosin-5′-diphosphate (GDP) for guanosin-5′-triphosphate (GTP), the β/γ dimer stimulates PLC-β2 to cleave the membrane lipid phosphatidyl inositolbisphosphate into inositoltrisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to its type III receptor to release Ca^{2+} from intracellular stores in the smooth endoplasmic reticulum. Ca^{2+} opens TRPM5 and the resultant cation flux depolarizes the cell to trigger the opening of hemichannels to release ATP. α-Gustducin decreases cyclic adenosine 5′-monophoaphate (cAMP) levels by an as yet unestablished mechanism. Low cAMP levels and a low activity of cAMP-dependent protein kinase (PKA) are required for adequate Ca^{2+} release through IP_3 receptor III.[170] Thus, α-gustducin sensitizes a taste receptor cell for a relevant response.

Type III cells are the only cell type in taste buds that engage in forming conventional synapses and thus show presynaptic specializations and express synaptic proteins.[144] Therefore, type III cells are also referred to as presynaptic cells.[127,128] This cell type does not respond to sweet, umami, or bitter stimuli. It does respond to depolarizing concentrations of extracellular K^+ with Ca^{2+} influx as well as the release of the neurotransmitters serotonin and, to a lesser extent, norepinephrine.[139,146] Type III cells specifically express a certain member of the transient receptor potential family, called PKD2L1.[147–149] Mice genetically engineered to express the diphtheria toxin specifically in the PKD2L1 cells are not only devoid of their PKD2L1 cells but also lost their nerve responses to acidic taste stimuli, thereby demonstrating that type III cells are sour-sensing cells.[147] This finding is further supported by observations that show that type III cells respond to acidic stimulation by Ca^{2+} influx and release of neurotransmitter.[150] However, the molecular identity of the sour receptor(s) still remains elusive, even though various candidates have been proposed, including PKD2L1 and its binding partner PKD1L3.[148,151] Type III cells also mediate the taste of carbonation or gaseous CO_2.[152] Experiments in

knockout mice have identified carbonic anhydrase 4, an enzyme that is attached to the cell surface of type III cells through a glycosylphosphatidylinositol anchor, as the principal CO_2 taste sensor. The experiments also show that synaptic transmission in type III cells is crucial for the taste response to CO_2.[152]

5.5. Type IV and Perigemmal Cells

Type IV cells display a shape similar to that of epithelial cells in the stratified squamous epithelium.[127,128] They are present at the basal region of taste buds and do not possess elongations that contact the taste pore. These cells express the developmental signaling protein sonic hedgehog, which indicates that they proliferate and is suggestive that they have a role as precursors of the other taste bud cells.[153] In this context it must be mentioned that taste cells are short-lived and are replaced after approximately 10 days. Perigemmal or type V cells are of similar appearance as type IV cells but, when they extend into the taste bud, they adopt a type I cell-like shape (Figure 8).[127,128] They are found in layers in the periphery of taste buds and form a network of keratin bundles. They do not contact the taste pore and are thought to support the taste bud or to form diffusion barriers that restrict the access of small molecules by lateral diffusion through the surrounding epithelium.[127,128]

5.6 Transmission of Gustatory Information to the Cerebral Cortex

Taste buds are innervated by afferent fibers of three cranial nerves.[127,128] Taste buds of the fungiform papillae of the anterior tongue and palatal taste buds are innervated by the Chorda tympani and the greater superficial petrosal branches of the VII cranial nerve, the facial nerve, respectively (Figure 11). Taste buds of the foliate and circumvallate papillae of the posterior tongue receive fibers from the lingual-tonsillar branch of the IX cranial nerve, the glossopharyngeus nerve. Taste buds of the pharyngeal and laryngeal epithelium are innervated by the X cranial nerve, the vagus nerve. The cell bodies of these fibres are found in three peripheral ganglia: the geniculate ganglion (VII), the petrosal (IX), and nodose ganglion (X). The fibers of the ganglionic neurons terminate in the medulla in a small region, referred to as the gustatory nucleus, which corresponds to the rostral central subdivision of the nucleus of the solitary tract (NTS), with the geniculate fibers ending most rostral followed caudally by the petrosal and nodose fibers.[154] Taste information is transmitted from the NTS in a descending pathway to various oromotor nuclei and the salivatory nucleus of the brain stem that govern jaw movements and salivation.[154] Thus, taste information appears to be used in local sensory-motor reflex pathways to control chewing, swallowing, salivation of palatable food, or opening of the mouth to eject hazardous toxic (bitter) compounds.[133] The ascending lemniscal pathway is used for the detection and discrimination of stimuli.[133,155] In humans and other primates, it connects the NTS taste neurons to relay neurons in the

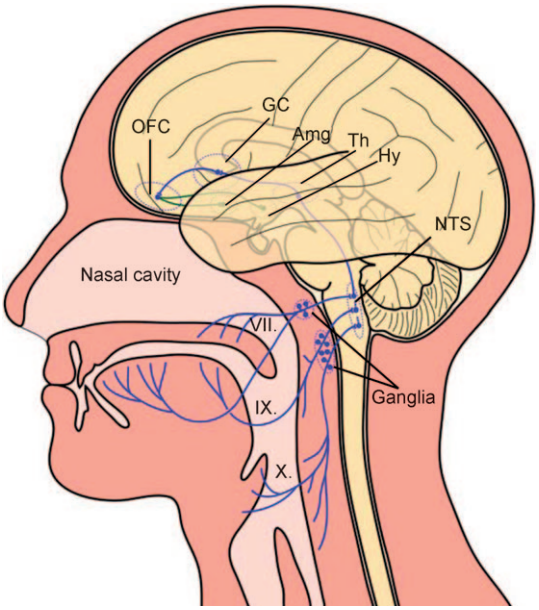


Figure 11. Transmission pathways of gustatory information. For details see main text. VII–X: cranial nerves; NTS: nucleus of the solitary tract; Th: thalamus; GC: gustatory cortex; OFC: orbitofrontal cortex; Am: amygdale; Hy: hypothalamus.

parvocellular division of the ventroposteromedial thalamic nucleus.[154] In rodents, however, this ascending pathway is obligatory relayed in the parabrachial nucleus of the pons. Taste information is further conveyed from the thalamic relay nucleus to the primary taste or gustatory cortex, that is, part of the cerebral cortex which includes two regions in the rostral frontal operculum and the adjacent insula. The activities of a relatively small proportion of taste neurons in this region represent the taste qualities.[156] This can be visualized in humans by using imaging techniques,[157] whereas the activities of numerous other neurons depend on other stimuli that relate to food, such as jaw or tongue movement, sight of food, and tactile stimulation of the mouth.[156] Gustatory information is transmitted from the primary taste cortex to the orbitofrontal cortex, also referred to as the secondary taste cortex. In this region, the sensory modalities of vision, olfaction, and taste converge to construct flavor representations.[156] Neurons in this region also appear to be involved in stimulus-specific satiety. The orbitofrontal cortex sends taste information to the amygdala and the lateral hypothalamus. These regions appear to impart hedonic value to taste or integrate taste information in the context of energy needs or reward.[156] In rodents, taste information is conveyed into the lateral hypothalamus and amygdala from the parabrachial nucleus—the obligatory relay that does not exist in primates.[154]

5.7. Link between Taste Cell Types and Behavior

The expression of sweet, umami, and bitter receptors (and also of the sour receptor candidate PKD2L1) in distinct subsets of receptor cells together with the observation that

receptor cells respond in vitro to only one category of taste stimuli (as discussed in Sections 5.2–5.5) suggest that taste cells form independent populations that are dedicated to the detection and transduction of only one taste quality. Several reports on genetically engineered mice provide definite evidence for this conclusion.[95,136,141,147,158] Mice with targeted deletions of the T1R1 or T1R2 gene selectively lost their nerve and behavioral responses to sweet or umami substances, whereas their responses to stimuli of the other basic tastes remained unaffected.[96] As expected, mice lacking T1R3 lost their sweet and umami taste but responded normally to salty, sour, and bitter stimulation.[96,159] The data clearly show that the receptors are specific for compounds of only the cognate taste quality.

In a sophisticated series of studies, mice having a targeted deletion of the PLC-β2 gene, were shown to lack responses to sweet, bitter, and umami compounds, in line with the central role of this signaling molecule in the transduction of these three tastes (Figure 10), while sour and salty tastes were normal.[95] When these mice were engineered to express PLC-β2 activity selectively in their bitter taste cells, their bitter taste was selectively recovered while they remained unresponsive to umami and sweet stimuli. The data clearly demonstrate that, in the periphery, a taste quality is encoded independently of the other taste qualities.[136]

Mice engineered to express a modified opioid receptor that responds only to a synthetic opiate in their sweet cells acquired strong attraction to the synthetic opiate. However, mice strongly avoided this opiate when the same receptor was expressed in their bitter taste cells.[95] The same observations were made when a human bitter taste receptor, for a compound that wild-type mice do not detect, was expressed in murine bitter or sweet taste cells by genetic manipulation. Depending on the site of expression in sweet or bitter cells, mice gained attraction or aversion.[141] These results indicate that sweet taste cells are "hard wired" to the nervous system to trigger attraction as a stereotypic behavioral output, whereas bitter cells trigger aversion.[136] Moreover, these studies demonstrate that the receptor molecules present in taste cells are the devices that determine their chemical specificity and convert chemical structures into biochemical and eventually neural responses. Finally, it should be stressed that the taste of a (sweet or bitter) chemical is defined by the subpopulation of taste cells that it excites.

6. Sweet Taste—A Metabolic Sense Involved in Energy Homeostasis

It appears conceptually logical that intake behavior and metabolic demands are somehow coordinated. For example, the ability of an organism to detect carbohydrates should be most sensitive in times of hunger or caloric need. Thus, under these conditions the sweet taste system should be sensitized. If energy stores are filled and the organism rests and digests, the sensitivity to sweet taste may drop. Moreover, sweet taste receptors could be used as fuel sensors in the alimentary canal to regulate gastrointestinal physiology or in the brain, where they could monitor adequate monosaccharide levels. In the following, a few examples are given to illustrate how the taste system is involved in metabolic control.

The hormone leptin is released from adipose tissue into the plasma in proportion to the fat mass, and thus signals the size of the body's energy stores.[160] The signal is detected by hypothalamic leptin receptors and, as a consequence, catabolic pathways are stimulated and food intake reduced if leptin levels are high.[161] If leptin levels are low, anabolic pathways and food intake are activated. The administration of leptin to normal, but not obese, and diabetic db/db (db = diabetes) mice which show defective leptin signaling reduced the peripheral gustatory neural and behavioral responses to sweet substances by about 30%, thus suggesting that the action of leptin on sweet taste requires functional leptin receptors.[162] Leptin inhibited the firing of the sweet-stimuli-induced action potential of isolated murine taste cells ex vivo, which demonstrates that it acts directly on the sweet taste cells themselves.[163] The role of leptin in regulating sweet taste sensitivity was also observed in humans.[163,164]

Leptin is released in a pulse fashion, which gives rise to diurnal variations in the leptin plasma concentrations, with about 30 peaks per day. However, the phase can be shifted by eating or fasting. It has been observed that the levels of plasma leptin and sensitivity to sweet taste synchronize.[165] The effect is specific for sweet taste and was not observed for the other taste qualities. More recently, it was also found that sweet taste is enhanced by the orexigenic endocannabinoids anandamide and arachidonoyl glycerol.[166] These compounds act through cannabinoid type 1 receptors in the hypothalamus and limbic forebrain structure to enhance appetite and feeding. Circulating endocannabinoid concentrations inversely correlate with plasma leptin levels. As expected, endocannabinoids selectively enhanced neural and behavioral responses to sweet taste stimuli without affecting the other basic tastes. It also boosted the electrophysiolgical responses of sweet taste cells. Specific inhibitors of the endocannabinoid type 1 receptor blocked the responses, which were also absent in mice with targeted disruption of the gene for this receptor.

The sense of sweet taste not only responds to metabolic needs as outlined above, but also regulates fuel homeostasis. Sweet taste receptors and their downstream signaling molecules were found to be present in the gastrointestinal tract of enteroendocrine L cells of the mouse and duodenum and small intestine in human.[167–169] Homogenates secreted glucagon-like peptide 1 (GLP-1) and gastrointestinal polypeptide (GIP) in response to glucose duodenal tissue, thereby giving rise to elevated plasma hormone concentrations. Subsequent to stimulation of L cells with glucose, adjoining enterocytes increased their mRNA levels for the sodium-dependent glucose transporter isoform 1 (SGLT1), which suggests that the glucose load led to enhanced absorptive capacity of the gut epithelium.[169] Both hormones are also known regulators of insulin release. Thus, sweet taste receptors are critically implicated in mediating the so-called incretin effect, that is, the observation that an oral glucose load augments insulin much stronger than a comparable intravenous glucose administration.[168,169] Although many more details need to be worked out in the future to fully understand the interplay of taste and metabolism, the present

data strongly suggest that there is interdependence of both systems in energy homeostasis.

7. Summary and Outlook

The array of sensory chemoreceptors provides valuable information on the overall flavor quality of the food we eat. However, the development of healthier food products, for example, reduced in fat, sugar, or salt, may sometimes induce unacceptable flavor defects in the products and has, thus, created unexpected flavor challenges for the food industry. In response to the consumers' demand for healthy but tasty foods, the discovery of novel ingredients as sweetness and umami enhancers has become essential to overcome such flavor challenges associated with the production of low-calorie beverages, salt-reduced foods, and food products without any MSG added. In particular, a vast number of highly sweet-tasting natural products, belonging to the chemical class of terpenoids, steroids, phenolics, and proteins, have been isolated from botanical sources and successfully identified in the past decades. Unfortunately, the low pH and temperature stability as well as the lingering bitter aftertaste of most of the high-potency sweeteners limit their use in food products. Academia as well as the global flavor and food companies are searching intensively for stable and safe high-potency molecules that modulate the perception of sweet and umami taste so as to make our daily diet more healthy, without compromising on the quality and palatability of the food.

The sensomics approach is currently mainly used for the activity-guided search for taste modulators in complex natural sources such as, for example, foods, botanicals, and biotechnological fermentation broths. Spurred on by the enormous progress made within the last decade on mammalian taste receptors and the increasing characterization of the binding sites for agonists and modulators by using approaches based on sophisticated chimeric receptors and mutagenesis, some companies have started to screen large sets of individual synthetic molecules by using post-genomic high-throughput array technologies similar to the drug-discovery strategies used for many years by the pharmaceutical industry. Although first hits have already led to the development of some intensely umami tasting, but artificial compounds that function at the lower ppm level, the umami taste profil of these compounds seem to be rather different from that of naturally occurring MSG, and the lingering aftertaste of these synthetic molecules is reminiscent of the very strong artificial sweeteners. It is anticipated that the molecular interactions between agonists/modulators and the corresponding TAS1Rs will be elucidated in the future in more and more detail. This process may finally allow the rational design of novel sweet and umami compounds without associated undesirable phenomena such as lingering aftertastes or side tastes. Furthermore, more detailed molecular analyses may also help to understand the differential nature of kinetics during the desensitization of sweet taste receptors.

Moreover, the molecular characterization of T1Rs is no longer restricted to taste research. As indicated by recent findings on T1R expression in the gastrointestinal tract of mammals, it has become an important aspect in research on the sensing of metabolic nutrients. Most interestingly, the sweet and, perhaps, also the umami receptor are not limited to the detection of nutrients present in the gut and, thereby, modulating the metabolic responses, the metabolic state also modulates the activation of T1Rs. It will be one of the future challenges to identify how the sensing of gastrointestinal nutrients, for example, by the sweet taste receptor, is coordinated with centrally processed information from the oral cavity to result in adequate adjustment of metabolic parameters. The answers to these important questions will again most likely stimulate the development of novel sweet and umami modulators which need to be designed in such a way that they hit their target receptor proteins in the oral cavity to enhance food palatability and, after ingestion and passage through the stomach, to activate or also to inhibit the same receptor in the gastrointestinal tract.

List of Abbreviations:

ara	L-arabinopyranosyl
glc	D-glucopyranosyl
glcA	D-glucuronopyranosyl
HPLC	high-performance liquid chromatography
MS	mass spectrometry
qui	D-quinovosyl
rha	L-rhamnopyranosyl
xyl	D-xylopyranosyl

We would like to thank the German Research Foundation for support of our original work refered to in this Review. We also thank Dr. Frauke Stähler, Jonas Töle, and Stephan Born for kindly providing images and illustrations.

Received: April 8, 2010
Published online: February 17, 2011

[1] G. Nelson, J. Chandrashekar, M. A. Hoon, L. Feng, G. Zhao, N. J. Ryba, C. S. Zuker, *Nature* **2002**, *416*, 199 – 202.
[2] A. D. Kinghorn, D. D. Soejarto, *Crit. Rev. Plant Sci.* **1986**, *4*, 79 – 120.
[3] T. H. Grenby, *Chem. Br.* **1991**, *27*, 342 – 345.
[4] B. V. Howard, J. Wylie-Rosett, *Circulation* **2002**, *106*, 523 – 527.
[5] V. B. Duffy, G. H. Anderson, *J. Am. Diet. Assoc.* **1998**, *98*, 580 – 587.
[6] Official Journal of the European Union, Commission Directive, **2009**/163/EU of 22.12.2009.
[7] N. Kim, A. D. Kinghorn, *Arch. Pharmacal Res.* **2002**, *25*, 725 – 746.
[8] J. E. Steiner, *Umami: A Basic Taste* (Eds.: Y. Kawamura, M. R. Kare), Marcel Dekker, New York, **1987**, pp. 97 – 123.
[9] D. K. Rassin, J. A. Sturman, G. E. Gaull, *Early Hum. Dev.* **1978**, *2*, 1 – 13.
[10] a) S. Yamaguchi, *J. Food Sci.* **1967**, *32*, 473 – 478; b) S. Yamaguchi, T. Yoshikawa, S. Ikeda, T. Ninomiya, *J. Food Sci.* **1971**, *36*, 846 – 849.
[11] a) M. Freeman, *J. Am. Acad. Nurse Pract.* **2006**, *18*, 482 – 486; b) L. Tarasoff, M. F. Kelly, *Food Chem. Toxicol.* **1995**, *33*, 69 – 72.

[12] R. A. Hussain, A. D. Kinghorn, D. D. Soejarto, *Econ. Bot.* **1988**, *42*, 267 – 283.
[13] G. E. DuBois, *Annu. Rep. Med. Chem.* **1982**, *17*, 323 – 332.
[14] A. D. Kinghorn, N. Kaneda, N.-I. Nam-In Baek, E. J. Kennelly, D. D. Soejarto, *Med. Res. Rev.* **1998**, *18*, 347 – 360.
[15] A. D. Kinghorn, D. Soejarto, *Med. Res. Rev.* **1989**, *9*, 91 – 115.
[16] C. M. Compadre, J. M. Pezzuto, A. D. Kinghorn, S. K. Kamath, *Science* **1985**, *227*, 417 – 419.
[17] A. D. Kinghorn, C. M. Compadre in *Alternative Sweeteners*, 3rd ed. (Ed.: L. Nabors O'Brien), Marcel Dekker, New York, **2001**, pp. 209 – 233.
[18] J. M. C. Geuns, *Phytochemistry* **2003**, *64*, 913 – 921.
[19] B. Crammer, R. Ikan, *Chem. Soc. Rev.* **1977**, *6*, 431 – 465.
[20] G. E. DuBois, R. A. Stephenson, *J. Med. Chem.* **1985**, *28*, 93 – 98.
[21] M. C. Carakostas, L. L. Curry, A. C. Boileau, D. J. Brusick, *Food Chem. Toxicol.* **2008**, *46*, S1 – S10.
[22] a) FDA GRAS Notice GRN No. 000253 and GRN No. 000252); b) JECFA, Joint FAO/WHO Expert Committee on Food Additives Monographs 5, Compendium of Food Additive Specifications from 69th JEFCA Meeting, Rome, June 17 – 26, **2008**.
[23] a) T. Tanaka, H. Kohda, O. Tanaka, F.-H. Chen, W.-H. Chou, J.-L. Leu, *Agric. Biol. Chem.* **1981**, *45*, 2165 – 2166; b) K. Ohtani, Y. Aikawa, R. Kasai, W.-H. Chou, K. Yamasaki, O. Tanaka, *Phytochemistry* **1992**, *31*, 1553 – 1559.
[24] T. Tanaka, O. Tanaka, Z. W. Lin, J. Zhou, *Chem. Pharm. Bull.* **1985**, *33*, 4275 – 4280.
[25] T. Takemoto, S. Arihara, T. Nakajima, M. Okuhira, *Yakugaku Zasshi* **1983**, *103*, 1151 – 1154.
[26] a) R. Kasai, R. Nie, K. Nashi, K. Ohtani, J. Zhou, G. Tao, O. Tanaka, *Agric. Biol. Chem.* **1989**, *53*, 3347 – 3349; b) K. Matsumoto, R. Kasai, K. Ohtani, O. Tanaka, *Chem. Pharm. Bull.* **1990**, *38*, 2030 – 2032.
[27] W. Jakinovich, C. Moon, Y.-H. Choi, A. D. Kinghorn, *J. Nat. Prod.* **1990**, *53*, 190 – 195.
[28] Y. Xia, M. E. Rivero-Huguet, B. H. Hughes, W. D. Marshall, *Food Chem.* **2008**, *107*, 1022 – 1028.
[29] D. J. Yang, Z. C. Zhong, Z. M. Xie, *Acta Pharmacol. Sin.* **1992**, *27*, 841 – 844.
[30] M. Yoshikawa, T. Morikawa, K. Nakano, Y. Pongpiriyadacha, T. Murakami, H. Matsuda, *J. Nat. Prod.* **2002**, *65*, 1638 – 1642.
[31] I. Kitagawa, *Pure Appl. Chem.* **2002**, *74*, 1189 – 1198.
[32] R. L. Smith, P. Newberne, T. B. Adams, R. A. Ford, J. B. Hallagan, *Food Technol.* **1996**, *50*, 72 – 78.
[33] European Commission, Opinion of the Scientific Committee on Food on Glycyrrhizinic acid and its Ammonuim salt (April 4**, 2003**).
[34] a) J. Jizba, V. Herout, *Collect. Czech. Chem. Commun.* **1976**, *32*, 2867 – 2874; b) J. Jizba, L. Dolejs, V. Herout, F. Sorm, *Tetrahedron Lett.* **1971**, *12*, 1329 – 1332.
[35] H. Yamada, M. Nishizawa, *Synlett* **1993**, 54 – 56.
[36] H. Yamada, M. Nishizawa, *J. Org. Chem.* **1995**, *60*, 386 – 397.
[37] J. Kim, J. M. Pezzuto, D. D. Soejarto, F. A. Lang, A. D. Kinghorn, *J. Nat. Prod.* **1988**, *51*, 1166 – 1172.
[38] A. D. Kinghorn, C. M. Compadre in *Alternative Sweeteners*, 2nd ed. (Eds.: L. O'Brien Nabors, R. C. Gelardi), Marcel Dekker, New York, **1991**, pp. 197 – 218.
[39] E. H. Rennie, *J. Chem. Soc. Trans.* **1886**, *49*, 857 – 865.
[40] R. M. Horowitz, B. Gentili in *Alternative Sweeteners* (Eds.: L. O'Brien, R. C. Gelardi), Marcel Dekker, New York, **1986**, pp. 135 – 153.
[41] F. Borrego, H. Montijano in *Alternative Sweeteners*, 3rd ed. (Ed.: L. O'Brien Nabors), New York, **2001**, pp. 87 – 104.
[42] H. Van der Wel, K. Loeve, *Eur. J. Biochem.* **1972**, *31*, 221 – 225.
[43] R. B. Iyengar, P. Smits, F. van der Ouderaa, H. van der Wel, J. van Brouwershaven, P. Ravestein, G. Richters, P. D. van Wassenaar, *Eur. J. Biochem.* **1979**, *96*, 193 – 204.
[44] C. M. Ogata, P. F. Gordon, A. M. de Vos, S.-H. Kim, *J. Mol. Biol.* **1992**, *228*, 893 – 908.
[45] P. Temussi, *Cell. Mol. Life Sci.* **2006**, *63*, 1876 – 1888.
[46] a) B. Muermann, *Ernaehr.-Umsch.* **1998**, *45*, 206 – 208; b) S. Salminen, A. Hallikainen in *Food Additives*, 2nd ed. (Eds.: R. L. Branen, P. M. Davidson, S. Salminen, J. H. Thorngate), Marcel Dekker, New York, **2002**, pp. 447 – 476.
[47] D. Ming, G. Hellekant, *FEBS Lett.* **1994**, *355*, 106 – 108.
[48] H. van der Wel, G. Larson, A. Hladik, C. M. Hladik, G. Hellekant, D. Glaser, *Chem. Senses* **1989**, *14*, 75 – 79.
[49] G. E. Inglett, J. F. May, *J. Food Sci.* **1969**, *34*, 408 – 411.
[50] Z. Bohak, S.-L. Li, *Biochim. Biophys. Acta Protein Struct.* **1976**, *427*, 153 – 170.
[51] J. A. Morris, R. H. Cagan, *Biochim. Biophys. Acta General Subjects* **1972**, *261*, 114 – 122.
[52] T. Masuda, N. Kitabatake, *J. Biosci. Bioeng.* **2006**, *102*, 375 – 389.
[53] Z. Hu, L. Peng, M. He, *Yunnan Zhiwu Yanjiu* **1985**, *7*, 1 – 10.
[54] Z. Hu, M. He, *Yunnan Zhiwu Yanjiu* **1983**, *5*, 207 – 212.
[55] M. Ding, Z. Hu, *Yunnan Zhiwu Yanjiu* **1986**, *8*, 181 – 192.
[56] D.-F. Li, P. Jiang, D.-Y. Zhu, Y. Hu, M. Max, D.-C. Wang, *J. Struct. Biol.* **2008**, *162*, 50 – 62.
[57] X. Liu, S. Maeda, Z. Hu, T. Aiuchi, K. Nakaya, Y. Kurihara, *Eur. J. Biochem.* **1993**, *211*, 281 – 287.
[58] H. Yamashita, S. Theerasilp, T. Aiuchi, K. Nakaya, Y. Nakamura, Y. Kurihara, *J. Biol. Chem.* **1990**, *265*, 15770 – 15775.
[59] A. Shimizu-Ibuka, Y. Morita, T. Terada, T. Asakura, K.-i. Nakajima, S. Iwata, T. Misaka, H. Sorimachi, S. Arai, K. Abe, *J. Mol. Biol.* **2006**, *359*, 148 – 158.
[60] a) K. Ikeda, *J. Tokyo Chem. Soc.* **1909**, *30*, 820 – 836; b) K. Ikeda, *Chem. Senses* **2002**, *27*, 847 – 849.
[61] N. Chaudhari, A. M. Landin, S. D. Roper, *Nat. Neurosci.* **2000**, *3*, 113 – 119.
[62] X. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller, E. Adler, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4692 – 4696.
[63] a) K. H. Ney, *Z. Lebensm.-Unters. Forsch.* **1971**, *146*, 141 – 143; b) J. Velíscaronek, J. Davídek, V. Kubelka, T. Tran Thi Bich, J. Hajscaronlová, *Food/Nahrung* **1978**, *22*, 735 – 743.
[64] T. Warendorf, H.-D. Belitz, U. Gasser, W. Grosch, *Z. Lebensm.-Unters. Forsch.* **1992**, *195*, 215 – 223.
[65] A. Dunkel, T. Hofmann, *J. Agric. Food Chem.* **2009**, *57*, 9867 – 9877.
[66] S. Yamaguchi, K. Ninomiya, *J. Nutr.* **2000**, *130*, 921S – 26S.
[67] H. Schlichtherle-Cerny, R. Amado, *J. Agric. Food Chem.* **2002**, *50*, 1515 – 1522.
[68] C. Winkel, A. de Klerk, J. Visser, E. de Rijke, J. Bakker, T. Koenig, H. Renes, *Chem. Biodiversity* **2008**, *5*, 1195 – 1203.
[69] M. Noguchi, S. Arai, M. Yamashita, H. Kato, M. Fujimaki, *J. Agric. Food Chem.* **1975**, *23*, 49 – 53.
[70] a) S. Arai, M. Yamashita, M. Noguchi, M. Fujimaki, *Agric. Biol. Chem.* **1973**, *37*, 151 – 156; b) K. Maehashi, M. Matsuzaki, Y. Yamamoto, S. Udeka, *Biosci. Biotechnol. Biochem.* **1999**, *63*, 555 – 559.
[71] S. Kaneko, K. Kumazawa, H. Masuda, A. Henze, T. Hofmann, *J. Agric. Food Chem.* **2006**, *54*, 2688 – 2694.
[72] a) S. Toelstede, T. Hofmann, *J. Agric. Food Chem.* **2008**, *56*, 2795 – 2804; b) S. Toelstede, T. Hofmann, *J. Agric. Food Chem.* **2008**, *56*, 5299 – 5307; c) J. C. Hufnagel, T. Hofmann, *J. Agric. Food Chem.* **2008**, *56*, 9190 – 9199.
[73] a) N. Rotzoll, A. Dunkel, T. Hofmann, *J. Agric. Food Chem.* **2005**, *53*, 4149 – 4156; b) N. Rotzoll, A. Dunkel, T. Hofmann, *J. Agric. Food Chem.* **2006**, *54*, 2705 – 2711.

[74] a) I. Schräder, K. Eichner, *Z. Lebensm.-Unters. Forsch.* **1996**, *202*, 474 – 480; b) T. Davidek, K. Kraehenbuehl, S. Devaud, F. Robert, I. Blank, *Anal. Chem.* **2005**, *77*, 140 – 147.
[75] E. Beksan, P. Schieberle, F. Robert, I. Blank, L. B. Fay, H. Schlichtherle-Cerny, T. Hofmann, *J. Agric. Food Chem.* **2003**, *51*, 5428 – 5436.
[76] H. Ottinger, T. Hofmann, *J. Agric. Food Chem.* **2003**, *51*, 6791 – 6796.
[77] H. Ottinger, T. Soldo, T. Hofmann, *J. Agric. Food Chem.* **2003**, *51*, 1035 – 1041.
[78] a) T. Soldo, I. Blank, T. Hofmann, *Chem. Senses* **2003**, *28*, 371 – 379; b) R. Villard, F. Robert, I. Blank, G. Bernardinelli, T. Soldo, T. Hofmann, *J. Agric. Food Chem.* **2003**, *51*, 4040 – 4045.
[79] a) T. Soldo, O. Frank, H. Ottinger, T. Hofmann, *Mol. Nutr. Food Res.* **2004**, *48*, 270 – 281; b) T. Soldo, T. Hofmann, *J. Agric. Food Chem.* **2005**, *53*, 9165 – 9171.
[80] E. de Rijke, N. Bouter, B. J. Ruisch, S. Haiber, T. König, *J. Chromatogr. A* **2007**, *1156*, 296 – 303.
[81] E. de Rijke, B. Ruisch, J. Bakker, J. Visser, J. Leenen, S. Haiber, A. de Klerk, C. Winkel, T. König, *J. Agric. Food Chem.* **2007**, *55*, 6417 – 6423.
[82] De Klerk, M. Baalbergen, R. Harry (Quest International Services B.V.), WO 2008/072963, **2008**.
[83] a) Y. Ueda, T. Tsubuku, R. Miyajima, *Biosci. Biotechnol. Biochem.* **1994**, *58*, 108 – 110; b) Y. Ueda, M. Yonemitsu, T. Tsubuku, M. Sakaguchi, R. Miyajima, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1977 – 1980; c) Y. Ueda, M. Sakaguchi, K. Hirayama, R. Miyajima, A. Kimizuka, *Agric. Biol. Chem.* **1990**, *54*, 163 – 169.
[84] a) S. Toelstede, A. Dunkel, T. Hofmann, *J. Agric. Food Chem.* **2009**, *57*, 1440 – 1448; b) S. Toelstede, T. Hofmann, *J. Agric. Food Chem.* **2009**, *57*, 3738 – 3748; c) A. Dunkel, J. Köster, T. Hofmann, *J. Agric. Food Chem.* **2007**, *55*, 6712 – 6719.
[85] T. Ohsu, Y. Amino, H. Nagasaki, T. Yamanaka, S. Takeshita, T. Hatanaka, Y. Maruyama, N. Miyamura, Y. Eto, *J. Biol. Chem.* **2010**, *285*, 1016 – 1022.
[86] R. S. Shallenberger, T. E. Acree, *Nature* **1967**, *216*, 480 – 482.
[87] a) P. A. Temussi, F. Lelj, T. Tancredi, *J. Med. Chem.* **1978**, *21*, 1154 – 1158; b) J. Kamphuis, F. Lelj, T. Tancredi, C. Toniolo, P. A. Temussi, *Quant. Struct. Act. Relat.* **1992**, *11*, 486 – 491.
[88] P. Temussi, *J. Mol. Recognit.* **2006**, *19*, 188 – 199.
[89] M. A. Hoon, E. Adler, J. Lindemeier, J. F. Battey, N. J. P. Ryba, C. S. Zuker, *Cell* **1999**, *96*, 541 – 551.
[90] a) A. A. Bachmanov, X. Li, D. R. Reed, J. D. Ohmen, S. Li, Z. Chen, M. G. Tordoff, P. J. de Jong, C. Wu, D. B. West, A. Chatterjee, D. A. Ross, G. K. Beauchamp, *Chem. Senses* **2001**, *26*, 925 – 933; b) M. Kitagawa, Y. Kusakabe, H. Miura, Y. Ninomiya, A. Hino, *Biochem. Biophys. Res. Commun.* **2001**, *283*, 236 – 242; c) E. Sainz, J. N. Korley, J. F. Battey, S. L. Sullivan, *J. Neurochem.* **2001**, *77*, 896 – 903.
[91] M. Max, Y. G. Shanker, L. Huang, M. Rong, Z. Liu, F. Campagne, H. Weinstein, S. Damak, R. F. Margolskee, *Nat. Genet.* **2001**, *28*, 58 – 63.
[92] J. P. Montmayeur, S. D. Liberles, H. Matsunami, L. B. Buck, *Nat. Neurosci.* **2001**, *4*, 492 – 498.
[93] G. Nelson, M. A. Hoon, J. Chandrashekar, Y. Zhang, N. J. Ryba, C. S. Zuker, *Cell* **2001**, *106*, 381 – 390.
[94] I. E. Lush, *Genet. Res.* **1989**, *53*, 95 – 99.
[95] Y. Zhang, M. A. Hoon, J. Chandrashekar, K. L. Mueller, B. Cook, D. Wu, C. S. Zuker, N. J. Ryba, *Cell* **2003**, *112*, 293 – 301.
[96] G. Q. Zhao, Y. Zhang, M. A. Hoon, J. Chandrashekar, I. Erlenbach, N. J. Ryba, C. S. Zuker, *Cell* **2003**, *115*, 255 – 266.
[97] S. Damak, M. Rong, K. Yasumatsu, Z. Kokrashvili, V. Varadarajan, S. Zou, P. Jiang, Y. Ninomiya, R. F. Margolskee, *Science* **2003**, *301*, 850 – 853.
[98] H. Xu, L. Staszewski, H. Tang, E. Adler, M. Zoller, X. Li, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14258 – 14263.
[99] P. Jiang, Q. Ji, Z. Liu, L. A. Snyder, L. M. Benard, R. F. Margolskee, M. Max, *J. Biol. Chem.* **2004**, *279*, 45068 – 45075.
[100] M. Winnig, B. Bufe, W. Meyerhof, *BMC Neurosci.* **2005**, *6*, 22.
[101] K. I. Nakajima, Y. Morita, A. Koizumi, T. Asakura, T. Terada, K. Ito, A. Shimizu-Ibuka, J. I. Maruyama, K. Kitamoto, T. Misaka, K. Abe, *FASEB J.* **2008**, *22*, 2323 – 2330.
[102] N. Ide, E. Sato, K. Ohta, T. Masuda, N. Kitabatake, *J. Agric. Food Chem.* **2009**, *57*, 5884 – 5890.
[103] M. Winnig, B. Bufe, N. A. Kratochwil, J. P. Slack, W. Meyerhof, *BMC Struct. Biol.* **2007**, *7*, 66.
[104] S. Zukerman, J. I. Glendinning, R. F. Margolskee, A. Sclafani, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2009**, *296*, R866 – R876.
[105] F. Zhang, B. Klebansky, R. M. Fine, H. Liu, H. Xu, G. Servant, M. Zoller, C. Tachdjian, X. Li, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4752 – 4757.
[106] G. Servant, C. Tachdjian, X. Q. Tang, S. Werner, F. Zhang, X. Li, P. Kamdar, G. Petrovic, T. Ditschun, A. Java, P. Brust, N. Brune, G. E. Dubois, M. Zoller, D. S. Karanewsky, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4746 – 4751.
[107] P. Jiang, M. Cui, B. Zhao, L. A. Snyder, L. M. Benard, R. Osman, M. Max, R. F. Margolskee, *J. Biol. Chem.* **2005**, *280*, 34296 – 34305.
[108] S. Vigues, C. D. Dotson, S. D. Munger, *Results Probl. Cell Differ.* **2009**, *47*, 187 – 202.
[109] a) X. Li, W. Li, H. Wang, J. Cao, K. Maehashi, L. Huang, A. A. Bachmanov, D. R. Reed, V. Legrand-Defretin, G. K. Beauchamp, J. G. Brand, *PLoS Genet.* **2005**, *1*, 27 – 35; b) X. Li, W. Li, H. Wang, D. L. Bayley, J. Cao, D. R. Reed, A. A. Bachmanov, L. Huang, V. Legrand-Defretin, G. K. Beauchamp, J. G. Brand, *J. Nutr.* **2006**, *136*, 1932S – 1934S.
[110] A. A. Fushan, C. T. Simons, J. P. Slack, A. Manichaikul, D. Drayna, *Curr. Biol.* **2009**, *19*, 1288 – 1293.
[111] Y. Nie, S. Vigues, J. R. Hobbs, G. L. Conn, S. D. Munger, *Curr. Biol.* **2005**, *15*, 1948 – 1952.
[112] P. Jiang, M. Cui, B. Zhao, Z. Liu, L. A. Snyder, L. M. Benard, R. Osman, R. F. Margolskee, M. Max, *J. Biol. Chem.* **2005**, *280*, 15238 – 15246.
[113] G. Morini, A. Bassoli, P. A. Temussi, *J. Med. Chem.* **2005**, *48*, 5520 – 5529.
[114] M. Cui, P. Jiang, E. Maillet, M. Max, R. F. Margolskee, R. Osman, *Curr. Pharm. Des.* **2006**, *12*, 4591 – 4600.
[115] a) M. Max, W. Meyerhof in *The Senses: A Comprehensive Reference*, *Vol. 4* (Eds.: A. I. Basbaum, A. Kaneko, G. M. Shepherd, G. Westheimer, S. Firestein, G. K. Beauchamp), Elsevier, San Diego, **2008**, pp. 197 – 218; b) X. Li, *Am. J. Clin. Nutr.* **2009**, *90*, 733S – 737S.
[116] N. Kunishima, Y. Shimada, Y. Tsuji, T. Sato, M. Yamamoto, T. Kumasaka, S. Nakanishi, H. Jingami, K. Morikawa, *Nature* **2000**, *407*, 971 – 977.
[117] a) X. Liu, Q. He, D. J. Studholme, Q. Wu, S. Liang, L. Yu, *Trends Biochem. Sci.* **2004**, *29*, 458 – 461; b) K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739 – 745.
[118] V. Galindo-Cuspinera, P. A. Breslin, *Cell. Mol. Life Sci.* **2007**, *64*, 2049 – 2052.
[119] V. Galindo-Cuspinera, M. Winnig, B. Bufe, W. Meyerhof, P. A. Breslin, *Nature* **2006**, *441*, 354 – 357.
[120] F. Zhang, B. Klebansky, R. M. Fine, H. Xu, A. Pronin, H. Liu, C. Tachdjian, X. Li, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20930 – 20934.
[121] E. Sainz, M. M. Cavenagh, N. D. LopezJimenez, J. C. Gutierrez, J. F. Battey, J. K. Northup, S. L. Sullivan, *Dev. Neurobiol.* **2007**, *67*, 948 – 959.
[122] O. Lugaz, A. M. Pillias, A. Faurion, *Chem. Senses* **2002**, *27*, 105 – 115.

[123] U. K. Kim, S. Wooding, N. Riaz, L. B. Jorde, D. Drayna, *Chem. Senses* **2006**, *31*, 599 – 611.
[124] Q. Y. Chen, S. Alarcon, A. Tharp, O. M. Ahmed, N. L. Estrella, T. A. Greene, J. Rucker, P. A. Breslin, *Am. J. Clin. Nutr.* **2009**, *90*, 770S-779S.
[125] M. Raliou, A. Wiencis, A. M. Pillias, A. Planchais, C. Eloit, Y. Boucher, D. Trotier, J. P. Montmayeur, A. Faurion, *Am. J. Clin. Nutr.* **2009**, *90*, 789S-99S.
[126] N. Shigemura, S. Shirosaki, K. Sanematsu, R. Yoshida, Y. Ninomiya, *PLoS One* **2009**, *4*, e6717.
[127] I. J. Miller, Jr. in *Handbook of Olfaction and Gustation* (Ed.: R. L. Doty), Marcel Dekker, New York, **1995**, pp. 521 – 547.
[128] M. Witt, K. Reutter, I. J. Miller, Jr. in *Handbook of olfaction and gustation* (Ed.: R. L. Doty), Marcel Dekker, New York, **2003**, pp. 651 – 678.
[129] I. J. Miller, Jr., *Anat. Rec.* **1986**, *216*, 474 – 482.
[130] A. Sbarbati, F. Merigo, D. Benati, M. Tizzano, P. Bernardi, F. Osculati, *Chem. Senses* **2004**, *29*, 683 – 692.
[131] H. Schmale, H. Holtgreve-Grez, H. Christiansen, *Nature* **1990**, *343*, 366 – 369.
[132] a) S. D. Roper, *J. Neurosci.* **1992**, *12*, 1127 – 1134; b) S. D. Roper, *Pfluegers Arch.* **2007**, *454*, 759 – 776.
[133] S. D. Roper, *Physiol. Behav.* **2009**, *97*, 604 – 608.
[134] a) D. L. Bartel, S. L. Sullivan, E. G. Lavoie, J. Sevigny, T. E. Finger, *J. Comp. Neurol.* **2006**, *497*, 1 – 12; b) D. M. Lawton, D. N. Furness, B. Lindemann, C. M. Hackney, *Eur. J. Neurosci.* **2000**, *12*, 3163 – 3171.
[135] A. Vandenbeuch, T. R. Clapp, S. C. Kinnamon, *BMC Neurosci.* **2008**, *9*, 1.
[136] a) J. Chandrashekar, M. A. Hoon, N. J. Ryba, C. S. Zuker, *Nature* **2006**, *444*, 288 – 294; b) D. A. Yarmolinsky, C. S. Zuker, N. J. Ryba, *Cell* **2009**, *139*, 234 – 244.
[137] a) R. A. Romanov, O. A. Rogachevskaja, M. F. Bystrova, P. Jiang, R. F. Margolskee, S. S. Kolesnikov, *EMBO J.* **2007**, *26*, 657 – 667; b) Y. J. Huang, Y. Maruyama, G. Dvoryanchikov, E. Pereira, N. Chaudhari, S. D. Roper, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 6436 – 6441.
[138] a) G. T. Wong, K. S. Gannon, R. F. Margolskee, *Nature* **1996**, *381*, 796 – 800; b) C. Hisatsune, K. Yasumatsu, H. Takahashi-Iwanaga, N. Ogawa, Y. Kuroda, R. Yoshida, Y. Ninomiya, K. Mikoshiba, *J. Biol. Chem.* **2007**, *282*, 37225 – 37231.
[139] R. A. DeFazio, G. Dvoryanchikov, Y. Maruyama, J. W. Kim, E. Pereira, S. D. Roper, N. Chaudhari, *J. Neurosci.* **2006**, *26*, 3971 – 3980.
[140] a) S. M. Tomchik, S. Berg, J. W. Kim, N. Chaudhari, S. D. Roper, *J. Neurosci.* **2007**, *27*, 10840 – 10848; b) R. Yoshida, A. Miyauchi, T. Yasuo, M. Jyotaki, Y. Murata, K. Yasumatsu, N. Shigemura, Y. Yanagawa, K. Obata, H. Ueno, R. F. Margolskee, Y. Ninomiya, *J. Physiol.* **2009**, *587*, 4425 – 4439.
[141] K. L. Mueller, M. A. Hoon, I. Erlenbach, J. Chandrshekar, C. S. Zuker, N. J. P. Ryba, *Nature* **2005**, *434*, 225 – 229.
[142] E. Adler, M. A. Hoon, K. L. Mueller, J. Chandrashekar, N. J. Ryba, C. S. Zuker, *Cell* **2000**, *100*, 693 – 702.
[143] T. E. Finger, V. Danilova, J. Barrows, D. L. Bartel, A. J. Vigers, L. Stone, G. Hellekant, S. C. Kinnamon, *Science* **2005**, *310*, 1495 – 1499.
[144] T. R. Clapp, K. F. Medler, S. Damak, R. F. Margolskee, S. C. Kinnamon, *BMC Biol.* **2006**, *4*, 7.
[145] Y. A. Huang, R. Dando, S. D. Roper, *J. Neurosci.* **2009**, *29*, 13909 – 13918.
[146] Y. A. Huang, Y. Maruyama, S. D. Roper, *J. Neurosci.* **2008**, *28*, 13088 – 13093.
[147] A. L. Huang, X. Chen, M. A. Hoon, J. Chandrashekar, W. Guo, D. Trankner, N. J. Ryba, C. S. Zuker, *Nature* **2006**, *442*, 934 – 938.
[148] Y. Ishimaru, H. Inada, M. Kubota, H. Zhuang, M. Tominaga, H. Matsunami, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12569 – 12574.
[149] a) N. D. LopezJimenez, M. M. Cavenagh, E. Sainz, M. A. Cruz-Ithier, J. F. Battey, S. L. Sullivan, *J. Neurochem.* **2006**, *98*, 68 – 77; b) S. Kataoka, R. Yang, Y. Ishimaru, H. Matsunami, J. Sevigny, J. C. Kinnamon, T. E. Finger, *Chem. Senses* **2008**, *33*, 243 – 254.
[150] Y. A. Huang, Y. Maruyama, R. Stimac, S. D. Roper, *J. Physiol.* **2008**, *586*, 2903 – 2912.
[151] a) H. Inada, F. Kawabata, Y. Ishimaru, T. Fushiki, H. Matsunami, M. Tominaga, *EMBO Rep.* **2008**, *9*, 690 – 697; b) S. Ishii, T. Misaka, M. Kishi, T. Kaga, Y. Ishimaru, K. Abe, *Biochem. Biophys. Res. Commun.* **2009**, *385*, 346 – 350.
[152] a) J. Chandrashekar, D. Yarmolinsky, L. von Buchholtz, Y. Oka, W. Sly, N. J. Ryba, C. S. Zuker, *Science* **2009**, *326*, 443 – 445; b) A. Dunkel, T. Hofmann, *Angew. Chem.* **2010**, *122*, 3037 – 3039; *Angew. Chem. Int. Ed.* **2010**, *49*, 2975 – 2977.
[153] a) H. Miura, Y. Kusakabe, H. Kato, J. Miura-Ohnuma, M. Tagami, Y. Ninomiya, A. Hino, *Gene Expression Patterns* **2003**, *3*, 427 – 430; b) J. M. Hall, M. L. Bell, T. E. Finger, *Dev. Biol.* **2003**, *255*, 263 – 277.
[154] M. C. Whitehead, T. E. Finger in *The Senses: A Comprehensive Reference*, *Vol. 4* (Eds.: A. I. Basbaum, A. Kaneko, G. M. Shepherd, G. Westheimer, S. Firestein, G. K. Beauchamp), Elsevier, Amsterdam, **2008**, pp. 237 – 260.
[155] A. C. Spector, J. I. Glendinning, *Curr. Opin. Neurobiol.* **2009**, *19*, 370 – 377.
[156] E. T. Rolls in *Handbokk of olfaction and gustation*, 2nd ed. (Ed.: R. L. Doty), Marcel Dekker, New York, **2003**, pp. 679 – 705.
[157] M. A. Schoenfeld, G. Neuer, C. Tempelmann, K. Schussler, T. Noesselt, J. M. Hopf, H. J. Heinze, *Neuroscience* **2004**, *127*, 347 – 353.
[158] J. Chandrashekar, C. Kuhn, Y. Oka, D. A. Yarmolinsky, E. Hummler, N. J. Ryba, C. S. Zuker, *Nature* **2010**, *464*, 297 – 310.
[159] S. Damak, M. Rong, K. Yasumatsu, Z. Kokrashvili, C. A. Perez, N. Shigemura, R. Yoshida, B. Mosinger, Jr., J. I. Glendinning, Y. Ninomiya, R. F. Margolskee, *Chem. Senses* **2006**, *31*, 253 – 264.
[160] R. V. Considine, *Semin. Vasc. Med.* **2005**, *5*, 15 – 24.
[161] a) J. K. Elmquist, E. Maratos-Flier, C. B. Saper, J. S. Flier, *Nat. Neurosci.* **1998**, *1*, 445 – 450; b) M. W. Schwartz, S. C. Woods, D. Porte, R. J. Seeley, D. G. Baskin, *Nature* **2000**, *404*, 661 – 671.
[162] a) K. Kawai, K. Sugimoto, K. Nakashima, H. Miura, Y. Ninomiya, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 11044 – 11049; b) N. Shigemura, R. Ohta, Y. Kusakabe, H. Miura, A. Hino, K. Koyano, K. Nakashima, Y. Ninomiya, *Endocrinology* **2004**, *145*, 839 – 847.
[163] N. Horio, M. Jyotaki, R. Yoshida, K. Sanematsu, N. Shigemura, Y. Ninomiya, *J. Pharmacol. Sci.* **2010**, *112*, 8 – 12.
[164] Y. Ninomiya, N. Shigemura, K. Yasumatsu, R. Ohta, K. Sugimoto, K. Nakashima, B. Lindemann, *Vitam. Horm.* **2002**, *64*, 221 – 248.
[165] Y. Nakamura, K. Sanematsu, R. Ohta, S. Shirosaki, K. Koyano, K. Nonaka, N. Shigemura, Y. Ninomiya, *Diabetes* **2008**, *57*, 2661 – 2665.
[166] R. Yoshida, T. Ohkuri, M. Jyotaki, T. Yasuo, N. Horio, K. Yasumatsu, K. Sanematsu, N. Shigemura, T. Yamamoto, R. F. Margolskee, Y. Ninomiya, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 935 – 939.
[167] a) D. Hofer, B. Puschel, D. Drenckhahn, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6631 – 6634; b) D. Hofer, D. Drenckhahn, *Histochem. Cell Biol.* **1998**, *110*, 303 – 309.
[168] H. J. Jang, Z. Kokrashvili, M. J. Theodorakis, O. D. Carlson, B. J. Kim, J. Zhou, H. H. Kim, X. Xu, S. L. Chan, M. Juhaszova,

M. Bernier, B. Mosinger, R. F. Margolskee, J. M. Egan, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15069 – 15074.
[169] R. F. Margolskee, J. Dyer, Z. Kokrashvili, K. S. Salmon, E. Ilegems, K. Daly, E. L. Maillet, Y. Ninomiya, B. Mosinger, S. P. Shirazi-Beechey, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15075 – 15080.
[170] T. R. Clapp, K. R. Trubey, A. Vandenbeuch, L. M. Stone, R. F. Margolskee, N. Chaudhari, S. C. Kinnamon, *FEBS Lett.* **2008**, *582*, 3783 – 3787.

