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The Pharmacology of Taste

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Preface

Everyone is interested in taste. To say that taste is centrally important to our lives is not hyperbole, just an underappreciated fact. Taste is a part of our daily experience. It guides our choices in what we eat and drink and thereby influences all activities relating to the procurement and preparation of food. One of the largest sectors of the world economy is fueled primarily by demand for taste – combined annual revenues from the flavor ingredients, food and beverage, and restaurant industries, all of which are driven by fierce competition to produce the most appealing tastes to consumers, easily range into the trillions of dollars.

It is commonly assumed that when humans were a foraging species, the sense of taste was critically important for survival. Taste likely served to identify and distinguish nutrient-rich ingestibles from perhaps similarly appearing articles that were nutrient-poor (thus a waste of effort), or even potentially poisonous or contaminated. That notion is so universally presupposed that it is almost trite. As our readers will find in this volume, the role of taste in guiding ingestion is not always so straightforward when scrutinized through an experimental lens. Nevertheless, there is little question that taste is tightly connected to ingestive behaviors.

But the sense of taste permeates our daily experience still more deeply. Taste is so ingrained in our psyche that even our language, our means of expressing our impressions and desires, is shaped by taste. Special judgements often are translated in terms of taste, as in demonstrating good taste in a choice or action or clothing, and the converse, disgust (from the Latin *gustus* or *gustare* – taste.) The words "sweet," "salty," "sour," "savory," and "bitter," not only are the labels given to sensory qualities (in western culture to the "basic tastes") but each also carries significant additional meanings generalizing to emotions, attitudes, or objects unrelated to actual taste stimuli.

Taste is familiar as one of the senses, the psychological representation of a particular aspect of the environment. As suggested by the etymology of the word – middle English and Latin *tastare* and *taxitare*, meaning to touch, test, gain direct knowledge by sampling – taste historically has been conceived as a connection between the outer environment and subjective consciousness, most evident in the epistemologies of the Enlightenment thinkers. As philosophies of a "rational mind" evolved into a science of psychology, the field of psychophysics arose to provide a framework of concepts and methodologies for an experimental analysis of sensory

perception. The psychophysics of taste has continued as the predominant approach to the study of taste into modern times, with the goal of quantitatively defining the relationship between the external stimuli impinging on the tongue and the consequent internal sensory experience.

However, that view began to change with the discoveries in the late 1990s and in the early 2000s of the G protein, transduction pathways, and receptors responsible for generating the signals ultimately interpreted as "taste." The moment triggered a paradigm shift, away from an exclusive emphasis on the subjective experience of taste toward efforts focused on gaining insight into the mechanisms by which chemicals interact with receptors to produce those experiences. In this context, taste stimuli, such as sucrose or quinine, now were thought of as agonists of tastant receptors, and inhibitors of taste such as lactisole were studied as receptor antagonists. The experimental questions and problem-solving approaches began to resemble those of the field of pharmacology, the science of receptors, and the biology they control. Within short order, a biotechnology industry rose up mirroring the pharmaceutical industry, with an overarching aim to revolutionize flavor ingredient discovery and development. Companies such as Senomyx and Redpoint Bio (Linguagen) were founded on a model similar to the process of drug discovery, where the newly identified receptors were isolated and incorporated into high throughput assays for rapid identification and characterization of novel taste-modifying compounds.

As a paradigm shift, though, the notion of taste as pharmacology has been relatively slow to develop. A pharmacological approach to the study of taste gained a foothold around 20 years ago, but its momentum has been gradual. The fundamental principles of pharmacology that formally relate taste responses to receptor function rarely have been applied to taste phenomena. Perhaps this is because pharmacology traditionally has been most closely associated with medical science and the development of therapeutics. Perhaps, taste has been overlooked as a serious concern of personal and public health. If so, this latter notion can readily be dispelled by pointing out the substantial obstacles that the aversive tastes of medicines pose for therapeutic compliance, particularly among children. Moreover, the behavioral and cognitive consequences of taste impairment can be severe. Loss of taste can interfere with normal food consumption resulting in unhealthy weight loss, and, furthermore, has been shown to deleteriously impact emotional well-being and overall quality of life, a fact that has gained greater public awareness due to the devastating effects of COVID-19 on chemosensory function.

If the embrace of taste as a subject proper to the field of pharmacology has been slow, its recognition as a pharmacological problem will remind all that pharmacology is not exclusively an applied science for the benefit of drug discovery. Pharmacology is a basic science with a firm theoretical basis and well-developed set of principles and methods of experimental analysis for elucidating the mechanisms of *any* receptor-mediated functional biology. This volume is devoted to that proposition.

With a goal of explicitly highlighting the pharmacodynamics attributed to the activation of taste receptors by sapid or other molecules, we wanted to make sure that

every part of this chemosensory system is captured and discussed; from ligand interactions with receptors, to recruitment of specific signaling cascades, to effects in the CNS and at the periphery and ultimately the impact of these receptor interactions on taste and other physiological and pathophysiological processes. Of note, notions of affinity, efficacy, specificity, selectivity, agonism, antagonism, and allosterism – important concepts precisely defining ligand–receptor interactions – are covered, and receptor structures and specific ligand binding sites are described, where possible.

The opening chapter, by Palmer, introduces the foundational concepts of pharmacology as the basis for an argument that taste *is* a proper subject matter for the field of pharmacology, and how the study of taste in turn helps to generalize the principles of pharmacology. Taste stimuli are presented as ligands that interact with their receptors in ways predicted by receptor occupancy theory, a lawfulness that clearly is evident in cell-based assays of tastant receptor function, and which also should be reflected by measures of the sensory perception of taste (but isn't always).

Molecular and physiological processes following taste receptor activation are more specifically addressed in the subsequent three chapters. Second chapter, by Banik and Medler, comprehensively reviews the most recent literature on post-receptor signal transduction events and presents new findings on a novel taste receptor-specific signaling pathway that challenges developing concepts on taste coding. Third chapter, by Roper, focuses on data accumulated over the last two decades aimed at shedding light on the highly disputed topic of taste coding; specifically, the possible mechanisms by which information is faithfully transferred from activated taste receptor cells to nerve afferents and on to higher brain centers. Roper articulately presents arguments supporting a combinatorial/population coding model that stands in contrast to the widely accepted labeled line model supported by the sophisticated and thought-provoking studies performed by the Zuker laboratory. The chapter is a highlight of an ongoing healthy debate in the field. In fourth chapter, Kinnamon and Finger present a review of the neurotransmitters present in taste buds and released following taste cell activation. Notably, the authors convincingly argue for a central role for adenosine triphosphate (ATP) as the main neurotransmitter and its corresponding receptors as necessary for taste signal progression to nerve afferents and taste perception. A conundrum still exists, however, as pointed out by the authors: if ATP is indeed necessary for sour taste, why is this neurotransmitter not detected following activation of Type III cells?

The reader then is introduced to the G protein-coupled receptors (GPCRs) that serve as detection mechanisms for umami, sweet, and bitter tastants. In the first of these five chapters, Servant and Frerot detail earlier work aimed at identifying umami taste receptors from the large family of metabotropic glutamate receptors, and subsequently the identification of a novel heterodimeric GPCR, T1R1/T1R3, exhibiting in vitro pharmacological properties that more precisely match psychophysical data on human umami taste. They review several potent synthetic or natural T1R1/T1R3 ligands, describe their effects on receptor modulation, and the correlation between assay potency values and taste potency values. Original data also are presented that suggest T1R1/T1R3 may serve as a receptor for kokumi taste, an

alternative to another current hypothesis that the calcium-sensing receptor mediates the taste of γ-L-glutamyl peptides.

To date, there are no structures of mammalian taste receptors available. We, therefore, wanted to make sure that advances in homology modeling, molecular docking, and molecular dynamics were included in this book. In sixth chapter, Spaggiari, Cavaliere, and Cozzini utilize such computational methods with the whole T1R1/R3 heterodimer umami receptor as a case study. Their in silico model faithfully recapitulates in vitro assay and mutagenesis data, showing that L-glutamate (MSG) binds preferentially to the extracellular domain of T1R1 over T1R3 to activate the receptor and that inosine monophosphate interaction with the extracellular domain of T1R1 stabilizes the interaction of MSG with T1R1. In seventh chapter, Behrens provides a thorough summary on the current state of knowledge on the sweet taste receptor, T1R2/T1R3, a closely related heterodimer, and the pharmacological properties of its ligands. Topics cover description of known sweeteners, novel positive allosteric modulators, the receptor's domains, and interactions sites, correlation between assay and taste data, canonical signaling pathways and some emerging pathways reminiscent of those used for glucose-induced insulin secretion by pancreatic β cells. The chapter further explores the evolutionary dynamics of the genes encoding T1R2/T1R3 across different species.

Bitter taste receptors and their role in taste physiology have been abundantly reviewed over the last 20 years. More recently, there has been mounting evidence that T2R bitter taste receptors play significant extraoral roles unrelated to taste sensation, most notably in innate immunity and respiratory functions. Medapati, Bhagirath, Singh, and Chelikani summarize in eighth chapter the T2Rs, their ligands, signaling properties, and expression in the oral cavity and other tissues. The authors expose a unique feature of these receptors – activation by quorum sensing molecules (QSMs) such as quinolones, *N*-acyl homoserine lactones, and small peptides, produced and excreted by bacteria. These QSMs activate bitter taste receptors expressed on nasal and gingival solitary chemosensory cells, on respiratory epithelial cells, and on leukocytes, promoting innate immunity responses such as the generation of nitric oxide, the release of antimicrobial β-defensin peptides, and increased phagocytosis by macrophages. In ninth chapter, Sharma, Conaway, and Deshpande provide a comprehensive view on the pharmacology of T2Rs and their emerging roles in airway physiology: promoting airway smooth muscle (ASM) cell relaxation, inhibiting ASM proliferation, enhancing ciliary motility, and regulating immune cells and cytokine production at inflammatory sites. These observations could lead to the development of novel therapies to alleviate symptoms of allergic asthma and other upper airway inflammatory disorders.

The molecular mechanism by which sour taste is mediated eluded researchers for decades, up until 4 years ago when the breakthrough discoveries from the labs of Emily Liman and Charles Zuker led to the identification of optometric 1 (OTOP1), a new proton-selective channel required for sour taste. In tenth chapter, Zhang, Lee, and Macpherson expertly articulate the discovery journey from the identification of Type III cells as solely responsible for sour taste sensation, through the pioneering work of Emily Liman's lab in hunting down a zinc-sensitive proton current in Type

III cells, to the identification of OTOP-1 by the Liman and Zuker labs using transcriptome profiling, and to its validation in knock-out mice. Zhang et al. further provide evidence for a distinct sour-sensing neuron population in the geniculate ganglion in rodents, strengthening the concept of a labeled line model for taste coding.

The type of oral sensation provided by dietary fats has been debated for years – whether "fatty" is a taste quality analogous to "sweet" and "bitter," for example, or the result of additional oro-sensory processes not directly related to tastant receptor function. In eleventh chapter, Hichami, Khan, and Khan present data and arguments supporting a basic fatty taste quality and candidate receptors that might underly its signaling. The authors describe several potential receptors, including GPCRs, and recruited signaling pathways in isolated taste receptor cells responsive to fatty acids. Agonists of one candidate receptor are even reported to elicit a fatty taste in humans. This intriguing chapter invites further investigation into the validation of candidate receptors for human fatty taste sensing.

The potential functional and physiological significance of tastant receptors and related taste signaling proteins expression in several non-gustatory tissues are reviewed in twelfth chapter by Wang, Matsumoto, and Jiang, with a focus on immune system responses. Solitary chemosensory cells (SCCs), epithelial cells found throughout the gut, the respiratory system, and various other tissues, often express components of the taste signaling machinery. The authors present a comprehensive summary of the ways in which genetic ablation of the different taste signaling proteins in SCCs impact immune responses to parasitic and bacterial infection, cell hyperplasia and inflammation. In several cases, the receptors involved in these effects still have yet to be identified.

The last two chapters take a more global perspective on taste, addressing taste impairments in a clinical setting and the teleonomic purpose of taste itself. In thirteenth chapter, Hummel reviews different taste disorders, including recent findings regarding the loss of taste following infection with SARS-CoV-2. Hummel also provides a comprehensive view on approaches and methods utilized to measure and quantify effects of sapid molecules in taste tests or on the tongue. In fourteenth chapter, Glendinning raises the question of whether taste qualities, as widely supposed, actually do convey information about nutritional composition and toxicity of foods. In almost every paper describing taste modalities, there is a paragraph stating that we are attracted to sweet tasting substances because they typically are carbohydrate-enriched, umami-tasting foods because they are protein-enriched, and that bitter and sour taste qualities signal the presence of toxins or spoilage to be avoided. While this may have been the case 1000s of years ago, is this really the case today? Glendinning performs a thorough analysis using products present in our modern diet, summarizes his findings, and offers an original insightful viewpoint.

Together, the 14 chapters of this work present a unique and valuable offering to the field of pharmacology as well as to more traditional perspectives on the study of taste. We are grateful to the world-renowned experts who contributed their time, effort, scientific acumen, and creative thought through their writings, gathered here for a truly exceptional volume, "The Pharmacology of Taste." We are equally

grateful to the series editors for inviting us to take on this task, and for closely working with us to produce this fine volume for the historic *Handbook of Experimental Pharmacology*.

Philadelphia, PA, USA — R. Kyle Palmer
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Why Taste Is Pharmacology

R. Kyle Palmer

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Abstract

The chapter presents an argument supporting the view that taste, defined as the receptor-mediated signaling of taste cells and consequent sensory events, is proper subject matter for the field of pharmacology. The argument develops through a consideration of how the field of pharmacology itself is to be defined. Though its application toward the discovery and development of therapeutics is of obvious value, pharmacology nevertheless is a basic science committed to

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examining biological phenomena controlled by the selective interactions between chemicals – regardless of their sources or uses – and receptors. The basic science of pharmacology is founded on the theory of receptor occupancy, detailed here in the context of taste. The discussion then will turn to consideration of the measurement of human taste and how well the results agree with the predictions of receptor theory.

Keywords

Pharmacology · Receptor theory · Taste · Taste discrimination · Taste intensity

1 Introduction: Paradigms for the Study of Taste

We see the world in terms of our theories.
– Thomas Kuhn, The Structure of Scientific Revolutions

In his landmark book *The Structure of Scientific Revolutions* (Kuhn 1962), Thomas Kuhn portrayed the advance of science as a process of transitioning through paradigms-models of the universe and how it operates. A scientific paradigm often is described as a world view, a lens through which natural phenomena are observed and interpreted. For the scientist, the paradigm defines the questions to be asked, the problems to be solved, how and what to measure – the independent and dependent variables – and how to analyze and interpret data. Thus, scientific "operations and measurements are paradigm-determined" (Kuhn 1962). Two scientists, each examining the same phenomenon from the perspectives of differing paradigms, potentially will arrive at very different conclusions about what they observe. Such an outcome might be due to different methods of measurement, different definitions of independent and dependent variables, or even more fundamentally, differing notions of causality.

Here, as elsewhere (Palmer 2007, 2019), the study of the collective phenomena conventionally referred to as "taste" will be presented as a study from the perspective of the paradigm of pharmacology. However, this is a relatively new approach to the study of taste, and currently not predominant. Taste always has been regarded as a sensory perceptual event, and the leading experimental paradigm for investigating sensory phenomena of any kind has been, and remains, psychophysics. Pharmacology studies the functional relationship between receptors and the ligands that occupy them, and how this relationship translates to changes in the functions of biological systems, including behavior. The goal of psychophysics is to obtain quantitative relationships between physical stimuli that impinge upon the nervous system and the subjective sensations and perceptions that follow. The paradigms of pharmacology and psychophysics are vastly different, each evolving from divergent epistemological lineages.

In some cases, the pharmacological and psychophysical techniques used to investigate an aspect of taste essentially are equivalent and, accordingly, generate

equivalent data; but the pharmacologist and the psychophysicist will have different explanations to account for the results. Taste discrimination experiments are representative of these cases. There are, however, situations where psychophysical experimental approaches to the study of taste result in datasets and conclusions that appear contradictory to expectations set by the paradigm of pharmacology. The psychophysics of taste intensity, particularly as it has been measured using intensity magnitude rating scales, exemplifies this latter case and will be addressed in this chapter.

2 The Purview of Pharmacology

It might strike the reader as peculiar that taste be called "pharmacology." After all, taste is a sensory perception, associated with enjoyment of foods and beverages, avoidance of unpleasant and potentially harmful substances, and quality of life. Taste guides ingestion. The ways in which taste has been studied have focused on the sensory event, whether it be detection or intensity of the experience.

Pharmacology, on the other hand, is associated with medical science. The history of pharmacology is tightly interwoven with the study of medicine. The first pharmacology programs at academic universities were founded on the study of therapeutics. John Jacob Abel, the first professor of pharmacology in the USA, formed his pharmacology department within the department of materia medica at the University of Michigan (Parascandola 1992). Moreover, application of the principles of pharmacology has built the entire engineering process of drug discovery in the pharmaceutical industry.

Nevertheless, it is the position of the editors of this volume, and indeed the raison d'etre for this volume, that the study of taste fits well within the domain of the field of pharmacology. To launch the argument, some definitions first are needed.

2.1 Definitions of Pharmacology

There is no shortage of differing opinions on what, or what should, constitute the field of pharmacology. The introduction of taste as a subject for pharmacological interrogation presents a prime opportunity to more clearly define what pharmacology is. In the current context, it would be most instructive to consider what a major pharmacology society considers to be the subject which unites its members. The American Society for Pharmacology and Experimental Therapeutics (ASPET) is a scientific society, founded by John Jacob Abel, dedicated to the science of pharmacology and its applications to therapeutics. The Society defines pharmacology as follows:

> Pharmacology is the science of how drugs act on biological systems and how the body responds to the drug. (https://www.aspet.org/aspet/education-careers/about-pharmacology, last accessed 2022 January 17)

Pharmacology is comprised of two major subdisciplines: pharmacodynamics and pharmacokinetics. Pharmacodynamics refers to the intermolecular reactions which underly the response to a drug, the level at which chemistry is joined to biology. Pharmacokinetics is the tracking of a drug through its time course of activity in the body, and thus ultimately how much of the drug originally administered will remain in its active form at the receptor compartment, the site of pharmacodynamics. Knowledge of the processes encompassed by both pharmacodynamics and pharmacokinetics is necessary to achieve a full understanding of "drug action." The receptors that mediate taste responses, expressed where they are in the microvilli of taste cells on the surface of the tongue (Yang et al. 2020), essentially are directly exposed to the chemicals with which they interact, and therefore "tastant action" is determined almost entirely by pharmacodynamics.

"Drug" is defined by the United States Food and Drug Administration as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" and "articles (other than food) intended to affect the structure or any function of the body of man or other animals." (https://www.fda.gov/industry/regulated-products/human-drugs#drug, last accessed 2022 January 17). Tastants generally are not used to remedy a disease state (though therapeutics certainly can generate a taste response), and the FDA distinguishes substances that are used for stimulating taste into a different, non-medical category. The FDA definition of drug also applies to recreational drugs, which are used intentionally to affect the function of the central nervous system (and consequently behavior), but explicitly excludes food, and by extension, taste stimuli. But the FDA's definitions are designed to clarify how categories of substances will be regulated for their specific marketed or recreational use.

If the chief objective of the field of pharmacology is to study exogenously applied chemicals that are defined as therapeutics and other agents of medical interest, then it is an applied science – the discovery and study of therapeutics to benefit medical science. There would be little room to include the study of taste in such a field.

However, the famous text *Goodman and Gilman's The Pharmacological Basis of Therapeutics* has acknowledged since its first edition (1941; Brunton et al. 2017) that "A drug may be broadly defined as any chemical agent that affects living protoplasm, and few substances would escape inclusion by this definition." (Rivera and Gilman 2017). Furthermore, the ASPET publication Journal of Pharmacology and Experimental Therapeutics, the first American pharmacology journal, also founded by John Jacob Abel, defines their mission as providing "broad coverage of all aspects of the interactions of chemicals with biological systems. . .," and then proceeds to list a multiplicity of biological systems and related areas of research in which the methods and principles of pharmacology are applied (which, importantly for this chapter, includes behavior; https://jpet.aspetjournals.org/content/jpet-faqs, last accessed 2022 January 17). These definitions and purposes for pharmacology lift its purview from an exclusive medico-centric perspective.

John Jacob Abel himself unequivocally held the view that pharmacology is a basic experimental science, the growth of which should not be encumbered by, in his

words, "the intrusive demands of practical utility." For Abel, pharmacology is the science that. . .

> . . .tries to discover all the chemical and physical changes that go on in a living thing that has absorbed a substance capable of producing such changes, and it also attempts to discover the fate of the substance incorporated. It is not therefore an applied science, like therapeutics, but it is one of the biological sciences, using that word in its widest sense. (quotes are from (Parascandola 1992)

Many histories of pharmacology emphasize its evolution as an experimental science. Often a path of discovery is traced from the work of Claude Bernard, who systematically narrowed down the site of action for curare to the neuromuscular junction (perhaps Alfred Vulpian deserves more credit, see Cousin, 2002), to John Newport Langley's "eureka moment" of the existence of a finite "receptive substance" that explains the pharmacological competition between curare and nicotine in muscle tissue (Changeux 2020; Limbird 2006; Maehle 2004; Rang 2006). Arrival at the receptor concept was the moment that pharmacology was born. The field of medicine benefited all along the way and has ever since.

Throughout the nineteenth century pharmacologists gained support from university departments of materia medica who were increasingly appreciative of the value of experimental pharmacology to the modernization of medical science (Lees et al. 2022; Lesch 1984). Though the history of pharmacology tightly interweaves with that of medicine, the scientific ancestors and founders of pharmacology primarily were interested in understanding the mechanisms by which chemicals changed physiology (Barrett et al. 2019; Scheindlin 2010). The drive to elucidate the mechanisms at play in the interface between chemistry and physiology produced the discipline of pharmacology, regardless of the sources of the chemicals or their intended use.

Currently there is good reason to regard pharmacology as a basic science which is valued for its broad applications, not just an applied science useful to the field of medicine. Often, the experimentation conducted by pharmacologists neither involves a therapeutic agent nor directly relates to the discovery of one. Instead, the focus of entire research programs in pharmacology can be solely on the mechanisms by which any chemical could directly alter physiology, observable at any level of experimental reduction, seeking lawful relationships of cause and effect. That relationship is the product of the function of receptors whose activities serve to translate chemical information from the external face of the cell membrane to the internal workings of the cell. It was the elucidation of a concept of "receptor" that created the theoretical foundation upon which a basic science of pharmacology has been built. The study of taste, a receptor-mediated biological event, fits well within this realization of pharmacology as a basic science.

2.2 Definitions of Taste

The word "taste" evokes many connotations, and consequently careful consideration must be given to a precise definition of the word as it is used to describe processes under scientific scrutiny. "Taste" has been studied from the most reductive examination of cellular and molecular events to emergent phenomena of conscious perception. There are different aspects of the concept of "taste" that determine the dependent variables to be systematically examined and how experimental results will be interpreted.

Perhaps the most familiar aspect of taste is that of a tastant's qualitative properties (Palmer 2019), exemplified by the question "what does this taste like?" The question implies a comparison between the substance of interest and a standard tastant previously experienced. By current consensus, "sweet," "bitter," "salty," "sour," and "umami" are the five taste qualities basic to the concept of "taste" (Beauchamp 2019; Erickson 2008), and common representative standards for these categories are sucrose, quinine, sodium chloride, citric acid, and glutamic acid, respectively (Palmer 2019; Palmer et al. 2021). G protein-coupled receptors (GPCRs) have been identified as the cognate receptors for the sweet, bitter, and umami categories of tastants (TAS1R2/R3, TAS2, and TAS1R1/R3 receptors, respectively, for each category, reviewed in Palmer 2007, 2019), and ion channel mechanisms have been elucidated that are thought responsible for the taste qualities of salty (Nomura et al. 2020; Roebber et al. 2019) and sour stimuli (Teng et al. 2019; Zhang et al. 2019, 2021). A growing body of evidence supports the distinction of a fat taste quality stimulated by long-chain fatty acids, with the scavenger receptor CD36 and the GPCR GPR120 as likely receptor candidates (Hichami et al. 2021). Recently, a taste cell mechanism has appeared to explain the sensory qualities associated with water (Zocchi et al. 2017) as a lingual stimulus (Rosen et al. 2010).

All behavioral assays (including human taste perception) of taste quality are, in one form or another, designed to measure the degree of discrimination or generalization between a sample tastant and a reference standard. At the cellular and molecular levels taste qualities are thought to arise from functionally segregated populations of cells within the taste bud that each are committed to signal one of the basic tastes (Caicedo et al. 2002; Yoshida et al. 2006). Each taste cell population selectively expresses receptors that are exclusively activated by tastants from one basic taste category. The taste cell signals resulting from receptor–tastant interaction in turn are faithfully propagated by independent sets of sensory neurons all the way to distinct locations in gustatory cortex (reviewed in Yarmolinsky et al. 2009). This "labeled line" hypothesis has predominated as the most widely accepted explanation to account for distinct taste qualities, compellingly supported by experiments in which molecular genetic techniques were used to redirect the expression of receptors for "sweet" agonists to "bitter" cells, and "bitter" agonists to "sweet" cells in mice. In a reversal of the consummatory behavior observed of wild-type mice, those genetically engineered mice avoided sucrose solutions and ingested solutions of substances considered bitter to humans (Mueller et al. 2005; Zhao et al. 2003). However, evidence contrary to a strict labeled line account of taste quality has been

present in the literature, where communication among ensembles of taste cells produces a combinatoric coding of taste signals (Roper 2021; Tomchik et al. 2007). More broadly, chemosensory discrimination apparently does not exclusively require a strict labeled line, as is evident in olfaction (Stettler and Axel 2009) and for psychoactive drugs, which act upon receptors distributed throughout the nervous system but still are behaviorally discriminated according to their stimulus properties (Porter et al. 2018).

Palatability is a term given to the preference for what is tasted, defined either as a measure of the consumption of a substance (reviewed in Palmer 2007, 2019) or, in human studies, the language subjects use to describe their preference for an ingestible substance (Wichchukit and O'Mahony 2015). Generally, palatability or preference is presented as a process that is dependent upon but distinct from taste quality. Preferences for substances taken into the oral cavity are acquired by associations between taste quality (and potentially other oro-sensory properties) and physiological consequences of ingestion. The associations are acquired through the experiential history of an individual organism (Chambers 2018; Reilly and Schachtman 2008) or are genetically determined (Diószegi et al. 2019). Measurements of palatability often are used to infer taste quality, particularly in animal experiments where the dependent variable is volume of consumption (Inoue et al. 2007; Tordoff and Bachmanov 2003) or the rate of licking from sipper tubes (Devantier et al. 2008; Long et al. 2010) or from 96-well plates (Palmer et al. 2013). In these cases the behaviors usually are referred to as "taste-guided" (Long et al. 2010; Spector 1995) to emphasize the distinction between processes exclusive to taste quality signaling and those of potentially additional physiological contributions to ingestive behaviors (Schier and Spector 2016).

The literature further distinguishes chemesthesis as a category of chemosensory responses to chemical irritants (Roper 2014; Slack 2016) that come into contact with receptors, such as transient receptor potential (TRP) channels, that are expressed in sensory nerve endings of the trigeminal nerve (Rhyu et al. 2021). The oro-sensory qualities associated with capsaicin through activation of TRPV1 (Long et al. 2010) and allyl isothiocyanate (AITC) and oleocanthal through TRPA1 (Des Gachons et al. 2011) are representative of chemesthesis.

Additional oro-sensory qualities that are thought to be due to processes separate from taste cell activity are recognized and studied, such as "astringency" (Green 1993; Schöbel et al. 2014), "mouth feel" (Simons et al. 2019), texture (Liu et al. 2017), and possibly sensory signals that result from osmotic changes (Gilbertson 2002; Lyall et al. 1999). Taste, defined as signals that result from the stimulation of taste cells, is one among many potential sources of sensory stimuli, also including olfactory (Djordjevic et al. 2004; Small and Prescott 2005), visual (Sakai et al. 2005; Spence et al. 2010; Zampini et al. 2007), auditory and verbal cues (Okamoto et al. 2008; Spence and Shankar 2010), and any other evoked cognitive associations (Liang et al. 2021; Noel and Dando 2015; Velasco et al. 2016) that contribute to the overall perceptual impression, or "flavor" (Auvray and Spence 2008; Prescott 1999), of substances taken into the oral cavity. It is important to reiterate and emphasize here that by scientific convention "taste" refers to the physiological and

perceptual phenomena that result specifically from the receptor-mediated functions of the specialized taste cells of the taste bud, distinct from other oro-sensory sensations that might be conflated into more inclusive notion of what is meant by "taste."

2.3 The Pharmacology of Taste

Certainly, any function of an organism that is affected by changes in tastant receptor activity can be, and has been, studied without reference to pharmacological principles when addressing experimental questions important to other scientific paradigms. However, the overarching goal of a pharmacological approach to the study of taste is the characterization of the relationships between tastants and all physiological consequences that follow from their effects on tastant receptors. It is the cause-and-effect association between tastant and taste response that is of interest to pharmacology. The lawfulness of that association should be reflected at every level of complexity, from the moment of signal transduction all the way to the subjective experience of the taste perception.

By the early 2000s the receptors that tastants act upon to generate the signals ultimately interpreted as sensory percepts of taste were discovered. They, generally, are the same molecular entities that had been the primary focus of the science of pharmacology since the early 1900s. Though the molecular objects underlying the phenomena under scrutiny were not known by pharmacologists until the second half of the twentieth century, the functional properties of receptors were understood and well-characterized prior to the time of their physical isolation. A set of principles emerged, coalescing into a general theory of receptor occupancy, that reliably accounts for the actions of receptors and the phenomena they control, including taste.

3 Essentials of Receptor Occupancy Theory

Careful measurement and experimental analysis of physiological changes caused by controlled administration of chemical agents to biological systems led to a concept of "receptor" that was defined not on its physical dimensions but on how it behaved (Barwich and Bschir 2017). Increasingly precise quantification of the effects of chemical agents on biological systems accumulating over the twentieth century led to formulation of principles that formed the theoretical basis of the scientific discipline of pharmacology. Certain physical properties of the previously unidentified receptor entity were deduced, and now with modern biophysical methodologies have been confirmed. The receptors have been isolated and identified as proteins (Grisshammer 2009; Lefkowitz 2013), their physical properties quantified (Hanson and Stevens 2009), and the molecular mechanisms underlying their activity determined (Rosenbaum et al. 2009).

Receptors are finite, countable objects, macromolecules that are structured such that they can accommodate a tight association with a second, usually small, molecule. The association, traditionally referred to as a "complex," is achieved by a complementarity between the ligand and a pocket in the surface of the receptor, both in terms of geometric shape and physico-chemical properties. Attractive intermolecular forces (for example, van der Waals forces, hydrogen bonds, ionic interactions) between the functional groups of the ligand and the R groups of the amino acids lining the pocket determine the duration of the receptor occupancy.

This first step describes the basics of a bimolecular reaction that is formalized with the notation of chemistry as follows:

$$L + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} LR$$

where L represents the ligand, R is the receptor, and LR is the ligand-receptor complex. The bidirectional arrows indicate a dynamic equilibrium between a forward reaction from reactants L and R to product LR, and a reverse reaction, the dissociation of the LR complex back to free L and R. Above and below the arrows are rate constants k_1 and k_2 for the forward and reverse reactions, respectively.

The rate constants are proportionality constants that relate the rate of reaction to concentrations of reactants. The rate, r, of the forward and reverse reactions are, respectively

$$r_1 = k_1[L][R] \quad (\text{forward reaction, the "on rate"}) \tag{1}$$

$$r_2 = k_2[LR] \quad (\text{reverse reaction, the "off rate"}) \tag{2}$$

At equilibrium the rate at which ligand binds receptor, the "on rate," is equal to the rate at which the ligand-receptor complex falls apart, the "off rate":

$$k_1[L][R] = k_2[LR] \tag{3}$$

Equations (1) through (3) are straightforward statements of the *law of mass action*, which posits that the rate of a chemical reaction is directly proportional to the product of the concentrations of the reactants, and further implies that the ratio of the concentrations of reactants to products will be constant at equilibrium.

From the information in Eqs. (1) through (3) a function can be derived that quantifies the fraction of a finite population of receptors that is occupied at any given concentration of ligand. The derivation, which is a series of simple algebraic manipulations (for examples, see Kenakin 2018; Limbird 2006), yields the following expression of receptor occupancy:

$$\frac{[L]}{\frac{k_2}{k_1} + [L]} = \widehat{p} \tag{4}$$

where $\widehat{p}$ is the fraction of occupied receptors at a given concentration of L. The ratio of the constant for the off rate, k_2, to that of the on rate, k_1, that appears in the denominator is also shown from the derivation to be equal to K_D, the dissociation constant, which is the concentration of L that achieves a fractional receptor occupancy of 0.5.

$$\frac{[L]}{K_D + [L]} = \widehat{p} \tag{5}$$

The K_D is the defining parameter of the affinity of a ligand for a specific receptor and is unique to each ligand-receptor pairing. The K_D also is the location parameter of the entire receptor occupancy function.

Equation (5) is formally equivalent to the Langmuir isotherm describing adsorption of gases to a surface (Langmuir 1918), and also to the Michaelis-Menten model for enzyme kinetics (Srinivasan 2021). The equation also was derived by Archibald Hill in the context of oxygen binding to hemoglobin (Hill 1910) and it is conventional to refer to the equation as the Hill-Langmuir equation in the context of receptor occupancy (Finlay et al. 2020; Neubig et al. 2003). A very similar form of this equation frequently is used as a quantitative model for analyzing concentration-response data (described below), and in that context is referred to simply as the Hill equation (Hill 1909; Neubig et al. 2003).

The equation describes a rectangular hyperbolic function. To account for cooperativity in binding the equation is modified by raising the ligand concentration variable to an exponent that reflects the slope of the function (Fig. 1). Under the conditions of a simple bimolecular association between ligand and receptor, the value for the exponent is 1, and accordingly, the slope of the function is 1. The slope exceeds a value of 1 if binding is positively cooperative – the formation of ligand-receptor complexes increases disproportionately with rising ligand concentrations.

Important characteristics of receptor behavior are immediately revealed by a plot of the Hill-Langmuir equation. When ligand concentration is plotted on a common logarithmic scale, the function is sigmoidal, a graphic presentation that enhances visual inspection of the quantitative characteristics of the ligand–receptor interaction (Fig. 1). Between, roughly, 15 and 80% of receptor occupancy, the function is practically linear. Beyond the linear portion of the curve is a region of saturation, a manifestation of the fact that progressively fewer receptors are open for ligand binding. From the function it can also be calculated that, under conditions of a simple, reversible bimolecular reaction, slope of 1, the ligand concentration required to occupy from 10 to 90% of the receptors will range by 81-fold (Figs. 1 and 2). Most of the concentration-occupancy function therefore is contained within a range of less than two $\log_{10}$ units, even less if binding is positively cooperative (Figs. 1 and 2). Functions that exceed this range imply additional complexities of ligand–receptor interactions, such as negative cooperativity, or the presence of multiple receptors in

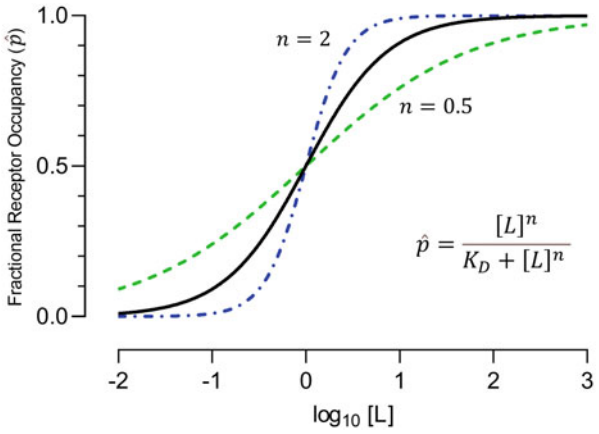

Fig. 1 Receptor occupancy function. The fraction of receptors occupied is plotted as a function of the ligand concentration ([*L*]) in common log units. In the figure, concentration in molarity is assumed and normalized to a value of 1 ($\log_{10} = 0$) for the purpose of generalizing the function to any range of concentrations. The concentration of ligand that occupies half of the total receptor population is equal to the K_D, the defining value for the affinity between ligand and receptor, and centers the domain for the function. Reversible bimolecular binding at equilibrium is assumed. Slopes greater than or less than unity, appearing in the exponent *n*, indicate cooperativity (positive or negative, respectively). The slope of the function does not impact the location of the K_D. The equation for the function defines the limits of the relationship between ligand concentration and receptor occupancy and sets the capacities for all concentration-response functions consequent to the formation of the ligand-receptor complex

the examined system that bind with differing affinities to the same ligand, all of which can be confirmed by further analysis with the right pharmacological tools and methods (if they are available; Christopoulos and Kenakin 2002).

3.1 The Concentration-Response Function and Its Relationship to Receptor Occupancy

The Hill-Langmuir equation presents a rigorous quantitation for the fraction of receptors that will be occupied at any given concentration of ligand, and is thus the centerpiece of the conceptual framework for all else that follows in the study of receptor-mediated functional biology. The general restrictions explicit in the concentration-occupancy function also are reflected in the *concentration-response* function. The ligand and receptor of interest here, of course, are a tastant agonist molecule and its cognate receptor. Since most of the concentration-occupancy function is contained within a range of less than two $\log_{10}$ units, the tastant concentration-response function that results from receptor occupancy also should be similarly restricted (Fig. 2). Functions that exceed these limits imply possible contributions of additionally activated tastant receptors, or perhaps other activities

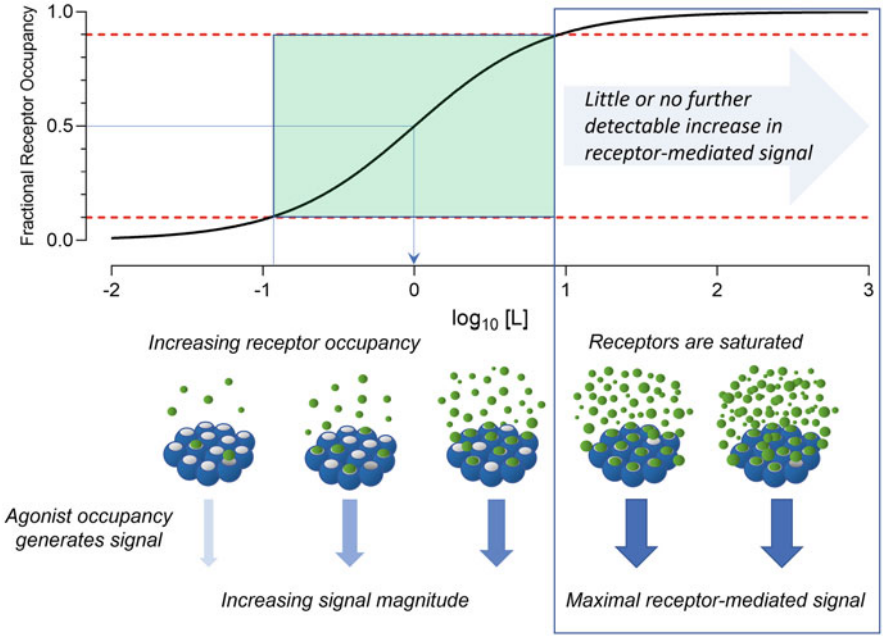

Fig. 2 The limits of receptor occupancy and implications for receptor signaling. Fractional receptor occupancy is plotted as a function of the concentration of ligand, here assumed to be an agonist. The function is defined by the Hill-Langmuir equation and assumes a slope of 1. Below the graph are drawings depicting receptors (blue ovals) increasingly occupied by agonist (green spheres) as agonist concentrations rise. Unoccupied receptors are indicated by a gray oval, representing open binding pockets, at the top of each receptor. The receptors are drawn to approximate correspondence with the portion of the occupancy curve above them. Signals that result from agonist occupancy accordingly increase in magnitude as agonist molecules occupy more and more receptors. The response capacity is maximized at saturation, beyond which no further increases in signaling or consequent response is possible through this receptor population. The dashed horizontal lines indicate fractional occupancy at 0.1 and 0.9. From the function it can be seen that most of the biological activity related to this receptor population occurs within an 81-fold range (<2 log units) of agonist concentration

unrelated to receptors that impact the dependent variable measured in any assay of taste.

Equation (5) also was derived by Alfred Joseph Clark (Clark 1927) as an attempt to generalize receptor occupancy to the magnitude of effect caused by any agonist. Later, Lloyd Beidler derived the same equation to quantitatively characterize concentration-dependence of gustatory nerve responses to tastants applied to rat tongue (Beidler 1954). Both Clark and Beidler assumed that the fraction of receptors occupied by agonist would be linearly related to the fraction of the maximum response. The additional property of efficacy (Stephenson 1956) was conceived to account for the observation that some agonists appeared to cause maximal effects while occupying a relatively small percentage of receptors, whereas other agonists never achieved maximal effect even at concentrations expected to saturate the

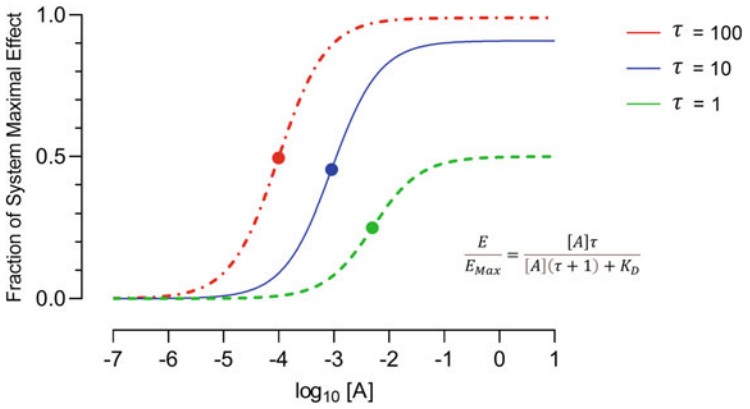

Fig. 3 The concentration-response function by the operational model of agonism. The curves in the figure are fit by the operational model of Black and Leff (1983), with a slope of 1 assumed, and the value of τ varied as shown. The y axis indicates the maximal range of capacity for change within the biological system under experimental scrutiny, and the x axis is arbitrary common log units of agonist concentration. The system could be a simple smooth muscle preparation or the response capacity of the population of cells in the tongue committed to sweet-taste signaling. The ability of agonist-receptor complexes to access system capacity is a function of receptor density and agonist intrinsic efficacy, both captured in the value of τ. The green curve shows a concentration-response function that results from relatively low access to system capacity by the agonist-receptor complexes, either because overall receptor density is below capacity, or the agonist-receptor complexes do not efficiently couple to the biological system. With a τ value of 1, the receptor-complexes can achieve a maximum of half of the system's capacity for change. Increases in receptor expression or agonist intrinsic efficacy move the effectiveness of agonist-receptor complexes progressively toward system capacity, as indicated by the blue and red curves (τ of 10 and 100, respectively). The closed circles at the inflection point of each curve indicate the position of the *EC*50 on the x axis below. For the green curve, the *EC*50 should closely approximate the agonist's affinity for its receptor

receptor pool. The term *intrinsic* efficacy refers to the ability of an agonist to "activate" a receptor (Clarke and Bond 1998; Kenakin 1985). High intrinsic efficacy agonists, relative to other agonists, require fewer receptors to cause changes in a biological system. In contrast, a low efficacy agonist, or partial agonist, fails to cause maximal effect even at saturating concentrations (Fig. 3). Evidence of partial agonism by tastants has been obtained from cell-based assays of murine TAS2R receptors (Lossow et al. 2016), but little or none has been reported for in vivo assays of taste, to date.

3.1.1 The Operational Model of Agonism

Manifestation of agonist intrinsic efficacy can be impacted by the numbers of receptors expressed in a given biological system. A maximal effect could result from a partial agonist if levels of receptor expression are sufficiently high. Thus, the magnitude of effect caused by an agonist is determined by properties intrinsic to the agonist as well as the tissue. Both factors also determine the location of the

concentration-response function – high expression and high intrinsic efficacy tend to shift concentration-response functions to the left (i.e., lower ranges of concentration).

A quantitative model of agonist activity that links receptor occupancy, intrinsic efficacy, and receptor density to the concentration-response function was derived by Black and Leff (1983) and Black et al. (1985). Essentially treating the agonist-receptor complex, *AR*, as the stimulus that launches the signal transduction chain of events, and from the former assumption of a direct correspondence between fractional receptor occupancy and physiological effect, Eq. (5) is reframed in the operational model as

$$\frac{E}{E_{\text{Max}}} = \frac{[AR]}{K_E + [AR]} \tag{6}$$

where E_{Max} is the maximal effect possible in the receptor-linked system, and K_E is the concentration of agonist-receptor complex, $[AR]$, that causes half-maximal effect. The operational model further introduces a "transducer ratio," $\boldsymbol{\tau}$, a measure of the efficiency of the transduction of signal from the agonist-receptor complex:

$$\tau = \frac{[R_T]}{K_E} \tag{7}$$

Here, the term $[R_T]$ represents the total population of receptors available to the agonist. Black and Leff (1983) incorporated these concepts into a step-by-step derivation that begins with Eq. (5) to arrive at the following relationship:

$$E = \frac{E_{\text{Max}}\tau[A]}{(K_D + [A]) + \tau[A]} \tag{8}$$

The equation emphasizes the impact of intrinsic efficacy and receptor density on the translation of receptor occupancy to a concentration-response function. Both the maximal response magnitude and the $EC50$ for an agonist are impacted by τ (Fig. 3).

The logistic form of the operational model is given by the equation:

$$E = \frac{E_{\text{Max}}[AR]^n}{K_E + [AR]^n} \tag{9}$$

which includes exponents to account for slopes differing from unity. In most practical applications, the Hill equation (or a closely related logistic version which allows determination of values for asymptotes) is used (Neubig et al. 2003):

$$\frac{E}{E_{\text{Max}}} = \frac{[A]^n}{[EC_{50}]^n + [A]^n} \tag{10}$$

Here, the exponent n is referred to as the Hill coefficient, again reflecting the slope of the concentration-response function. The main difference between Eqs. (9)

and (10) is that the former conceptualizes the agonist-receptor complex as the unit of stimulus, whereas the latter equation frames the concentration-response function in terms of the agonist, the independent variable that is under direct control of the investigator.

4 Conclusions to be Drawn from Receptor Theory: What Is to be Expected of Taste?

The equations detailed above provide a well-reasoned progression from the chemical event of receptor binding to its consequent physiological effect. The theory quantitatively traverses the interface of chemistry and biology, and by doing so defines the agonist concentration-dependence of any physiological action that is linked to receptors. Scientific theories are best when they set clear limitations to what is possible for the natural phenomena they purport to explain. Taste is universally acknowledged to be mediated by receptors, and so also should operate within the bounds set by receptor theory.

The relationship between tastant concentration and magnitude of taste response should assume a hyperbolic (or related logistic) function. Most of the function should be contained within a span of approximately two $\log_{10}$ units. There must be an upper limit to the concentration-dependence of tastant responses as the population of tastant receptors saturate with tastant. Additional increases in tastant concentration beyond that limit should not result in any further measurable increase in taste (Fig. 2). One potential nuance to this rule is that higher concentrations could result in a faster onset of action (by the forward rate of the ligand binding reaction, r_1, defined above), which could be a detectable cue incorporated into the taste response. However, rate of onset also will soon reach a limit to its potential as a discriminable cue.

The location of the concentration-response function, indicated by the $EC50$ parameter, is determined by the agonist's affinity for its receptor (K_D) and factors controlling receptor density and coupling efficiency (factors represented by τ, Fig. 3). Potentially, then, genetic variations that impact tastant receptor structure in the binding site, in the domains that couple the receptor to signaling, or in the promoters for receptor (or G protein) expression, could shift the concentration-response functions for taste to lower or higher ranges across individuals. Threshold measurements for taste, a common focus of psychophysical studies, would be affected by shifts in tastant potency. However, differences among individuals in thresholds for taste also could be due to other physiological processes that are not directly related to the functionality of tastant receptors. Especially where inter-subject differences in thresholds are small, analysis of the entire concentration-response relationship would bolster correlations of taste-sensitivity phenotypes with specific genetic variants of tastant receptors.

Efficiency of coupling also is partly determined by the agonist, and it is possible that some tastants are more effective than others in translating receptor occupancy into receptor signaling (the property of intrinsic efficacy). In vivo demonstration of a

low efficacy tastant agonist would, by itself, be an important discovery; but a partial tastant agonist would be particularly useful for identifying receptor density as a determinant of taste-sensitivity phenotype.

The above equations and reasoning rest upon an assumption of equilibrium conditions. A question then naturally arises over whether receptor occupancy theory is directly applicable to taste, which is a rapid response occurring within milliseconds of contact with a tastant agonist (Stapleton et al. 2006). As of yet, molecular assays capable of directly determining the kinetics of tastant receptor occupancy are not available. However, there should be a correspondence between response magnitude and a specific fraction of occupied receptors, even if tastant binding equilibrium has not been reached within the timeframe of a taste response.

Concentration-response analysis of data from cell-based assays of heterologously expressed tastant receptors provides an appropriate test of the predictions of receptor theory on transient responses that are likely to occur under hemi-equilibrium conditions (Charlton and Vauquelin 2010; Kenakin et al. 2006).

4.1 Characteristics of the Concentration-Response Functions from Cell-Based Assays of Recombinant Tastant Receptors

Cell-based assays of heterologously expressed TAS2R (Meyerhof et al. 2009), TAS1R2/R3 (Li and Servant 2008; Servant et al. 2010), and TAS1R1/R3 (Servant and Frerot 2021) receptors have been in use for over two decades, both for basic research and for commercial purposes. The assays record tastant-stimulated mobilization of intracellular calcium through the use of fluorescent dyes and imaging devices such as FLIPR (Woszczek et al. 2021). Calcium responses occur within seconds of addition of tastant, and in that regard cell-based assays serve as a suitable model system for pharmacological comparison with similarly rapid in vivo tastant responses.

The characteristics of the concentration-response functions obtained from recombinant tastant cell-based assays are quite consistent with the operational model and receptor occupancy theory upon which it is based. An abundance of data is available from human TAS1R2/R3 assays of concentration-dependent responses to sucrose and other sweet tastants to serve as a useful illustration. Concentration-response functions for sucrose (Li et al. 2002; Servant et al. 2010; Xu et al. 2004; Zhang et al. 2010) are anchored at the low end by concentrations of approximately 3 to 10 mM, after which the functions rapidly accelerate through a phase that is essentially linear. The midpoint of the linear portion, the *EC*50 value representing sucrose potency, is explicitly stated in some papers with values of 62, 52 (Servant et al. 2010), and 19.4 mM (Xu et al. 2004), and where not explicitly stated can be seen from inspection of graphs to range between approximately 30 and 60 mM (Li et al. 2002; Xu et al. 2004; Zhang et al. 2010; summarized in Table 1 of Palmer 2019). The curves also can be seen to approach an asymptote as concentrations reach or exceed 100 mM, where no further increases in responsiveness occur. Thus, the entire concentration-response functions for sucrose obtained from cell-based assays for

recombinantly expressed TAS1R2/R3 are contained within approximately 1.5 to no greater than two $\log_{10}$ molar units of concentration range. The steepness of the curves plausibly is explained by the hemi-equilibrium kinetics of receptor occupancy expected of calcium signaling assays (Charlton and Vauquelin 2010). Similar characteristics for concentration-response functions for the TAS1R2/R3 agonist sucralose in cell-based assays also are evident, but shifted approximately 1,000-fold to the left of that for sucrose; the median of multiple *EC*50 determinations by Servant et al. (2010) was 61 μM, and their functions saturated as concentration approached 1 mM.

4.2 Concentration-Dependence of Human Taste

4.2.1 Power Functions for Taste Intensity

Concentration-dependent measure of suprathreshold tastant responses has been conducted for many decades through the use of scales of taste "intensity." Historically, most of the development of scales for rating sensory intensity developed out of experiments involving manipulation of the energy output of visual or auditory stimuli (Stevens 1957), but eventually scales of taste intensity also were designed (Stevens 1969). For taste intensity, subjects are instructed to report the magnitude of their resulting sensory experience in terms of numbers, as in scales of magnitude estimate (Stevens 1969), or verbal labels which have been equated with a numeric scale, as in the labeled magnitude scale (LMS; Schifferstein 2012) and generalized (or general) labeled magnitude scale (gLMS; Bartoshuk et al. 2004).

Taste intensity rating scales are considered to remedy both logistical and conceptual limitations of threshold measurements, which focus only on the lowest detectable concentrations of tastant. Statistical resolution of thresholds requires many trials of samples containing tastant and "blanks" (vehicle alone), and the results do not necessarily inform on responsiveness to suprathreshold concentrations that normally are encountered by humans as they sample sources of tastant from their environment (Keast and Roper 2007). Concentration-dependence of taste intensity is described as progressing from thresholds of detection through increases in magnitude to a hypothetical asymptote, where further increases in tastant concentration of tastant no longer cause increases in perceived taste intensity (Low et al. 2014). Nominally this description would be consistent with the predictions of receptor occupancy. However, empirical results from studies of the relationship between taste intensity and tastant concentration often do not agree with this description; in particular, taste intensity frequently has been shown to continue increasing without saturation as tastant concentration rises. The range of taste-active concentrations also appears to exceed the theoretical limits predicted by receptor theory.

From the earliest studies of suprathreshold taste intensity measurements, the resulting concentration-intensity relationships were fit to a power function. Such results were viewed as an expected generalization of the model promoted by Stanley S. Stevens (Stevens 1957), which purported that the relationship between the intensity of a physical stimulus and the perceived magnitude of sensation is best fit

by a power function for all sensory modalities. The function is described by the equation:

$$\psi = kS^n$$

where ψ is the subjective sensation experienced, S is the stimulus (in the context of taste, the variable represents tastant concentration), k is a constant, and n is the empirically determined exponent for the power function that fits the data.

A defining characteristic of power functions is that they can be linearized by a log transformation as follows:

$$\log \psi = n \log S + \log k$$

A plot of the function on a log-log scale produces a straight line with a slope defined by the power exponent, n.

Stevens applied the power function model to data obtained from subjects who were instructed to assign numbers to the magnitude of sensation they experienced across a range of sucrose concentrations (Stevens 1969). The results of Steven's experiment are shown in Fig. 4 (redrawn from the original publication). The slope of the plot yields a power of 1.3 for the function. A power function with an exponent >1 quantitatively describes an accelerating function (it does not saturate).

Steven's results, which he reported to have replicated in additional experiments, are not explained by receptor theory. Receptor occupancy must saturate, and therefore any response that is functionally related to receptors also must saturate. Stevens acknowledged that eventually a saturation might be expected as the capacity of the nervous system for processing incoming sensory information was approached, but it is the saturation of receptor occupancy that matters in setting the limits to generating any signals that result from tastant agonist activity.

Not all investigators have reported an exponent of 1.3 from the power function obtained for sweetness intensity rating of sucrose. A paper by Meiselman (Meiselman 1971) addressing questions over the potential effects of tastant presentation procedure (sip, anterior dorsal mouth flow, whole mouth flow) on taste intensity functions summarized in a table the exponents of power functions from taste intensity studies of NaCl, quinine, sucrose, saccharin, HCl, and citric acid. A wide range of exponent values, above and below 1, for all tastants is evident. The table suggests a tendency for stimulus presentation by flow methods to result in lower power function exponents in comparison to sip methods. However, exponents obtained from each of the methods separately considered also vary widely. For example, sip methods from 11 studies of sucrose taste intensity produced power functions with exponents that range from 1.8 to 0.62. More recently reported values for sucrose sweet taste intensity continue to range below and above 1 (for example, values of 0.78 from Green et al. (1993), and 1.3 from Wee et al. (2018)).

There are different versions of scales and methods of their use, and there have been continuous debates over the merits and shortcomings of each (Schifferstein 2012). It often has been argued that the scale design and attendant methods of its

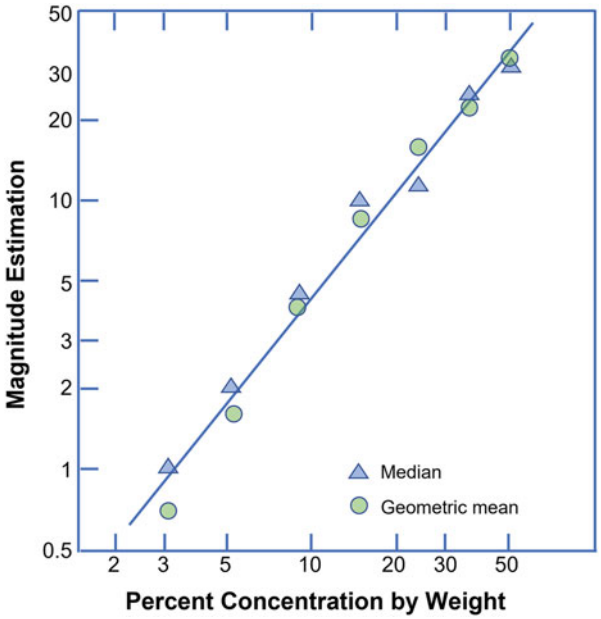

Fig. 4 Sweet taste intensity magnitude estimation. The figure is redrawn from Fig. 2 of Stevens (1969). The figure shows a log-log plot of the magnitude of subjectively experienced taste intensity experienced (y axis) from varying concentrations of sucrose solutions (expressed as percent W/W on the x axis, ranging from 3% (87 mM) to 50% (1,450 mM)). Subjective intensity was estimated by each subject using a scale for magnitude estimates. Prior to testing the scale was calibrated by establishing a standard of sweet taste intensity. The standard was created by giving each subject a single concentration of sucrose to taste and instructing them that the intensity experienced should be a predetermined value (10, for example). Subjects were then further instructed to assign numbers expressed as ratios of intensity relative to the standard for all subsequently presented sucrose concentrations. Instructions included detailed explanation of how to assign ratios. The data were fit by a power function with an exponent of 1.3 (the slope of the line in the log-log plot), indicating continuous acceleration of the function over the range of sucrose concentrations tested. In some instances, subjects did not report their sensory magnitude estimates as ratios, and such cases suggested to Stevens a failure of the subject to "grasp the concept of proportionality"

administration could bias the experimental outcome (Lawless et al. 2000; Meiselman 1971; Running and Hayes 2017). However, there is no accounting for such wide shifts from negative to positive in the exponents of power functions fit to the various datasets, nor even why a concentration-response function for receptor-governed responses should be fit by a power function (particularly non-saturating power functions) instead of a hyperbolic function. In a recent report (Wee et al. 2018) of a concentration-response analysis performed on 16 different sweeteners, sweetness intensity rating data were fit both to a power function model and also to the Hill equation. The sweetness intensity rating data for sucrose fit by a power function returned an exponent of 1.3 indicating positive acceleration throughout. Curiously, the same dataset analyzed by the Hill equation yielded saturating functions. The Hill

analysis of sucrose taste intensity by Wee et al. is consistent with the results reported by Antenucci and Hayes (2015), a similar analysis performed on sucrose intensity ratings from a group of 401 subjects. In stark contrast, the results of the power function analysis of sucrose taste intensity by Wee et al. are inconsistent with the Hill analysis of Antenucci and Hayes (2015) and also with their own Hill analysis.

Ultimately there is no theoretical basis for a power function model to quantitatively describe tastant–agonist interactions. There is no need, therefore, to rely on them for analyzing taste intensity data. Concentration-intensity relationships for tastants have been graphically represented and statistically treated without power function curve fits (or any other model, for that matter), and by doing so achieve experimental objectives without wading into the difficulties of interpretating the curve fits outlined above. For example, the concentration-intensity relationship for sucrose using the gLMS, plotted as a simple point-to-point graph, demonstrated the perceptual effect of antagonizing the TAS1R2/R3 receptor with clofibrate (Kochem and Breslin 2017).

4.2.2 The Relationship of Taste Intensity to Taste Thresholds

There is, however, another question that arises from the results of many taste intensity experiments regardless of curve fitting models. Relative to measurements of taste detection thresholds, taste intensity ratings often occur across concentrations that range greater than would be expected for a receptor-mediated phenomenon. The range of taste-active concentrations should be anchored at the low end by thresholds. Most threshold measurements are achieved by presenting two or more samples to the subject in a randomized or stepwise pattern, one with a "blank" (usually water) and the others with tastant solution. The lowest concentration of tastant that is statistically determined to be correctly distinguished from water represents the threshold.

Despite the likely impact of a variety of conditions and subject-dependent variables on taste sensitivity (Trius-Soler et al. 2020), the values obtained for sucrose detection generally range around 5 to 10 mM (reviewed in Palmer 2019, and Trius-Soler et al. 2020). For example, average sucrose thresholds of 6.8 and 10.83 mM in healthy adults have been reported by Petty et al. (2020) and Zhang et al. (2008), respectively. A recent application of signal detection analysis to generate *d'* values (a measure of discriminability) from a method of constant stimuli experiment (Palmer et al. 2021) indicated that, on average, adults could discriminate 5 mM sucrose from water. Collectively, these results strongly suggest that concentrations of sucrose near 5 to 10 mM also should anchor the low end of the concentration-response function for sucrose taste.

In contrast, sweetness intensity ratings of sucrose typically begin to register at higher concentrations, apparent in Steven's data (see Fig. 3) extrapolated to a low concentration of approximately 2% w/w, or 58 mM, and also in those of Kochem and Breslin (2017) mentioned above. In the latter study, sweetness intensity ratings were anchored for sucrose (in the "neat," or without antagonist, condition) at the lowest concentration tested, 30 mM, where the numeric value for intensity would be equivalent to a label of "barely detectable" by the gLMS (Bartoshuk et al. 2004). In both studies, sweetness intensity continued to rise with sucrose concentration up to

the highest concentration tested of approximately 1,500 mM, at which point saturation was not evident. Increasing taste intensity as sucrose concentrations exceed 1 M is reported in many studies (reviewed in Palmer 2019). Recently, sucrose intensity ratings obtained by the gLMS in a group of type 2 diabetics were shown to continue increasing at 2.02 M (Vidanage et al. 2022).

Possibly the conundrum of ratings that translate to "barely detectable" in taste intensity scales for sucrose concentrations that are readily detectable in threshold procedures is explained by contrast effects, a suppression of taste intensity perception when judging low tastant concentrations in a test which includes trials of substantially greater concentrations (Lawless et al. 2000; Shepard et al. 2017). More importantly, however, the range of taste-active concentrations spanning from threshold detection through those of taste intensity measures exceeds the range set by the limits of receptor capacity. In the case of sucrose, there is a 300-fold range of taste-active concentrations, from approximately 5 at threshold to 1,500 mM, with no indication of response saturation in most studies. The problem is even more pronounced with sucralose; the span of taste-active concentrations appears to begin with thresholds of 11.9 uM (Breslin et al. 2021) but sucralose sweetness intensity ratings continue to increase at 100 mM (Kochem and Breslin 2017), or a 10,000-fold range of concentrations. Data generated by sweetness intensity measurement are difficult to reconcile with the predictions of receptor theory. Perhaps something more than TAS1R2/R3 receptor function is involved in the perception of sweet taste intensity. If taste is defined by taste receptor signaling, which is determined by tastant receptors, then taste intensity is a more comprehensive oro-sensory experience.

4.3 Dependent Variables in Human Taste Measurement

There is no doubt that a causal relationship exists between tastant concentration and measures obtained by taste intensity scales – as concentration increases, subject ratings of taste intensity predictably increase. However, the issues raised here over the range of concentration-dependence and the quantitative characteristics of the functional relationship suggest a fundamental question over what, precisely, defines "taste intensity" as a dependent variable to be measured. The nature of sensations – what they are and how they represent the external world to the subject – has a long history of philosophical discourse that is central to Western thought. Here, however, the focus will remain limited to the practical aspects of defining taste as a dependent variable for measurement and how the resulting data are to be interpreted.

A different approach to measuring human taste as a function of concentration recently was reported (Palmer et al. 2021) that was based on a taste discrimination procedure. In contrast to most discrimination experiments, where the focus is on threshold determination, the procedure of Palmer et al. (2021) trained subjects through an automated game-like operant task to compare a range of sucrose concentrations (from 3.9 to 500 mM, randomly presented) to two standards, water and 200 mM sucrose. The solutions were self-administered to the tongue in aliquots of 200 μl from an electronic pipette. The datum was a binary "sucrose-like" or

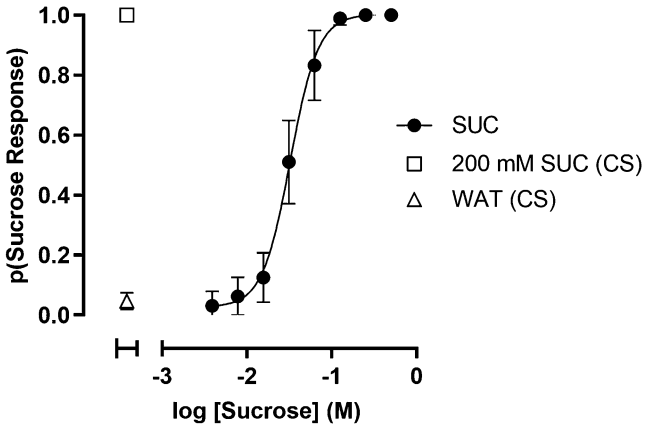

Fig. 5 Concentration-response function for sucrose taste discrimination. The figure is from Palmer et al. (2021), its use here is permitted through the Creative Commons License. A cohort of 8 subjects was trained through a game-like interactive algorithm to associate coordinates on a touch-sensitive laptop display with two standards (control stimuli, CS), water (WAT) and 200 mM sucrose (SUC), automatically drawn in 200 μl aliquots from a 96-well plate and self-administered to the tongue. Each of the 96 trials in a session was occasioned by a consequence – a virtual poker chip appeared on the display that represented actual monetary value if the correct target was touched, or a reduction of value if an incorrect choice was made. Once a criterion of test-readiness (90% correct out of 96 trials) was achieved, subjects were tested with a 96-well plate containing multiple replicates of the standards and of 8 sucrose concentrations ranging in two-fold dilutions from 500 mM to 3.9 mM. All responses, regardless of target, resulted in a poker chip reward on trials from the sucrose concentration range, but only correct responses on standard trials were rewarded (errors were penalized). The resulting data set was analyzed by nonlinear regression using a logistic model based on the Hill equation. An *EC*50 of 33 mM was returned from the curve fit, remarkably similar to values reported for recombinant TAS1R2/R3 cell-based concentration-response analyses of sucrose. The function saturates between 125 and 500 mM, and the entire range of taste-active sucrose concentration spans <1.5 log units of molarity. The results are consistent with receptor theory

"water-like" choice recorded by touches to a "sucrose" target and a "water" target on a touch-sensitive laptop display. The resulting dataset, averaged across 8 subjects, was fit by a logistic equation based on the Hill equation and related operational model of agonism. The concentration-response function plotted as proportion of sucrose-like responses, shown in Fig. 5, is anchored at the low end by a minimum of responses made on the "sucrose" target (i.e., most of the responses occurred on the "water" target). The frequency of sucrose target responses increases rapidly with rising sucrose concentrations approaching a limit of essentially 100% between 125 and 500 mM.

The steep slope of the curve (Hill coefficient of 2.64) indicates a cooperative effect on the relationship between tastant concentration and taste response, with the majority of the curve contained well within a span of <1.5 $\log_{10}$ units. Saturation is readily apparent from the sigmoidal shape of the semi-log plot. The quantitative characteristics of the sucrose taste discrimination curve are completely consistent

with the function obtained from a receptor-mediated process as predicted by theory. Though the subjects in this experiment initially were trained to discriminate between 200 mM sucrose and water, perhaps regarded as a qualitative categorization, the results from this experiment clearly indicate that their discriminations also were based on concentration – an operation on magnitudes.

Ostensibly, measurement of taste intensity also must entail a discrimination between two concentrations of tastant; a discrimination process must occur or there would be no report of a difference in intensity magnitudes. Taste intensity measures are subjective measures, meaning that they are a kind of operation performed to quantify an event which necessarily can be witnessed by only a single observer – the subject who experiences the sensation produced by the tastant (Tourinho 2006). It is the subject who performs the measurement and reports the result back to the investigator. The investigator might provide examples of stimuli and how they might be rated to "calibrate" the subject (Olabi and Lawless 2008), but the dimensions of the sensory experience still are defined in the private "privileged access" (Heil 1988) of the subjective world. The subject defines the limits of what is to be considered a sensation and the dimensions of its magnitude.

Given the task of estimating the magnitude of a taste sensation, the subject is free to use any and all information available from the sensory input that obtains from oral contact with a substance. Rate of receptor occupancy is concentration-dependent (by Eq. (2)), and likely to contribute to concentration-dependence of onset of taste stimulus (Garrido et al. 2001; Yamamoto et al. 1985; Yamamoto and Kawamura 1981). The on-rate potentially could serve as the basis for discriminating between concentrations beyond those required for occupancy saturation; but on-rate also soon would reach a limit and consequently have little or no further impact on perceived intensity at much higher tastant concentrations. Clearance of tastant from the oral cavity (Luke et al. 1999; Sreebny et al. 1985) and by implication, the receptor compartment, also would be expected to be concentration- and time-dependent; potentially a discriminable cue. Furthermore, the physical properties of a tastant can change substantially as concentrations increase. A pertinent example is sucrose, the viscosity of which increases by more than ten-fold across concentrations ranging from 10 to 50% (292 to 1,462 mM; Telis et al. 2007). Chemical and physical properties of a tastant at high concentrations quite possibly could be detected by other sensory mechanisms unrelated to tastant receptors to enhance the perception of a taste stimulus already present at its maximum. These additional sources of sensory information suggested here are only conjecture, but whether they can shape the subject's estimation of magnitude is an experimentally approachable question. However, these are not pharmacological questions, which are limited to the analysis of taste defined as output of taste cell activity under the control of tastant receptors.

5 Concluding Remarks

The idea that taste is pharmacology, though perhaps currently a minority view, is not a new one. Decades ago the behavioral pharmacologist Robert Balster (Balster 1988) observed the similarities between psychoactive drugs, which generate "interoceptive" discriminative stimuli, and the exteroceptive stimulus properties of odorants and tastants:

> . . .it should be remembered that drugs are chemicals. Detection of drug stimuli could be viewed as a type of chemoreception. There are important similarities in receptor theory for drug action and current theories of olfactory and gustatory stimulus transduction.

Balster further lamented a lack of cross-fertilization between the fields of chemoreception and pharmacology. Missing at the time was a clear understanding of the molecular mechanisms of chemoreception signal transduction. Now that the molecular mediators of taste and olfaction have been identified as GPCRs and ion channels, the link to pharmacology is obvious.

A pharmacological approach to the study of taste however must remain limited to the operations of tastant receptors and those processes that are under their control. Until the ambiguities of taste intensity measurements and their relationship to taste receptor activation are resolved, their application to elucidation of receptor functionality must be accepted cautiously. For the time being, taste discrimination appears to be more in line with receptor pharmacology, and therefore might be a better choice of assay for establishing relationships between taste phenotypes to variants of receptor structure that determine receptor density and tastant affinity. This would in turn help to distinguish tastant sensitivities that are due to receptor function from those that result from physiological factors.

Pharmacological analysis of taste discrimination might further help to refine some concepts traditional to psychophysics, such as "taste intensity." In vivo demonstration of a partial agonist for taste responses would be most useful in this regard. No matter how high the concentration, the maximal effect of a partial agonist should be perceived to produce the same intensity as a submaximal concentration of a tastant of higher intrinsic efficacy. A partial agonist also should mitigate the taste intensity of a high efficacy tastant in a binary mixture if the two share the same receptor binding site. Antagonism by a partial agonist should be pronounced under conditions that promote taste receptor desensitization, as has been demonstrated for the human P2Y1 receptor low efficacy agonist ATP in a cell-based transient calcium mobilization assay (Palmer et al. 1998). Desensitization of sweet taste intensity ratings following prolonged exposure to agonists of sweeteners has been reported (Schiffman et al. 1994), presumably a consequence of time- and concentration-dependent tachyphylaxis of agonist occupied receptors.

For those who still require a medicinal application for inclusion under the purview of pharmacology, there is ample occasion for the study of taste to meet such a demand. Taste long has been associated with palatability of foods and beverages and dietary choices (Costanzo et al. 2021; Kourouniotis et al. 2016;

Yeomans 1998) and would seem an obvious driver of overconsumption. Despite years of very active research, the connection between taste and obesity still is not clear (Ribeiro and Oliveira-Maia 2021). On the other hand, loss of taste, due to damage and disease (Dawson et al. 2020; Heckmann et al. 2005; Ibekwe et al. 2020; Nakanishi et al. 2019), chemotherapy and medication (Kan et al. 2021; Kumari et al. 2017; Rademacher et al. 2020), and aging (Kaneda et al. 2000; Schiffman and Graham 2000) has clear negative impact on food intake (Risso et al. 2020), emotion (Dudine et al. 2021), and quality of life (Jeon et al. 2021; Kaizu et al. 2021). Taste also is an important factor in the adherence of orally administered therapeutic regimens, particularly among pediatric patients (Baguley et al. 2012; Walsh et al. 2014). Better understanding of the interactions between active pharmaceutical ingredients and tastant receptors that mediate aversive tastes should help toward improving the acceptability of oral formulations. There are important unmet medical needs involving taste that can be addressed through the application of pharmacological principles, as has been done for many other health-related conditions.

Taste is a unique system for in vivo pharmacologic analysis. In contrast to the pharmacology of systemically administered drugs, the impact of pharmacokinetics on tastant responses is greatly diminished. The receptors are expressed on the apical microvilli of taste cells, localized to the surface of the tongue where they are exposed to administered tastant agonists with no obvious barrier to access. The link between pharmacodynamics at the receptor compartment and the behavioral outcome should be quite direct. Taste responses are rapid and relatively easy to record, and data can be generated quickly and at low risk to subjects. Taste presents an ideal experimental system for exploring the concepts central to the paradigm of pharmacology, further suggesting a broadening of the scope of pharmacology to other types of chemoreceptor systems such as olfaction.

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Taste Receptor Signaling

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Abstract

All organisms have the ability to detect chemicals in the environment, which likely evolved out of organisms' needs to detect food sources and avoid potentially harmful compounds. The taste system detects chemicals and is used to determine whether potential food items will be ingested or rejected. The sense of taste detects five known taste qualities: bitter, sweet, salty, sour, and umami, which is the detection of amino acids, specifically glutamate. These different taste qualities encompass a wide variety of chemicals that differ in their structure and as a result, the peripheral taste utilizes numerous and diverse mechanisms to detect these stimuli. In this chapter, we will summarize what is currently known about the signaling mechanisms used by taste cells to transduce stimulus signals.

Keywords

Bitter · Peripheral taste cells · Salt · Signal transduction · Sour · Sweet · Umami

1 Introduction

The taste system evolved to detect nutrients that are needed for survival as well as a defense mechanism to avoid potentially harmful compounds. There are five basic taste qualities: sweet, umami, salt, bitter, and sour. Sweet, umami, and salt taste are appetitive and are used to identify compounds that are necessary for survival. Sweet taste is elicited by carbohydrates (sugars) and allows for the detection of calorie-rich food items. Umami, the "savory taste," detects amino acids, particularly glutamate, and identifies proteins in food. Salt taste detects sodium and other ions which are needed to maintain ionic homeostasis. Bitter and sour identify chemicals that are potentially harmful and are considered aversive taste qualities. Toxic compounds are often bitter while sour taste detects acidic compounds that may be unsafe for consumption. The detection of these different types of chemicals by the taste system informs the brain about the content and the quality of the potential food items which allows for either ingestion or rejection to occur. Without the taste system, an organism could not detect nutrients or avoid ingesting toxins, making the taste system critical for survival.

2 Organization of the Peripheral Taste System

Chemicals in potential food items are detected by taste receptor cells (TRCs) which are grouped together in taste buds located in the oral cavity. In mammals, taste buds are localized in specialized grooves or bumps called papillae on the dorsal surface of the tongue. Additional taste buds are located on the soft palate and scattered throughout the oral cavity (Finger and Simon 2000).

Most taste buds in the oral cavity are housed in one of three different papillae on the tongue: fungiform (Fun), foliate (Fol), and circumvallate (CV) papillae. Fun

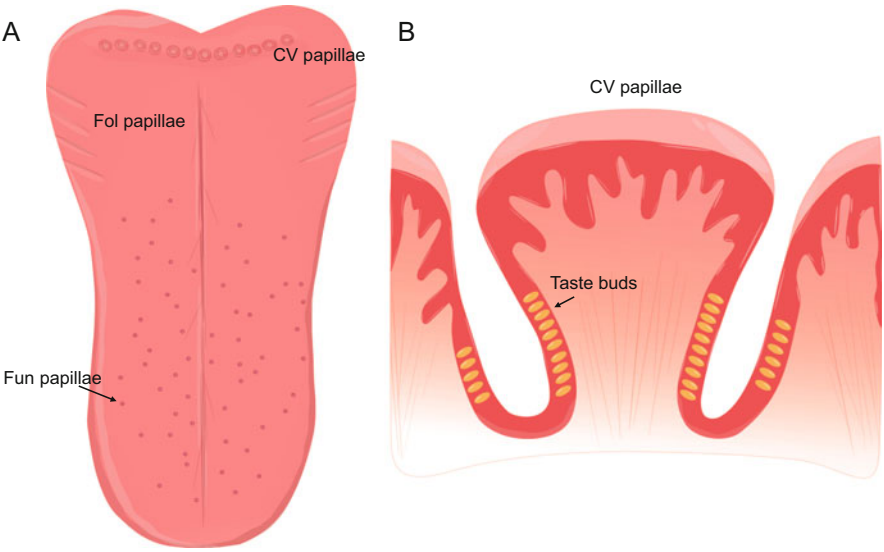

Fig. 1 Anatomy of the human tongue and taste papillae. (**a**) Fungiform (Fun) papillae are located on the anterior 2/3rd of the tongue and house 1–2 taste buds each. The foliate (Fol) papillae are located on the posterior-lateral side of the tongue and house hundreds of taste buds. The circumvallate (CV) papillae are located in the back of the tongue and house hundreds of taste buds each. (**b**) Cross-sectional view of a CV papilla. Each CV has two crypts that are each lined with hundreds of taste buds

papillae are present on the tip and the lateral margins of the tongue. Each Fun papilla contains 1–2 taste buds and is innervated by the chorda tympani branch of the facial nerve (cranial nerve VII). CV papillae are located in the posterior tongue, forming a U-shaped invagination on the dorsal epithelium. These papillae house hundreds of taste buds and are innervated by the lingual branch of the glossopharyngeal nerve (cranial nerve IX). Fol papillae are formed from vertically oriented folds on the lateral side of the posterior tongue and contain hundreds of taste buds. The Fol papillae are innervated by both the glossopharyngeal nerve, which innervates the posterior area, and the chorda tympani nerve, which innervates the anterior Fol papillae (Fig. 1) (Lindemann 1996; Finger and Simon 2000).

There are some taste buds located in other areas of the oral cavity, including the soft palate. These taste buds are most dense in a region of the palate just caudal to the hard palate called the geschmacksstreifen or "taste stripe." Palate taste buds are innervated by the greater superficial petrosal nerve, another branch of the facial nerve (El-Sharaby et al. 2001). The epiglottis, larynx, and upper esophagus also contain taste buds that are innervated by the vagus nerve (cranial nerve X). These taste buds primarily contribute to the detection of airway chemicals, bacterial secretions, and/or water (Bradley 2000; Finger et al. 2003; Tizzano et al. 2010).

3 Taste Receptor Cells

Mammalian taste buds are comprised of heterogeneous populations of 50–150 TRCs. TRCs are spindle shaped cells that project microvilli into the oral cavity through the taste pore which is a small opening at the apical end of the taste bud. Chemicals are dissolved in saliva in the oral cavity and activate the taste receptors located on the microvilli of the TRCs. Stimulated TRCs generate signals that activate the gustatory nerves which form synapses and other atypical connections with the basolateral portion of the TRCs. The gustatory nerves transmit the signals to the central taste system (Fig. 2) (Lindemann 2001; Roper and Chaudhari 2017).

The primary function of TRCs is to detect and translate a chemical signal from the external environment into a signal that is then sent to the brain. Because these chemicals have diverse structures, TRCs have evolved multiple signaling pathways to detect and respond to multiple types of compounds. TRCs are grouped into different cell types called basal cells, Type I, Type II, and Type III cells. These cell types differ both in their morphology and function within the taste bud. Because TRCs are in direct contact with the external environment, they are routinely replaced throughout an organism's lifetime, approximately every 10–14 days (Farbman

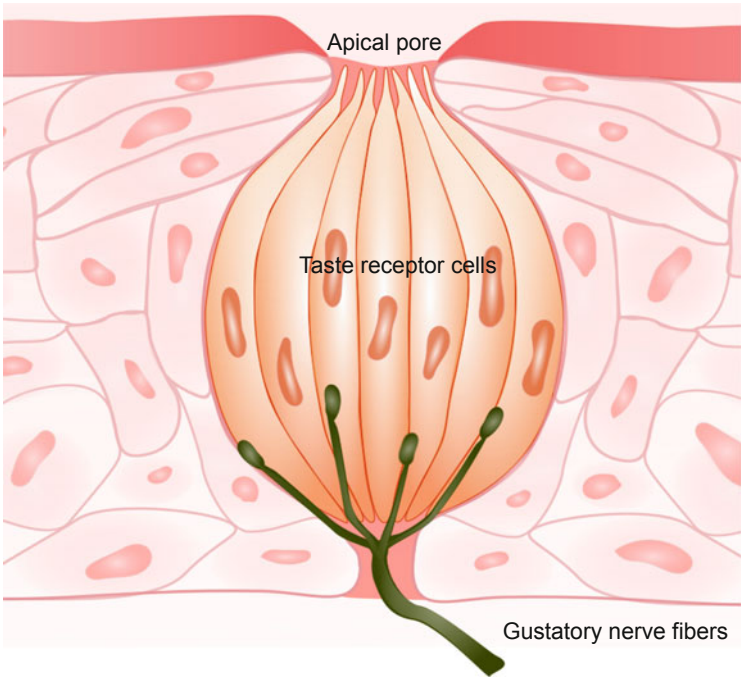

Fig. 2 A cross-sectional view of a mammalian taste bud. Individual taste buds consist of 50–150 taste receptor cells which extend their cilia into the oral cavity through the apical pore of the bud. Individual taste cells are innervated by gustatory nerve endings that transmit the taste signal to the brain

1980). The basal cells are the progenitor cells that give rise to new TRCs and are located in the basolateral portion of each taste bud (Miura and Barlow 2010). The other cell types are the signal transducers (Type II and III) or function to maintain an appropriate signaling environment within the taste bud (Type I). After a brief description of the cell types, we will discuss the current understanding of the signaling pathways that are used by TRCs to translate chemical signals into the output signals that are sent to the brain.

4 Type I TRCs

Type I TRCs are the most abundant taste cell type in taste buds and are thought to primarily serve as support cells for other TRCs (Murray 1993). Type I cells have extensive cellular processes that tend to wrap around other TRCs (Royer and Kinnamon 1988; Pumplin et al. 1997) and express NTPDase2 (Ectonucleoside Triphosphate Diphosphohydrolase 2) and GLAST-1 (Glutamate Aspartate Transporter 1) which hydrolyze ATP and glutamate, respectively (Lawton et al. 2000; Bartel et al. 2006). Since both ATP and glutamate act as neurotransmitters in TRCs (Finger et al. 2005; Vandenbeuch and Kinnamon 2016), it is thought that Type I cells play a role in neurotransmitter clearance within the taste bud. Type I TRCs in Fun papillae can respond to salt stimuli (Vandenbeuch et al. 2008; Baumer-Harrison et al. 2020), so it is possible these TRCs contribute to salt transduction, but further work is needed to confirm this initial finding.

5 Type II TRCs

Type II TRCs express the identified receptors for bitter, sweet, and umami stimuli and have well-established roles in the detection of these stimuli (Adler et al. 2000; Chandrashekar et al. 2000; Nelson et al. 2001, 2002; Zhang et al. 2003; Zhao et al. 2003). When bound by ligand, the taste GPCRs activate a phospholipase C (PLC) signaling cascade to cause neurotransmitter release (Zhang et al. 2003). Interestingly, these TRCs lack conventional synapses and use a novel mechanism to communicate to the gustatory nerve (Taruno et al. 2013a, b; Ma et al. 2018; Romanov et al. 2018). These cells can also respond to aversive salt stimuli (high concentrations), though the signaling mechanism is not clear (Oka et al. 2013; Tordoff et al. 2014; Roebber et al. 2019; Larson et al. 2020). Type II taste cells are narrowly tuned and individual cells generally respond to a single bitter, sweet, or umami stimulus (Zhang et al. 2003; Zhao et al. 2003; Mueller et al. 2005). Loss of these cells results in significantly reduced bitter, sweet, and umami taste (Matsumoto et al. 2011; Larson et al. 2020), confirming their importance in detecting these taste stimuli.

6 Type III TRCs

Unlike Type II TRCs, Type III TRCs form conventional chemical synapses with afferent gustatory nerve fibers. They express neuronal proteins such as synaptosomal-associated protein 25 (SNAP-25) (Yang et al. 2000; Clapp et al. 2006) and neural cell adhesion molecule (NCAM) (Nelson and Finger 1993), and are the only TRCs to express voltage-gated calcium channels (VGCCs) (Medler et al. 2003; Roberts et al. 2009; Rebello et al. 2013). These cells detect sour stimuli (Richter et al. 2003; Huang et al. 2006; Tu et al. 2018; Teng et al. 2019; Zhang et al. 2019) as well as some salt (Oka et al. 2013; Lewandowski et al. 2016).

7 Broadly Responsive (BR) Taste Cells

Earlier studies reported that some TRCs can be broadly tuned (Kimura and Beidler 1961; Ozeki 1971; Ozeki and Sato 1972; Sato and Beidler 1982; Tonosaki and Funakoshi 1984; Sato and Beidler 1997; Gilbertson et al. 2001; Caicedo et al. 2002; Tomchik et al. 2007; Yoshida et al. 2009b), but to date this idea has not gained wide acceptance, instead some suggest that all TRCs are selectively responsive to a single stimuli (Yarmolinsky et al. 2009). This may, in part, be due to the lack of characterization of individual TRCs that are broadly tuned. These earlier studies were performed in intact taste buds and some concluded that these broadly tuned cells likely receive input signals from neighboring cells (Tomchik et al. 2007). However, a recent study has shown that individual TRCs can be broadly responsive and transmit bitter, sweet, and umami taste signals using a PLCβ3 signaling pathway (Dutta Banik et al. 2020). These broadly responsive cells are a subset of Type III cells and respond to sour as well as bitter, sweet, and umami stimuli. The presence of these broadly responsive TRCs suggests a level of complexity within taste signaling that to date has not been characterized.

8 Transduction Pathways in TRCs

Taste transduction involves the binding of the stimulus to either G protein-coupled receptors (GPCRs) or ion channels present on TRCs to initiate a signaling event that culminates in the generation of an output signal that is sent to the brain. Due to the complex structure of many bitter, sweet, and umami chemicals, these stimuli are thought to primarily activate GPCRs which are able to bind to larger and/or more complex ligands. The interactions between tastants and their receptors are of low affinity compared to other GPCRs, with binding affinities in the high μM to mM range so they are not activated unless a high concentration of stimulus is present. To date, two classes of taste GPCRs have been identified, T1Rs and T2Rs. T1Rs mediate sweet and umami taste, whereas T2Rs detect bitter stimuli. In contrast, salt and sour stimuli interact with and activate ion channels. Identifying these

channels has been somewhat challenging and several candidate receptors have been proposed for both salt and sour transduction.

9 GPCR Signaling

Long before the identification of the GPCR taste receptors, a taste specific G protein, Gα-gustducin, was identified (McLaughlin et al. 1992) that is required for the normal detection of bitter, sweet, and umami stimuli (Wong et al. 1996a, b; Ruiz-Avila et al. 2001; Caicedo et al. 2003; Ruiz et al. 2003; Glendinning et al. 2005). When gustducin is absent, taste responses to all three of these stimulus types are severely compromised, however, the physiological role of gustducin is still unclear. Gustducin is very similar to transducin which activates a phosphodiesterase (PDE) in photoreceptor cells to reduce cytosolic cGMP levels (Fung 1983; Fung and Nash 1983; Manning and Gilman 1983; Stryer 1983; Stryer et al. 1983; McLaughlin et al. 1992, 1993; Spickofsky et al. 1994) and can activate a PDE in vitro (Wong et al. 1996b) supporting the idea that gustducin regulates cyclic nucleotide levels in TRCs. What is not clear, however, is how cyclic nucleotides contribute to taste signaling. One study using gustducin knockout mice found that basal cAMP levels were higher when gustducin was absent, indicating that gustducin is tonically active. The authors suggest that gustducin normally functions to activate a PDE to keep cytosolic cAMP levels low in order to reduce protein kinase A (PKA) activity. When gustducin is absent, PKA phosphorylates key signaling proteins in the phospholipase C pathway to inhibit their activity (Clapp et al. 2008).

While gustducin appears to have a key role in regulating the signaling environment, these events are not the primary transduction pathway used to transmit taste information in Type II cells. Type II cells use a common signaling pathway to transduce bitter, sweet, and umami stimuli. The heterotrimeric G protein complex in TRCs contains specific beta-gamma (βγ) subunits (β3γ13) which activate phospholipase C β2 (PLCβ2) (Huang et al. 1999; Rossler et al. 2000; Miyoshi et al. 2001). PLCβ2 cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the second messenger 1,4,5-inositol triphosphate (IP_3). IP_3 binds to the IP_3 receptor type III (IP_3R3) and causes calcium (Ca^{2+}) release from the internal stores to activate transient receptor potential melastatin family members 4 and 5 (TRPM4 and TRPM5) (Zhang et al. 2007; Dutta Banik et al. 2018). Sodium influx through TRPM4 and TRPM5 depolarizes the membrane and causes ATP release through the Ca^{2+} homeostasis modulator 1/Ca^{2+} homeostasis modulator 3 (Calhm1/3) complex (Taruno et al. 2013b; Ma et al. 2018). Type II cells do not have conventional chemical synapses, but instead use this unique synaptic mechanism to release ATP onto the gustatory nerve (Finger et al. 2005; Romanov et al. 2018). The specificity of the response is controlled by the taste receptor that activates the signaling pathway (Zhang et al. 2003) (Fig. 3a).

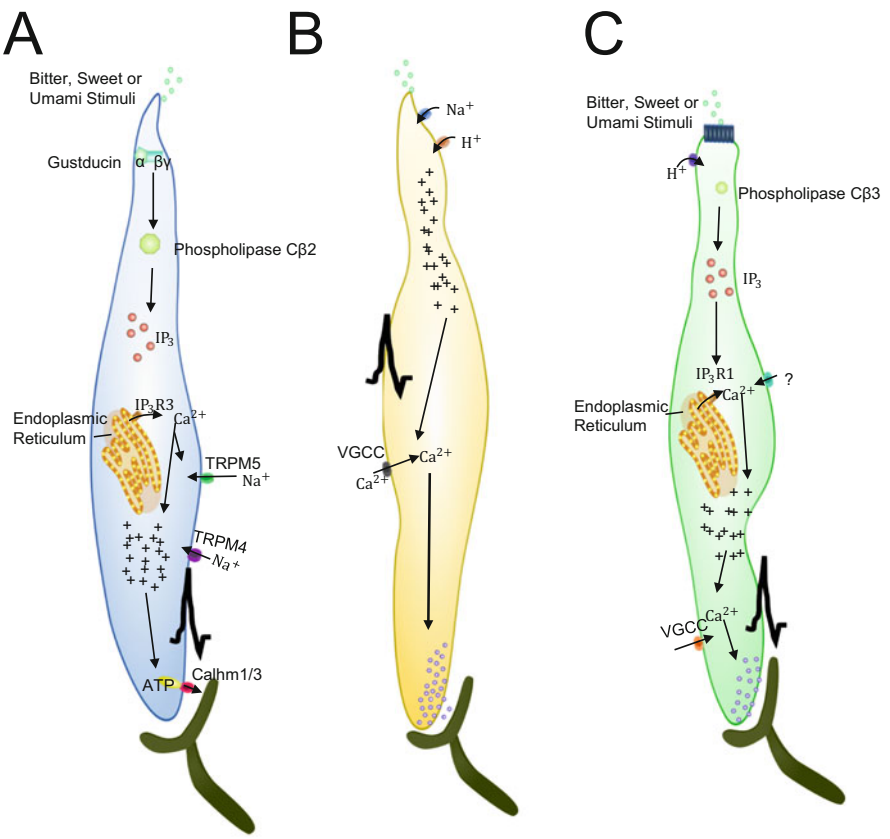

Fig. 3 Signaling pathways in different taste cell types. (**a**) The current signaling model in Type II TRCs. Bitter, sweet, or umami stimuli bind to taste receptors to activate a PLCβ2/IP_3R3 signaling pathway which causes calcium release from the internal stores. This calcium activates sodium-selective TRP channels TRPM4 and TRPM5. The sodium influx through these channels depolarizes the cell to fire an action potential. This opens Calhm1/Calhm3 channels to cause release of the neurotransmitter ATP in a non-vesicular manner. (**b**) The current signaling model in Type III TRCs. Sour and salty stimuli enter the cell through ionotropic receptors which causes a depolarization. This cell depolarization causes firing of an action potential which activates voltage-gated calcium channels (VGCCs) to cause vesicular release of neurotransmitter. (**c**) The current signaling model in Broadly Responsive (BR) cells. Bitter, sweet, and/or umami stimuli activate a PLCβ3/IP_3R1 signaling pathway in a subpopulation of Type III cells. The G proteins in this pathway have not been identified. The downstream events of this signaling pathway are still unknown but it is postulated that this signaling pathway depolarizes the cell to activate calcium influx through VGCCs to cause neurotransmitter release. Further studies are needed to confirm this hypothesis

10 Bitter Taste Receptors

The family of GPCRs called T2Rs, encoded by the Tas2R gene family (Adler et al. 2000; Chandrashekar et al. 2000) are bitter taste receptors. While there are more than 40 identified Tas2R genes in mice, only 30 of those genes encode functional proteins which are thought to detect all bitter compounds (Bachmanov et al. 2014). This family of GPCRs are diverse in their structure, individual receptors share 30–70% amino acid identity (Adler et al. 2000) which likely reflects the needs of these receptors to identify structurally diverse bitter ligands. Some of these receptors are very specific to particular compounds, while others are broadly tuned and respond to multiple bitter stimuli (Meyerhof et al. 2010; Behrens and Meyerhof 2018).

While it is generally accepted that all bitter taste is mediated by T2Rs through the PLCβ2 signaling pathway (Zhang et al. 2003), there is data to suggest that specific bitter compounds can activate other signaling pathways (Dotson et al. 2005; Damak et al. 2006). Nicotine, a bitter compound, binds to the nicotinic acetylcholine receptor (nAchR) in the TRCs to generate an intracellular response (Oliveira-Maia et al. 2009), while denatonium, another bitter compound, can directly modulate ion channels in addition to activating T2Rs (Sawano et al. 2005). Caffeine activates the adenosine receptor in TRCs but can also directly modulate inositol triphosphate receptor type 3 (IP_3R3) and ryanodine receptor (RyR) activity to generate a cellular response (Maes et al. 1999; Guerreiro et al. 2011; Gees et al. 2014; Mustard 2014; Poole and Tordoff 2017). Thus, while T2Rs are important bitter receptors to generate a cellular response to bitter compounds, a variety of pathways appear to contribute to the detection of bitter.

11 Sweet and Umami Taste Receptors

The second known family of taste receptors is the T1Rs which consist of three genes: TasR1, Tas1R2, and Tas1R3 (Nelson et al. 2001, 2002; Montmayeur and Matsunami 2002; Zhao et al. 2003). All members of Tas1R gene family are GPCRs and form obligate heterodimers to transduce either sweet or umami stimuli. Tas1R3 encodes the T1R3 protein which is the common member for both sweet and umami receptors. T1R3 heterodimerizes with T1R2 to form the sweet receptor, which is activated by sugars, D-amino acids, artificial sweeteners, and some sweet proteins (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Li et al. 2002). Separately, T1R3 heterodimerizes with T1R1 to form the umami receptor (Nelson et al. 2001; Li et al. 2002). While some studies have concluded that these receptors detect all sweet and umami stimuli (Adler et al. 2000; Chandrashekar et al. 2000; Nelson et al. 2001, 2002), the loss of T1R3 does not abolish all sweet and umami responses (Damak et al. 2003; Delay et al. 2006; Ohkuri et al. 2009; Zukerman et al. 2009), indicating that additional signaling mechanisms likely exist.

The T1R2 + T1R3 dimer is thought to couple to the PLCβ2 signaling pathway to cause Ca^{2+} release in response to sweet stimuli (Zhang et al. 2003). One study reported that T1R2 does not co-localize with gustducin, suggesting that gustducin is

not a critical component of this pathway (Hoon et al. 1999). Conversely, a separate study found some gustducin co-expression with T1R2 positive TRCs (Kim et al. 2003). This co-localization varied across the different papillae types but suggests that gustducin could be linked to the T1R2 + T1R3 receptor in some taste cells. Since gustducin null mice have severely reduced sweet taste (Ruiz-Avila et al. 2001; Danilova et al. 2006), it is possible that sweet taste does not depend entirely on the T1R2 pathway. Earlier studies demonstrated that cyclic nucleotides have a role in sweet taste transduction (Striem et al. 1989; Naim et al. 1991; Cummings et al. 1993, 1996; Nakashima and Ninomiya 1999; Krizhanovsky et al. 2000; Trubey et al. 2006) which agrees with the need for gustducin signaling in sweet taste. However, little research has followed up on this question and the specifics of this pathway in TRCs, including the receptor that activates cyclic nucleotide signaling in TRCs are unclear. Interestingly, glucose transporters have been implicated in detecting sweet stimuli (Yee et al. 2011; Yasumatsu et al. 2020), and one study suggests that sweeteners directly regulate cell responses by reducing a potassium current (Cummings et al. 1996); however, how these signaling events relate to gustducin activity or cyclic nucleotides is unknown. Thus, it is likely that multiple pathways are used to detect sweet stimuli, but further studies are needed to characterize these signaling events.

There is also evidence that the T1R1 + T1R3 heterodimer is not the only receptor that detects umami stimuli (Delay et al. 2006, 2009; Eddy et al. 2017). Metabotropic glutamate receptors, mGluR1 and mGluR4 have been proposed as possible umami receptors (Toyono et al. 2003; San Gabriel et al. 2005, 2009; Maruyama et al. 2006; Nakashima et al. 2012; Yasumatsu et al. 2014; Pal Choudhuri et al. 2016). mGluR4 is expressed in taste cells and mGluR4 agonists generate behavioral and neural responses that are comparable to umami stimuli (Chaudhari et al. 1996; Chaudhari and Roper 1998; Yasumatsu et al. 2014). Further analysis revealed that the mGluR4 has a truncated extracellular N-terminal domain that contains the glutamate binding site which requires a much higher glutamate concentration to be activated. This truncated N-terminal domain likely evolved as an adaptation to only allow receptor activation by the high glutamate concentrations that occur in food (Chaudhari et al. 2000). Likewise, a truncated mGluR1 receptor has also been implicated in transmitting umami stimuli (San Gabriel et al. 2005; Nakashima et al. 2012). At the cellular level, it is still not clear what signaling pathway is activated by these receptors or how they contribute to umami taste (Damak et al. 2003; Delay et al. 2006, 2009; Shigemura et al. 2009; Yoshida et al. 2009c; Nakashima et al. 2012; Yasumatsu et al. 2014; Eddy et al. 2017). These receptors may be expressed in distinct cell populations or they may be co-expressed within the same TRCs. Future studies are needed to address this question.

12 Ionotropic Signaling

Salt and sour tastes depend on the detection of ions, either protons in acids or cations such as Na^+, K^+, or Li^+ for salts. These ions directly interact with and activate ionotropic receptors. Activation of these channels leads to cell depolarization and

neurotransmitter release. Despite this simplicity in the overall signaling events, neither salt nor sour transduction is completely defined (Fig. 3b).

13 Salt Transduction

Salt taste involves at least two separate signaling mechanisms: one that is amiloride-sensitive (AS) and one that is amiloride-insensitive (AI). The AS channel is an epithelial sodium channel (ENaC) and is responsible for detecting lower, appetitive NaCl concentrations (<100 mM) that drive salt consumption (Avenet and Lindemann 1988; Kretz et al. 1999; Lin et al. 1999; Liu et al. 2003; Yoshida et al. 2009a; Chandrashekar et al. 2010). At higher concentrations (>300 mM NaCl), salt taste becomes aversive and recruits additional signaling pathways that are AI (Oka et al. 2013; Lewandowski et al. 2016; Roebber et al. 2019). The identity of the AI receptor is currently unknown, and it seems likely that multiple receptors/transduction pathways are involved in detecting aversive salt taste (Oka et al. 2013; Lewandowski et al. 2016).

Multiple studies investigating where the AS ENaC channel is expressed have reported that this channel is present in Type II TRCs, a subset of Type III TRCs, some Type I TRCs and/or a unique taste cell population (Vandenbeuch et al. 2008; Chandrashekar et al. 2010; Baumer-Harrison et al. 2020; Nomura et al. 2020). A recent study evaluated the expression patterns of the 3 subunits (α,β,γ) that comprise the fully functional AS ENaC channel in different taste cell types. Interestingly, they found that the α subunit was exclusively expressed in Type III TRCs while the β subunit was primarily expressed in Type I TRCs. The γ subunit was most frequently expressed in Type II TRCs with some expression in the other cell types and there was very little overlap in the expression of these different subunits (Lossow et al. 2020). This is surprising since it has been assumed that all three subunits are forming the AS ENaC channel. However, these data suggest that the AS salt response may be due to different ENaC subunit assemblies that vary by cell type. Further work is needed to the role of these ENaC subunits in AS salt taste.

Not only is the location and composition of the AS ENaC channel uncertain, the transduction events that occur after this channel is activated are also unclear. Salt stimuli have been shown to generate an internal Ca^{2+} signal within TRCs (Chandrashekar et al. 2010) but recently, it was reported that ENaC-mediated sodium influx activates a voltage-dependent neurotransmitter release through Calhm1/3 channel complex that is strictly electrical in nature and does not rely on cytosolic Ca^{2+} (Chandrashekar et al. 2010; Nomura et al. 2020). These different pathways were reported to be located in different TRC populations, which suggests that AS salt transduction events likely encompass different signaling pathways within distinct TRC populations.

Aversive salt taste is mediated through an AI channel, which to date has not been identified (Huang et al. 2006; Chandrashekar et al. 2010; Oka et al. 2013; Lewandowski et al. 2016). The molecular mechanisms responsible for aversive salt detection are also not clear. One study proposed that high salt activates bitter

pathways in Type II cells and sour signaling in Type III cells, concluding that these cell types act as general aversive detectors. However, this study did not characterize any downstream signaling events for aversive salt in these cell types (Oka et al. 2013). A separate study identified two populations of Type III cells that generated AI salt responses (Lewandowski et al. 2016). Both of these cell populations were subpopulations of sour-sensitive cells, though they differed in their sensitivity to anion size (Lewandowski et al. 2016). It has also been reported that the anion of NaCl is critical in the detection of aversive AI salt responses by Type II cells (Roebber et al. 2019). This is somewhat controversial since blocking known chloride channels in TRCs does not affect aversive salt taste (Lewandowski et al. 2016; Roebber et al. 2019) so follow-up studies are needed. Taken together, these data suggest that salt taste signaling utilizes several receptors in multiple TRC populations that activate different signaling pathways. The apparent level of complexity within salt signaling has made it challenging to understand how salt signals are transduced by TRCs. Several questions remain, including the identity of the AI channel, the subunit composition of the AS ENaC channel (or channels), as well as characterizing the downstream signaling events in the different TRC populations that are salt sensitive.

14 Sour Transduction

Understanding sour taste transduction has been challenging. To date the identity of the sour receptor is not definitive, even though several candidate channels have been proposed. Early reports postulated that acid-sensing ion channels (ASICs) were sour receptors (Ugawa et al. 1998; Lin et al. 2002), but inhibition of these channels did not affect sour responses and they are not thought to be involved in sour taste (Richter et al. 2003, 2004). The transient receptor potential (TRP) heterodimer, polycystic kidney disease 2-like 1/polycystic kidney disease 1-like 3 (PKD2L1/PKD1L3) was also proposed to be the sour receptor (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006; Kataoka et al. 2008; Ishii et al. 2009). This dimer is expressed in Type III cells which transduce sour responses (Richter et al. 2003; Huang et al. 2008; Kataoka et al. 2008; Horio et al. 2011), further supporting the idea that this dimer is the sour receptor. However, follow-up studies found that sour taste was reduced, but not abolished by the loss of PKD2L1, while the loss of PKD1L3 had no effect on sour responses (Nelson et al. 2010; Horio et al. 2011). These data suggest that this dimer is not the sour receptor but that PKD2L1 has a role in sour taste.

These earlier studies characterized channels that were activated by protons but pass other cations to cause cell depolarization. In 2010, a proton current was identified in sour-sensitive TRCs (Chang et al. 2010) which suggested that sour transduction may be due to a proton channel. Follow-up studies identified this proton channel as otopterin1 (Otop1) which directly depolarizes the cell when opened (Tu et al. 2018; Teng et al. 2019; Zhang et al. 2019). The H^+ ions also block the Kir2.1 channel to enhance cell depolarization in response to acids (Ye et al. 2016).

This cell depolarization then activates voltage-gated channels and causes vesicular release of neurotransmitter. The loss of Otop1 inhibits both the cellular and gustatory nerve responses to sour stimuli, further supporting the idea that Otop1 is the sour receptor. However, the same study reported that sour driven taste behaviors were not impaired by loss of Otop1 in TRCs (Teng et al. 2019), which suggests that Otop1 is not critical for sour taste. A separate study reported similar findings; however, they postulated that the sour responses in TRCs work in conjunction with the somatosensory system to regulate sour driven taste behaviors (Zhang et al. 2019). If they are correct, this would explain why loss of Otop1 did not impair sour taste behaviors in the Teng et al. study. Clearly more work is needed to understand how sour signals are transmitted to the brain for processing.

15 Signaling in BR Cells

BR cells are a subset of Type III cells that respond to sour stimuli but also use a PLCβ signaling pathway to respond to bitter, sweet, and umami stimuli. Unlike Type II cells, individual BR cells are broadly tuned and respond to multiple stimuli across different taste modalities (Dutta Banik et al. 2020). BR cells use a PLCβ3/IP_3R1 signaling pathway to release Ca^{2+} in response to bitter, sweet, and umami stimuli (Hacker et al. 2008; Dutta Banik et al. 2020), but there is much about this signaling pathway that is still unknown. Neither the taste receptors that activate this pathway nor the downstream signaling components have been identified in these cells, although TRPM4 expression coincides with PLCβ3 expression in Type III cells (Sukumaran et al. 2017). Since TRPM4 has been shown to be a critical downstream effector in the transduction of bitter, sweet, and umami stimuli in Type II cells (Dutta Banik et al. 2018), it may have a role in the transduction of these stimuli by BR cells. Future studies are needed to characterize the signaling events in these newly identified cells (Fig. 3c).

16 Conclusions

The need to detect diverse chemicals in potential taste stimuli has resulted in the evolution of multiple signaling pathways and receptors to detect these stimuli within TRCs. This has resulted in a complex signaling environment within taste buds that is still not well-defined. The ability to accurately detect nutrients and avoid harmful compounds is critical to survival so it is not surprising that the mechanisms used to detect environmental chemicals are widely encompassing and as a result, complex in nature. Future studies are needed to fully understand the signaling events that are used by TRCs to translate environmental chemicals into signals the brain can interpret.

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Encoding Taste: From Receptors to Perception

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Contents

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Abstract

Taste information is encoded in the gustatory nervous system much as in other sensory systems, with notable exceptions. The concept of *adequate stimulus* is common to all sensory modalities, from somatosensory to auditory, visual, and so forth. That is, sensory cells normally respond only to one particular form of stimulation, the *adequate stimulus,* such as photons (photoreceptors in the visual system), odors (olfactory sensory neurons in the olfactory system), noxious heat (nociceptors in the somatosensory system), etc. Peripheral sensory receptors transduce the stimulus into membrane potential changes transmitted to the brain in the form of trains of action potentials. How information concerning different aspects of the stimulus such as quality, intensity, and duration are encoded in the trains of action potentials is hotly debated in the field of taste. At one extreme is the notion of *labeled line/spatial coding* – information for each different taste quality (sweet, salty, sour, etc.) is transmitted along a parallel but separate series of neurons (a "line") that project to focal clusters ("spaces") of neurons in the gustatory cortex. These clusters are distinct for each taste quality. Opposing this are concepts of *population/combinatorial coding* and *temporal coding*, where taste information is encrypted by groups of neurons (circuits) and patterns of impulses within these neuronal circuits. Key to population/combinatorial and temporal coding is that impulse activity in an individual neuron does not provide unambiguous information about the taste stimulus. Only populations of neurons and their impulse firing pattern yield that information.

Keywords

Adaptation · Gustatory cortex · Sensory coding · Sensory ganglia · Taste

"Taste coding" is often interpreted to mean how the gustatory nervous system discriminates sweet, sour, bitter, salty, umami, and perhaps fat tastes (Roper and Chaudhari 2017; Running et al. 2015). However, gustatory stimuli have other properties/features that the sensory nervous system encodes, including stimulus intensity, duration, and hedonic value (or "valence," i.e., pleasant vs unpleasant). The following pages attempt to guide the reader how the gustatory nervous system processes and encodes taste signals, beginning with initial sensory transduction at the level of membrane channels and receptors on taste bud sensory cells, and progressing to higher order brain centers in the gustatory cortex. The significance of understanding how the nervous system encodes sensory information in general, and taste in particular, is highlighted, for example, by successful efforts in vision, where images can be elicited by applying electrical pulses with an appropriate "code" to the retina (Brackbill et al. 2020) (http://med.stanford.edu/artificial-retina.html), or perceived images can be decoded and reconstructed from electrical signals recorded from the visual cortex (Tripathy et al. 2021). However, perhaps most impressive are the advances in decoding CNS language circuits and signals. Here,

biomedical researchers have constructed brain–computer interfaces to restore the ability of individuals with severe speech impairments to communicate (Moses et al. 2021). All these endeavors have required a fundamental understanding of how and where the brain had encoded the sensory signals.

1 Information Coding in the Peripheral Nervous System

1.1 Exteroreceptors

Before focusing on taste, *per se*, some basic principles and common themes in the sensory nervous system are in order. The following is a brief overview of how sensory signals are received and transduced in peripheral sensory organs and transmitted to the brain (Fig. 1).

Sensory stimuli for the body's *exteroreceptors* consist of some form of external energy or external force, such as a photon (vision), or gravitational pull (balance), or the chemical energy released when an odorant binds to its receptor (smell), or a physical distortion of the cell membrane (touch), and so forth. Every sensory

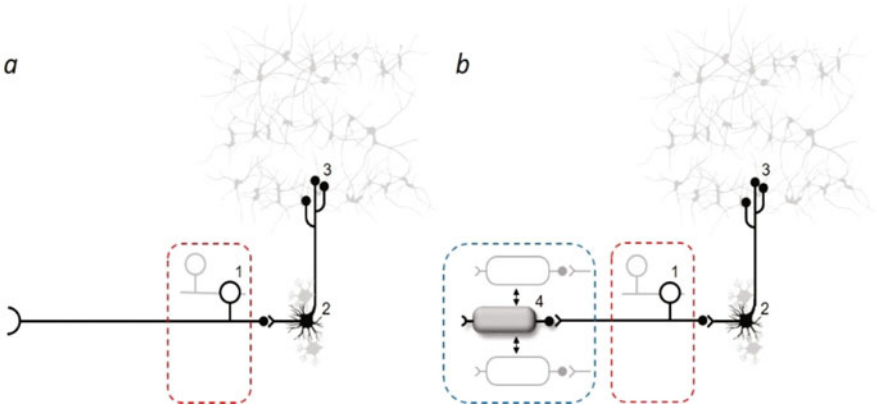

Fig. 1 Schematic diagram of sensory receptor pathways. (**a**) Drawing showing receptor cells and their central connections in the somatosensory nervous system (touch, proprioception, pain, itch). Sensory neurons (1) reside in ganglia (red dashed line) that lie alongside the spinal cord and brain. These neurons send sensory afferent fibers to the periphery (to the left). These peripheral processes express molecular receptors for the cell's *adequate stimulus*. The central processes of sensory neurons (to the right) enter the CNS and synapse with neurons in the spinal cord and hindbrain (2). These CNS neurons in turn project to higher brain centers (3). (**b**) In the sensory end organs for hearing, balance, vision, and taste (cochlea, vestibular apparatus, retina, and taste buds, respectively) (blue dashed line), the sensory cells (4) communicate synaptically with sensory ganglion neurons (1). The sensory cells may have cell–cell interactions within the peripheral end organ itself (double arrows in 4). Ganglion neurons (1) for these senses transmit signals into the CNS, as in (**a**). Olfactory sensory neurons (not depicted) are somewhat a "hybrid" of these two structures (**a**, **b**). Olfactory sensory neurons reside in the peripheral tissue (olfactory epithelium in the nasal cavity) and send their axons directly into the brain (olfactory lobe)

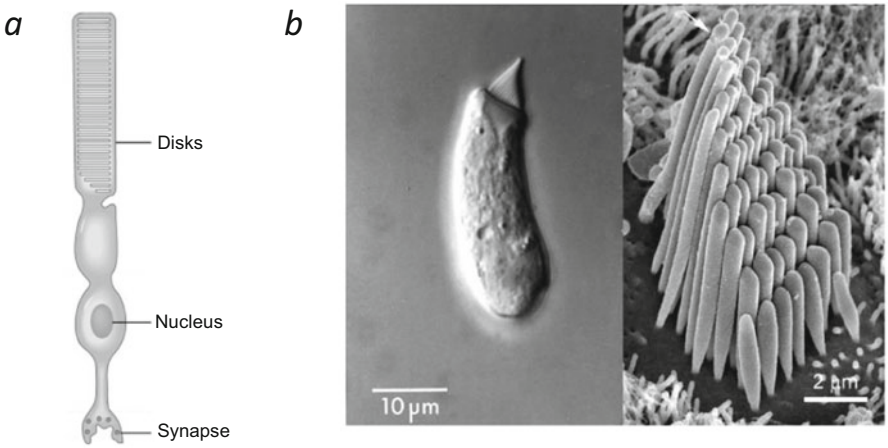

Fig. 2 Sensory receptor cells have specialized regions designed to capture their adequate stimulus. (**a**) rod photoreceptor, showing stacks of specialized structures (intracellular disks) that contain the photosensitive protein, rhodopsin. Reproduced with permission from Wikimedia (DžiugilėMED – Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=43488131). (**b**) cochlear hair cell illustrating the specialized apical stereocilia that transduce mechanical perturbations. Courtesy of J. Hudspeth, with permission

receptor cell has a specialized region where membrane proteins designed to capture a particular form of energy are embedded. For example, the distal ends of rod photoreceptors are pancaked with flattened intracellular compartments ("discs") whose membranes are rich in rhodopsin, a G protein coupled receptor protein (GPCR) that absorbs photons (Fig. 2a). Or, cochlear hair cells possess an apical tuft of specialized, elongate stereocilia that express mechanosensitive channel proteins on their tips (Fig. 2b). These mechanosensitive channels are tugged open/shut as the stereocilia on hair cells are pushed to and fro by acoustical vibrations. Thus, sensory receptor cells for each modality (vision, hearing, touch, taste, smell, etc.) are characterized by the presence of specific membrane receptor proteins located in particular regions of the cell, with these receptor proteins being "tuned" to the appropriate energy source for that modality. This energy source is often termed the *adequate stimulus*. Interactions between an adequate stimulus and its cognate membrane receptors are converted into *generator potentials* within the sensory receptor cell, described below.

In brief, the "code" for stimulus modality at the level of peripheral sensory receptor cells is the expression of appropriate membrane receptor proteins. This is under transcriptional control by the sensory receptor cell genome – i.e., the code for sensory "modality" is a genetic code.

1.2 Stimulus Intensity

The interaction between an adequate stimulus and its membrane receptor protein in most instances is not an all-or-none event. That is, apart from certain GPCRs such as rhodopsin, ligand binding/receptor activation is not believed to act in the manner of an on/off switch. Rather, as described by Kobilka and Deupi (2007), "GPCRs behave more like rheostats." Thus, encoding stimulus intensity at the level of single membrane receptor proteins, certainly for GPCRs and perhaps also for ionotropic receptors, is carried out by an increase in the receptor active state. Put simply, the stronger the stimulus energy, the more likely the receptor protein will be in an active state.

At the level of the sensory cell, an increased stimulus intensity will recruit an increased number of membrane receptor proteins, each of which contributes to a varying degree depending on its activation state. In certain cases where the adequate stimulus itself is an ion (e.g., for nociceptors, K^+ released by damaged cells), an increased stimulus intensity (increased extracellular K^+ concentration) drives a larger ion influx (larger electrical current) through membrane ion channels. There is no agonist-triggered "active state"; the relevant channels are constitutively open. However, in most cases, an increased stimulus intensity (mechanical, thermal, chemical, etc.) recruits an increasing population of membrane receptors or channels, each of which (especially for GPCRs) is in an elevated state of activity, i.e., their "rheostat" is turned up.

The net effect of stimulating receptor proteins on sensory receptor cells is to initiate a flux of ions across the receptor cell membrane (i.e., electrical current), either *directly* in the case of ionotropic receptors and ion channels (as just described above for nociceptors) or *indirectly* as a consequence of activating effector ion channels downstream of GPCRs. This ionic flux produces a change in the voltage across the peripheral sensory receptor cell membrane, termed a *generator potential* (Fig. 3).

All this is to say that the cumulative activity of activated GPCRs and their downstream ion channel effectors, or the activity of ionotropic receptors leads to a *generator potential* (sometimes called "*receptor potential*"). The generator potential is graded and encodes increased stimulus *intensity* by an increased membrane potential change; the greater the number of individual receptor events, the larger the ion flux, and hence the larger the change in membrane potential.[1]

[1] As stated above, few GPCRs act as all-or-none switches. The exceptions include rhodopsin, where all-or-none quantal receptor generator potentials have been recorded in response to a single photon (Baylor et al. 1979). Similar observations have also been in olfactory receptor neurons which are capable of responding to single odor molecules (Bhandawat 2005; Menini et al. 1995).

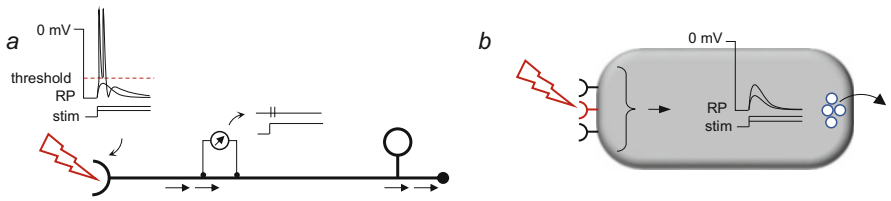

Fig. 3 Stimulus intensity and duration are encoded by the graded amplitude and variable duration of generator potentials in somatosensory ganglion neurons (**a**) and in receptor cells of sensory end organs (**b**). (**a**) increasing the stimulus (✎) strength (stim) produces larger generator potentials which, if they reach threshold, elicit action potentials (inset, above left). Note that although the stimulus might be maintained (inset), the generator potential declines, or adapts. Action potentials are propagated (inset, right) to the neuronal soma in a sensory ganglion and into the central nervous system. (**b**) similarly, increasing the stimulus strength to a receptor cell in a sensory end organ recruits additional membrane receptors and produces a larger generator potential. This graded generator potential, in turn, causes graded release of synaptic transmitter (vesicles at base of sensory receptor cell, right). Note that the generator potential here also adapts, despite a maintained stimulus

1.3 Stimulus Duration

Generally speaking, the precise *duration* of a sensory stimulus is only approximately encoded by the time course of the generator potential it produces. Passive electrical properties of sensory receptor cells (capacitance, conductance) determine how quickly the generator potential tracks the ion fluxes (transmembrane currents) initiated by the sensory stimulus. Importantly, *adaptation* limits the duration of the generator potential in most sensory receptor cells. Adaptation is the dissipation of the generator potential even as the sensory stimulus remains constant (Fig. 3, insets).

Cellular and molecular events underlying adaptation of the generator potential are complex and vary greatly among the different types of receptor cells. Adaptation of the generator potential can be produced by: (a) relaxation of the proximate stimulus due to properties of *accessory tissues* surrounding the receptor cell. For example, in mechanosensitive Pacinian corpuscles, non-sensory cells that surround and encapsulate the sensory neuron terminal dissipate/filter the physical deformations of the sensory organ (Mendelson and Lowenstein 1964). This is a classic example of how accessory tissues mediate adaptation; (b) receptor protein *desensitization* caused by intracellular biochemical processes, such as phosphorylation of GPCRs followed by binding of inhibitory, "arrestin," proteins (Gurevich and Gurevich 2020); (c) inhibition of effector ion channels downstream of GPCRs, caused by Ca^{2+} influx (Nakatani et al. 2002); and (d) actions of Ca^{2+} upon key enzymes in the transduction pathway, resulting in inhibitory feedback (Fain et al. 2001). Finally, generator potentials are shaped by the intrinsic biophysical properties of the sensory receptor cell itself, such opening/closing of membrane ion channels triggered by membrane depolarization and hyperpolarization. There is no one-size-fits-all explanation for sensory receptor cell adaptation.

In brief, the time course of a generator potential does not necessarily accurately depict the precise time course of the sensory stimulus.[2] In most instances the generator potential fades before the sensory stimulus disappears (adaptation). Interestingly, in certain sensory receptor cells, specifically nociceptors, the opposite occurs; positive feedback mechanisms can prolong and even amplify the generator potential (Woolf and Salter 2000).

1.4 Transmitting Sensory Information from the Periphery to the CNS

Once the sensory stimulus produces a generator potential in the sensory receptor cell, there are two possible outcomes for encoding this information. In *primary sensory afferent fibers* of the somatosensory system (i.e., touch, temperature, pain, itch, proprioception) and sensory receptor neurons in the olfactory system, generator potentials elicit action potentials that are propagated directly from the periphery into the CNS. By contrast, in peripheral sensory organs such as the cochlea, vestibular apparatus, retina, and taste buds, generator potentials elicit synaptic neurotransmitter release from local sensory receptor cells onto sensory neuron terminals and these (secondary) sensory neurons transmit the "code" into the CNS.

1.5 Stimulus Modality

In peripheral sensory afferent fibers, information about the sensory *modality* (i.e., touch, temperature, taste, itch, pain, etc.) is encoded by the activation of discrete neural pathways. That is, touch receptors are part of a specific neuroanatomical pathway that ultimately terminates within a particular brain cortical region and activates neuronal circuits in the CNS that are dedicated to touch; thermoreceptors participate in a separate pathway that terminates in somewhat different cortical regions and activates different neural circuits, and so forth. For the somatosensory nervous system, topographical maps of these distinct regions in the brain form miniature representations of the body projected onto the cortical surface to form an *homunculus* (Fig. 4). Other sensory systems (auditory, visual, gustatory, olfactory) have varying degrees of topographical or other systematic mapping from the periphery onto the cortical surface, from highly ordered (auditory, visual) to lack of a precise mapping (olfactory).

[2] An exception is the generator potential in cochlear hair cells (auditory sensory cells). Up to a point, the generator potential in these cells accurately tracks the time course of the stimulus. Thus, an acoustical perturbation (sinusoidal vibration) at, say, 440 Hz (a tone equal to the musical note, concert A) produces a generator potential that oscillates at 440 Hz in specific cochlear hair cells.

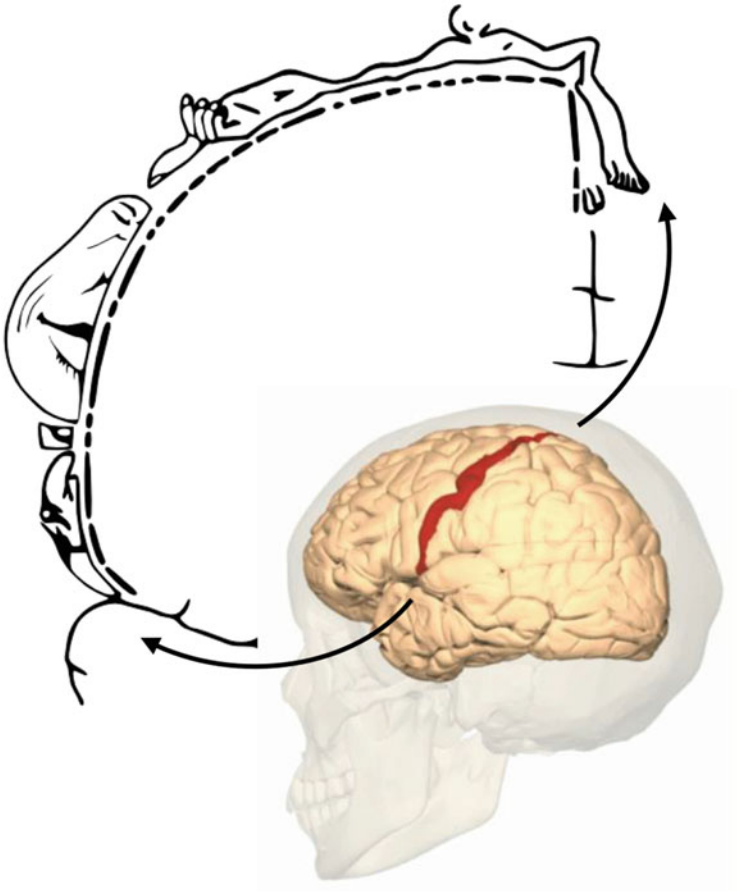

Fig. 4 The sensory homunculus. Somatosensory information is encoded as a topographical map of the body onto the primary sensory cortex (below, red). Cortical neurons here occupy an area proportional to their sensory field (receptive field) in the periphery. Thus, body regions having a dense somatosensory innervation such as the face and lips take up an enlarged cortical surface relative to less-densely innervated regions (e.g., neck, trunk). The result is a distorted topographical body map, or homunculus, on the primary sensory cortex (Penfield and Rasmussen 1950). Reproduced and modified, with permission, from https://upload.wikimedia.org/wikipedia/commons/e/e6/BA312_-_Primary_Somatosensory_Cortex_-_lateral_view.png and https://commons.wikimedia.org/wiki/File:Homunculus-ja.png

1.6 How Do Afferent Fibers Encode Intensity?

The intensity of sensory stimulation is encoded by the firing rate of action potentials in an individual sensory afferent fiber (*frequency coding*) and by the number of afferent fibers carrying action potentials (*population coding*). In brief, the more

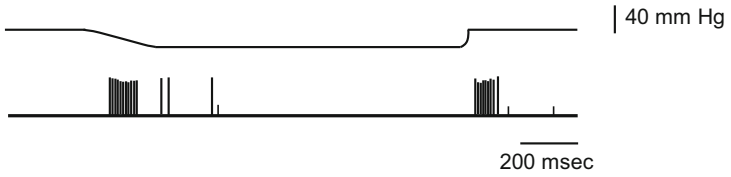

Fig. 5 Sensory afferent fiber "on response" and "off response." Action potentials in a single afferent fiber from a rapidly adapting receptor in the isolated trachea of an anesthetized cat. Upper record, tracheal pressure; lower record, action potentials. N.B. how this rapidly adapting sensory fiber encodes the onset ("on response") and offset ("off response") of stimulation, but not the entire duration. Redrawn, from Widdicombe (1954)

intense the stimulation, the higher the frequency of action potentials transmitted by a sensory afferent fiber and the greater the number of fibers activated.

1.7 Sensory Afferent Fibers Only Partially Encode Stimulus Duration

The duration of a sensory stimulus is only approximately coded by the evoked action potentials in many afferent fibers. Often there is an initial burst of activity ("on response") followed by a decrease or even cessation of impulses even in the face of a maintained stimulus, particularly for mechanosensors.[3] Adaptation of the receptor potential (above) largely explains the decline of impulse traffic in afferent fibers even when the sensory stimulus is maintained. The rate of adaptation varies among different types of sensory afferent fibers, with some being rapidly-adapting and others being slowly-adapting. Furthermore, for some afferents, particularly rapidly-adapting somatosensory afferents, there is a second burst of impulses at the offset of the stimulus ("off response") that encodes termination of stimulation (Fig. 5). Biophysical mechanisms underlying off responses in sensory afferent fibers are not well described. Presumably, off responses in mechanoreceptor sensory organs involve displacement of the surrounding tissues when the physical stimulus is withdrawn (Iggo and Ogawa 1977). Overall, adaptation of impulse activity in sensory afferent fibers limits the ability of these fibers to precisely encode the kinetics of a stimulus.[4]

[3] Exceptions include the sensory afferent fibers that innervate cochlear hair cells which fire in synchrony (phase) with the auditory tone stimulus, up to 1 to 5 kHz (Crawford and Fettiplace 1980; Rose et al. 1967), thus providing an accurate record of the stimulus "duration." In this context, "duration" means the time course of stereocilia motion on the cochlear sensory cells (inner hair cells), not how long an auditory tone is presented.

[4] Anecdotally, sensory adaptation can be recognized as how, when one enters an unfamiliar room, the initial odor fades over time (though the odorant is still present). Or, the tactile sensation from one's clothing is not a constant presence when one remains motionless. (Of course, adaptation of impulse firing in sensory afferent fibers is only a partial explanation of the complex phenomenon of perceptual adaptation and habituation).

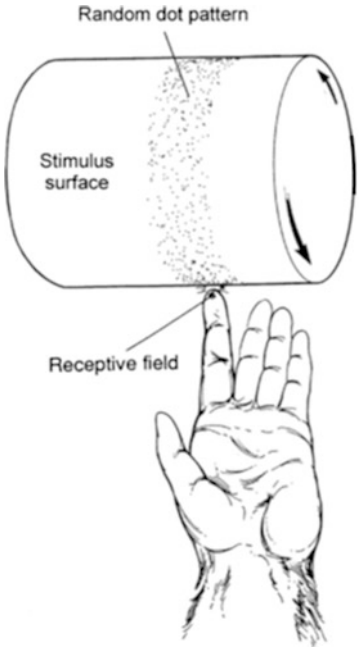

Fig. 6 Receptive fields on a monkey fingertip. The stimulus is a field of raised dots on the surface of a rotating drum. Illustrated here is a typical area on the finger where tactile stimulation would excite an underlying somatosensory receptor terminal, i.e., the terminal's receptive field. Modified from DiCarlo et al. (1998), with permission

1.8 How Do Sensory Afferent Fibers Signal the Location of Stimulation?

The location of a stimulus is encoded according to the notion of *receptive fields*. The receptive field for somatosensations (touch, temperature, pain, itch) refers to the specific surface area on the body where an afferent fiber terminates. This area is the field of skin (for exteroceptors) where the stimulus is effective (e.g., a minute area of skin on the hand for touch receptors there) (Fig. 6). Each afferent fiber has its own "personal" receptive field. Receptive fields of neighboring afferents of the same modality overlap such that adjacent fibers share portions of their receptive fields. By comparing signals coming from two or more adjacent afferent fibers, the nervous system can achieve higher spatial acuity for discriminating the position of a stimulus.

As mentioned above, somatosensory receptive fields are mapped topographically onto brain structures such as the somatosensory cortex. Every region of this cortex responds to a specific part of the body, producing a map of the body on the cortex, the "homunculus." This map is a distorted representation of the body according to the relative densities of the receptive fields. Thus, regions such as the hands and fingers that are densely populated with sensory afferents and their receptive fields command a larger cortical area relative to input from other body regions.

Comparable receptive fields and their topological mapping in vision also occur in the retina, thalamus, and primary visual cortex.

The concept of receptive fields only loosely applies to olfaction. Although olfactory sensory neurons are distributed in broad stripes or zones along the

olfactory epithelium inside the nasal cavity (Ressler et al. 1993), these are not receptive fields, *per se*. Spatial localization of odor stimuli has more to do with behavioral responses such as sniffing and turning one's head than it has to do with olfactory "receptive fields." There is no topographical map or homunculus in the brain for odors[5]; indeed, how odors, let alone their localization in space, are represented in the brain appears to be as "dispersed ensembles" of neurons without any obvious topological pattern (Stettler and Axel 2009).

In taste, gustatory receptive fields resemble somatosensory receptive fields and describe a spatial localization of the stimulus on the tongue. Indeed, localizing taste stimuli is assisted by tactile (somatosensory) receptive fields.[6] That is, a sapid stimulus (e.g., food particle, crystal of salt) is often accompanied by tactile stimulation. Nonetheless, when somatosensations are carefully controlled, human subjects can identify the location of taste stimuli on the tongue (Lim and Green 2008). There are bona fide taste receptive fields. Curiously, the tongue has higher "acuity" for some tastes (e.g., sweet, salty) compared to others (e.g., bitter) (Lim and Green 2008).

2 Information Coding in the Central Nervous System

2.1 Sensory Modalities

Every sensory modality (vision, touch, hearing, smell, taste, etc.) has specific features or qualities that can be deciphered. Thus, visual stimuli consist of different colors, shapes, and contrasts. Our somatosensory system readily discriminates light touch versus pressure versus stretch. Humans can distinguish different tones within the range of ~20 Hz to ~20 kHz. There are five or more basic tastes and perhaps more than a trillion distinctive odors (Bushdid et al. 2014, but see Gerkin and Castro 2015). How the brain identifies and discriminates among different qualities within a given modality is a major question in sensory coding.

Impulses from the peripheral sensory afferent fibers for a given modality (discussed above) synapse with specific clusters of neurons (nuclei) embedded in the spinal cord or hindbrain. Those neurons then send axons to the thalamus, a central sensory processing structure in the brain (Fig. 7). The thalamus is subdivided into different anatomical regions for each modality – vision, touch, audition, somatosensations (i.e., touch, temperature, itch, pain, others) and so forth.

[5] This is not to say there is no map at all for odors. Indeed, researchers believe there is some form of "odor map" in the olfactory bulb (Uchida et al. 2000). However, this "map" is not a representation of where an odor is located in space.

[6] Somatosensory (e.g., tactile) receptor neurons for the oral cavity comprise a quite separate neural pathway from gustatory receptor neurons. Somatosensory neurons are located in the trigeminal ganglion and enter the brain via the fifth cranial nerve, whereas gustatory sensory neurons are found in geniculate, petrosal, and nodose ganglia and enter the brain via the seventh, ninth, and tenth cranial nerves.

Fig. 7 The thalamus collects information from peripheral sensory receptors and distributes it to overlying cortical structures. The thalamus is a large, bilateral ovoid structure in the middle of the brain (dark shaded area). The overlying somatosensory cortex is shown here in green. For clarity, other sensory cortices (e.g., auditory, gustatory, visual) are not depicted. Reproduced and modified, with permission, from https://www.kenhub.com/en/library/anatomy/parietal-lobe, with permission

From the thalamus, sensory information is radiated to the appropriate, overlying primary sensory cortex (Fig. 7), distinct for each modality. For instance, visual information is routed from the retina to the thalamus and then to the primary visual cortex in the occipital lobe; auditory information from the cochlea to hindbrain, then thalamus, and on to primary auditory cortex in the temporal lobe; taste information from taste buds to the hindbrain, then thalamus, then primary gustatory cortex in the insula and operculum.

A striking exception to this general pattern of sensory nervous system organization is olfaction. Here, sensory information travels from the olfactory epithelium in the nose to its first relay in the CNS, the olfactory bulb. From the olfactory bulb, olfactory signals are transmitted directly to the primary olfactory (piriform) cortex and other CNS regions, bypassing the thalamus.

As a general principle, stimulus identity, intensity, and duration are encoded by ensembles of neurons (neural circuits) in the CNS, and not by individual neurons *per se*. Ensembles of CNS neurons can be relatively compact and highly organized, as in the cortical columns found in the somatosensory, auditory, and visual cortices (Linden and Schreiner 2003; Mountcastle 1997). Or, the neurons that process

sensory information in the CNS can be widely dispersed with no obvious anatomical relationships, as in the olfactory (piriform) cortex (Stettler and Axel 2009).

The point is that in the brain, the neural code for identifying a stimulus, its intensity, and duration is no longer a simple construct of a generator potential and series of action potentials in one neuron or one nerve fiber. Consequently, notwithstanding the tremendous advances made with single channel microelectrode recordings in the somatosensory, auditory and visual systems, to understand CNS coding of sensory signals, especially in taste and olfaction, requires technologies for recording from large ensembles of neurons simultaneously, either with multi-electrodes, high-resolution fMRI, or optical methods.

The reader is referred to any number of modern neuroscience textbooks for our current understanding of how the brain encodes sensory signals in the auditory, visual, vestibular, somatosensory, and olfactory nervous systems. Such an overview is beyond the reach of this chapter; the remainder will focus on sensory coding in the gustatory nervous system.

3 Sensory Coding in the Gustatory Nervous System: From Taste Buds to Cortex

Food and beverages contain compounds ("tastants") that either (a) bind to membrane receptors on the apical tips of taste bud cells, (b) permeate ion channels and generate ion flux across the taste cell membrane, or (c) penetrate taste bud cells and modulate the intracellular (cytoplasmic) face of membrane ion channels. The "taste code" at this initial stage of signal generation is which of these membrane proteins is/are involved, and of course, which taste bud cell expresses that receptor/ion channel.

3.1 How Do Gustatory Membrane Receptors Identify Taste Stimuli?

Sugars, artificial sweeteners, and sweet-tasting proteins are agonists for two class C GPCRs – TAS1R2 and TAS1R3 (abbreviated hereafter as T1R2 and T1R3). These GPCRs form a heterodimer having several different and somewhat independent binding sites for different sweet-tasting agonists. These sites are illustrated in Fig. 8. Thus, the "code" can be somewhat ambiguous even at the earliest stage of signal transduction. How different sweet-tasting agonists are differentiated is still not well understood, and may involve differences in duration of ligand occupancy, interactions of the ligands with receptors other than T1R2 + T1R3, and ligand interactions with sugar transporters on the taste cell surface (which themselves may contribute to conscious perceptions, although the jury is still out on this notion) (Glendinning et al. 2017; Yee et al. 2011). For example, (a) some sugars such as fructose activate T1R2 + T1R3 and elicit sweetness; (b) others such as glucose activate T1R2 + T1R3 and are taken up by taste cells (via sugar transporters) to activate K_{ATP} mechanisms and cephalic phase insulin release (CPIR) (Glendinning

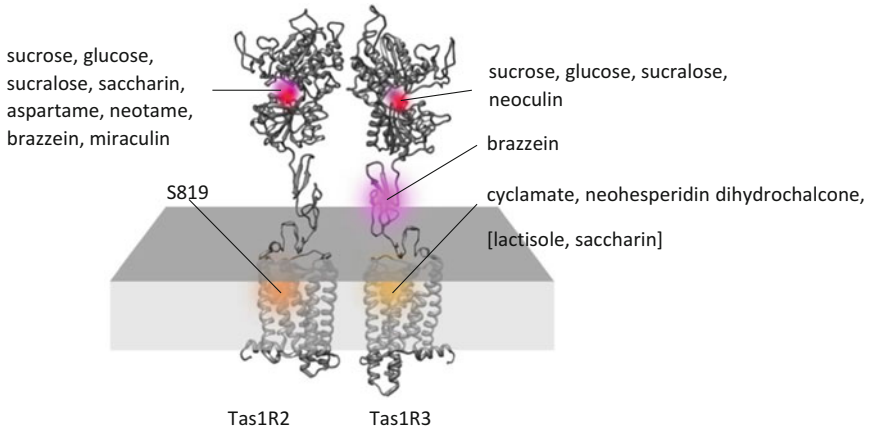

Fig. 8 The G protein-coupled sweet taste receptor heterodimer, T1R2 + T1R3, has multiple sites where ligands can bind. Approximate sites where several sweet taste compounds bind the receptor are shown. Reproduced here with permission from Roper (2020)

et al. 2017); and finally (c), some compounds (e.g., starch) elicit CPIR but without sweet taste. Yet, sugar transporters and T1R2 + T1R3 are present in the same taste cell (Yee et al. 2011). Thus, what is the taste receptor cell "code" for sweetness versus the "code" for CPIR? The signals seem to arise from the same taste bud cells.

T1R2 + T1R3 dimers are expressed by a specific set of taste bud cells belonging to the Type II category (Fig. 9). Ligand binding to T1R2 + T1R3 initiates a cascading series of intracellular events that culminates in specific downstream ion channels opening, thereby producing an inward (depolarizing) current (generator potential) and triggering action potentials (Roper and Chaudhari 2017). This represents the conversion of the chemical signal (tastant) into an electrical signal (generator potential), a key step in sensory coding, as previously discussed.

A different combination of T1R receptors, namely TASA1R1 + TAS1R3 (T1R1 + T1R3) is activated by savory ("umami") tastants (e.g., amino acids, notably glutamate). Umami tastants also activate other Class C taste GPCRs, namely truncated forms of metabotropic glutamate receptors, mGluR1 and mGluR4 (Chaudhari et al. 2000; Nakashima et al. 2012; San Gabriel et al. 2009; Yasumatsu et al. 2015). Little is known about multiple binding sites in all these umami taste receptors. However, T1R1, T1R2, and T1R3 are co-expressed in some taste bud cells, generating possible ambiguity in the encoding of sugars ("sweet") versus amino acids ("umami"). In fact, under certain conditions, rodents confuse sweet and umami tastants (Saites et al. 2015; Stapleton et al. 1999).

Bitter-tastants activate yet another family of GPCRs, named TAS2Rs (hereafter, T2Rs), with 25 members in humans to date (Meyerhof 2005). T2Rs most closely resemble Class A GPCRs (Di Pizio et al. 2016). Each T2R in this family has a ligand binding site buried in the transmembrane domain of the GPCRs. The binding pockets of the different T2Rs vary in their breadth of ligand selectivity. In

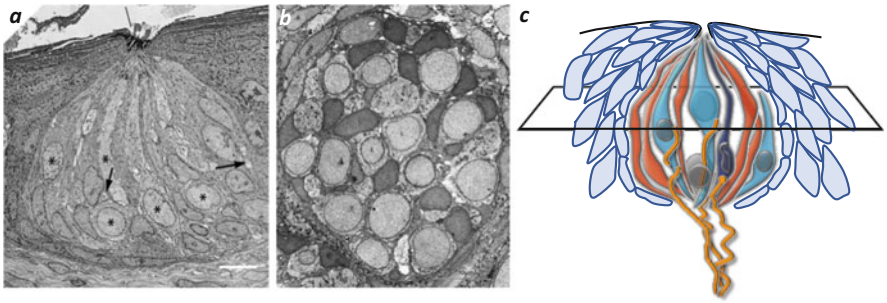

Fig. 9 Taste buds are populated by several types of cells. (**a**) Electron micrograph of a rabbit foliate taste bud showing cells with dark or light cytoplasm, and nerve profiles (arrows). Asterisks mark Type II (receptor) cells. (**b**) Electron micrograph of cross section through a rat vallate taste bud, illustrating distribution and proportion of Type I (here, "D" for dark) and Type II (here, "L" for light) cells. [In this early study it was not possible to distinguish Type III cells, which requires identifying synapses]. Note that the dark Type I cells enwrap surrounding taste cells with lamellar processes. (**c**) schematic diagram of taste bud depicting relative proportion of Type I (red), Type II (cyan), Type III (blue), and Type IV (grey) cells. Rectangle through middle of taste bud shows approximate plane of cross section in (**b**). Reproduced here with permission from Roper (2020)

experiments designed to explore the specificity of different T2R receptors, Meyerhof et al. (2010) challenged each of the family of 25 human T2Rs with a catalog of 58 natural and 46 synthetic bitter compounds. Certain of the T2Rs were rather broadly selective yet others were "tuned" to a much narrower spectrum of bitter tastants. Although one might argue that this narrow tuning of certain T2Rs is a product of the limited number ($N = 104$) of compounds tested (Palmer 2019), these studies nonetheless suggest that there are differences in the *relative* selectivity among T2Rs.

Parenthetically, recent cryo-electron microscopy and functional investigations of a unique insect odorant receptor that has broad chemical tuning, carried out on its bound and unbound states, may be illuminating here. These studies revealed that the ligand binding pocket for the odorant receptor is not a tightly organized, "lock-and-key" site shaped to fit specific chemical or molecular features of a given ligand. Rather, the pocket is a more flexible "promiscuous binding site that recognizes the overall physicochemical properties" of multiple ligands (del Mármol et al. 2021). Whether binding sites in taste T2Rs have similar features awaits comparable detailed structural analyses.

Further, individual taste bud cells express multiple different T2Rs (Behrens et al. 2007). The combination of individual T2Rs being somewhat promiscuous regarding their ligand selectivity (above), along with the fact that individual taste bud cells express multiple members of the T2R family, results in a rather broad range of bitter tastants that can activate any given bitter-sensing taste receptor cell. Indeed, when it has been tested, rodents cannot easily discriminate different bitter tastants (Spector and Kopka 2002). Nonetheless, taste bud cells in mice distinguish among different bitter compounds (Caicedo and Roper, 2001) – bitter-sensing taste bud cells do not

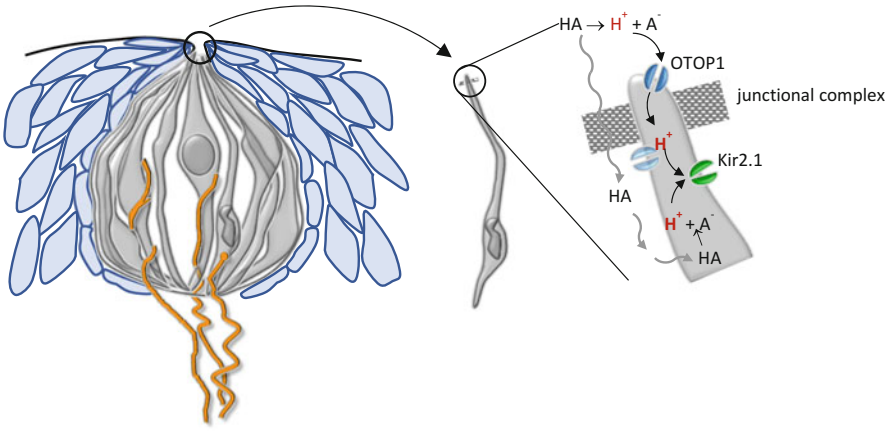

Fig. 10 Transduction pathways for sour (acid) taste in Type III taste bud cells. Protons permeate the apical tips of Type III taste cells through OTOP1 channels. This generates a small depolarizing (inward) current. Undissociated acid molecules (HA, e.g., acetic acid) penetrate apical junctional complexes (stippled bar) and permeate cell membranes to acidify the cytosol. Intracellular H^+ from HA permeation and from influx via OTOP1 bind to and block potassium channels (Kir2.1), thereby depolarizing Type III cells. Reproduced here with permission from Roper (2020)

form a uniform class that responds identically to all bitter compounds. When human subjects distinguish among bitter compounds, this likely also involves other sensory input such as olfaction and chemesthesis.

T2Rs are also expressed by Type II taste bud cells. However, the T1 and T2 classes of taste GPCRs are not often found in one and the same Type II taste bud cell. Sweet/umami taste receptors (T1Rs) and bitter taste receptors (T2Rs) only very occasionally co-localize in Type II cells (Dando et al. 2012; Sukumaran et al. 2017; Yamada et al. 2021), if at all (Adler et al. 2000).

Acids stimulate sour taste. Acid molecules (e.g., acetic acid, citric acid) permeate cell membranes and acidify the cytosol (Lyall et al. 2001; Richter et al. 2003). In Type III taste bud cells, cytosolic acidification blocks potassium channels ($K_{IR2.1}$) that establish the resting potential. By doing so, they depolarize the cell (Ye et al. 2016). Protons in solution also enter Type III cells via specific ion channels (OTOP1), generating an inward proton current and depolarizing the membrane (Teng et al. 2019; Zhang et al. 2019) (Fig. 10). These two mechanisms are specific to acidic stimuli but cannot readily discriminate among different acids. Thus, at the membrane receptor level, the code for sour taste appears to be generalized across all acids.

Far less is known about salt (NaCl) taste transduction, particularly in humans. Thus, the code for salty is still somewhat obscure at the level of membrane mechanisms (Roper and Chaudhari 2017). Intriguingly, a recent study concluded that coincident activation of both Na^+ and Cl^- receptor pathways "encodes" salt taste, reinforcing a longstanding view that sodium and chloride ions alike contribute

to the taste of NaCl (Roebber et al. 2019). Further, an important new study identified the taste cells and ion channels that contribute to salt taste preference in rodents (Nomura et al. 2020). These authors reported that NaCl-sensing cells secrete the neurotransmitter ATP via CALHM1/3 channels.

In summary, at the initial event in taste reception, gustatory signals are encoded by specific membrane receptor proteins, transporters, or ion channels for the 5 basic tastes (sweet, umami, bitter, sour, salty) and perhaps fat (Roper and Chaudhari 2017; Running et al. 2015). A small minority of taste bud cells co-express receptors for more than one taste quality.

3.2 Gustatory Stimulus Intensity

For GPCR-mediated tastes, the intensity of the stimulus is encoded by the number of receptors occupied, which in turn determines the number of downstream effectors activated and thus the amplitude of the eventual membrane depolarization (generator potential). Similarly, for tastes transduced by ion channels, the stimulus intensity (tastant concentration) is encoded by the magnitude of the ionic flux across the membrane, and in the case of acid stimuli, by the proportion of $K_{IR2.1}$ channels that are blocked by intracellular acidification.

3.3 Gustatory Stimulus Duration

Unlike other senses, and especially hearing, precise timing of the stimulus signal is not as critical in taste. Although under certain experimental conditions, rats can recognize a tastant within 250 ms of stimulation (Halpern and Tapper 1971), in most situations, taste response latency and duration are quite variable. For instance, onset of the stimulus depends on several factors, such as enzymatic digestion in the oral cavity (e.g., lipase-mediated release of fatty acids from triglycerides), mastication, solubilization of food chemicals, and so forth. Further, some compounds, such as quinine, are lipophilic and remain active long after the initial stimulus has been rinsed away. Lastly, little information is available on taste GPCR desensitization or inactivation. In short, apart from its obviously important role in food science and for understanding lingering tastes, little attention has been paid to molecular mechanisms of stimulus duration and adaptation in taste.

3.4 How Do Taste Bud Receptor Cells Discriminate Gustatory Stimuli?

Considerable controversy surrounds taste coding at the level of receptor cells and their connections with the CNS. At one extreme, proponents of *labeled line coding* propose that individual taste bud cells are "tuned" to specific qualities (sweet, salty, sour, etc.) and transmit these signals to the brain via dedicated primary afferent fibers

(i.e., the peripheral processes of gustatory ganglion neurons in the petrosal and geniculate ganglia) (Barretto et al. 2015; Yarmolinsky et al. 2009). Countering this, proponents of *combinatorial/population coding* propose that the identity of taste quality emerges from signal processing among taste bud cells and multiple connections with primary afferent sensory fibers (Erickson 1963; Ohla et al. 2019; Wu et al. 2015).

It is generally accepted that certain cells in the taste bud, namely Type II taste cells, express specific GPCR taste receptors and are tuned to respond either to sweet, bitter, or umami taste stimuli (see above). Nonetheless, as state previously, there is some "noise" in the expression of taste-specific GPCRs. Although it is not pronounced, single cell analyses have shown mouse Type II taste bud cells can express multiple diverse taste GPCRs (i.e., sweet, umami, and bitter) (Dando et al. 2012; Sukumaran et al. 2017; Yamada et al. 2021). Further, recordings from mouse taste buds show that although most Type II taste bud cells are tuned to one tastant, some respond to two or more tastes (i.e., they are broadly tuned) (Tomchik et al. 2007; Yoshida et al. 2009). Importantly, those same studies showed that although *isolated* Type III taste bud cells may specifically be tuned to acid taste stimuli, *in intact taste buds* Type III taste cells respond to several (Type II cell) taste stimuli, in addition to sour. These data have led to the postulate that there is cell–cell communication between taste bud cells (specifically, between Types II and III cells) (Roper 2021; Roper and Chaudhari 2018; Tomchik et al. 2007) (Fig. 11). In sum, the notion that individual gustatory cells are strictly dedicated to a single taste stimulus (i.e., the basis for labeled line coding) is questionable even at the level of the taste bud.

Parenthetically, the notion of multiply-responsive, broadly tuned taste bud cells is linked to the concept of entropy and the information content in transmitted signals. The concept of entropy in signal transmission is derived on the classic studies of Shannon and Weaver (1949). Taste researchers quantify the breadth of tuning in taste bud receptor cells and taste neurons in terms of H, or signal *entropy*. Quantification of entropy in studies on taste yields H values that vary between 0 and 1 (Smith and Travers 1979). The greater the number of tastants to which of a cell responds (i.e., the greater the breadth of tuning), the greater the entropy and the higher the value of H (up to a max of 1.0). Conversely, a cell that is tuned to a single taste compound has no entropy and $H = 0$. Counterintuitively, in signal transmission, the greater the entropy, the greater the information content in the signal (e.g., see https://machinelearningmastery.com/what-is-information-entropy/). That is, a cell "tuned" to a single taste quality (i.e., $H = 0$) encodes less information than a neuron that responds to multiple taste stimuli ($H > 0$). Tomchik et al. (2007) reported that the average H for mouse Type II taste bud cells was low, 0.07; Yoshida et al. (2009) also found entropy was similarly low in mouse Type II taste cells, mean $H = 0.09$. These findings are both consistent with Type II cells being relatively narrowly (but not completely) tuned to single taste qualities. By contrast, Type III taste cells have much higher entropy, mean $H = 0.12$ (Yoshida et al. 2009) to 0.47 (Tomchik et al. 2007) in the mouse.

In summary, Type II taste bud cells fairly accurately, but not without some ambiguity or "noise," encode sweet, bitter, salty, or umami taste stimuli. These

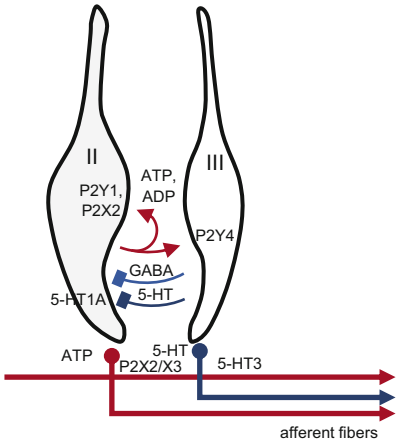

Fig. 11 Schematic diagram summarizing feedforward and feedback signaling in mammalian taste buds. The diagram shows cell–cell interactions between Type II and Type III taste bud cells. Type II cells express G protein–coupled taste receptors for sweet, bitter, or umami taste compounds. Taste stimulation evokes adenosine triphosphate (ATP) secretion from Type II cells. ATP excites (a) gustatory primary afferent fibers (shown at bottom), (b) neighboring Type III taste bud cells, and (c) via autocrine feedback, Type II cells, as shown here in red. ATP released during taste stimulation is degraded to ADP and adenosine (Ado), both of which, along with ATP, serve as autocrine positive feedback signals. Type III cells make synaptic contacts with nerve fibers and secrete serotonin (5-HT) and norepinephrine (not shown). Type III cells also release γ-aminobutyric acid (GABA) when stimulated by acids (sour tastants). GABA and 5-HT from Type III cells, shown here in blue, inhibit Type II cells. Receptors for ATP, adenosine diphosphate (ADP), adenosine, GABA, and 5-HT are identified at their respective target sites. Reproduced here with permission from Roper and Chaudhari (2018)

cells are not all tuned to a single taste stimulus. Type III taste bud cells encode acid tastants as well as other taste stimuli and consequently respond more broadly to multiple tastes. Cell–cell communication between Types II and III taste bud cells may underlie the ability of Type III cells to encode multiple taste stimuli. Thus, a strict labeled line does not accurately describe taste coding at the level of the taste bud. More realistically, it is likely that while some taste cells respond only to one taste quality and appear to be "labeled," others have a dominant, though not exclusive, tuning. Thus, a given prototypic basic taste stimulus (sweet, salty, sour, bitter, or umami) activates an ensemble of taste cells, some highly tuned to that taste and others less so, with the combinatorial effect being to signal the dominant taste.

3.5 Stimulus Intensity and Duration Coding in Taste Bud Receptor Cells

Where it has been measured, the concentration/response relationship for taste stimuli in taste bud receptor cells shows a steep, monotonically increasing plot that fits a conventional sigmoidal ligand binding curve (Caicedo et al. 2002; Caicedo and

Roper 2001; Roebber et al. 2019). Intensity appears to be encoded at the level of taste cells simply as a function of the amplitude of the response (e.g., generator potential).

Measurements of response duration in taste bud cells using taste-evoked $\Delta[Ca^{2+}]_{intracellular}$ as a surrogate for generator potentials approximately track the time course of brief presentations of taste stimuli, although this has not been examined extensively. Where it has been tested, NaCl-evoked responses display marked adaptation to prolonged stimulation (Caicedo et al. 2002; Roebber et al. 2019).

3.6 Do Gustatory Sensory Ganglion Neurons Encode Taste?

Taste bud receptor cells communicate with the peripheral processes (sensory afferent terminals) of gustatory sensory neurons located in the geniculate, petrosal, and nodose ganglia and transmit their signals to these processes for propagation into the hindbrain. Although labeled line coding had long been discussed as one possibility for taste coding in these gustatory neurons, this notion was renewed and given new impetus by in vivo functional imaging studies carried out by Barretto et al. (2015) in mice. These investigators stimulated the animal's tongue with sapid solutions and recorded geniculate ganglion neuron activity. They reported that 2/3 of the sensory neurons in the ganglion were tuned to only one of the five basic taste stimuli (i.e., had low entropy, $H = 0$). On this basis, they concluded that the coding of taste signals transmitted from taste buds was via labeled lines. Perhaps supporting this notion were earlier findings on human subjects carried out by Von Bekesy (1964, 1966). Von Bekesy (ibid.) reported that stimulating single taste buds electrically with a fine metal probe, or chemically with microscopic droplets of taste solutions elicited singular taste qualities (sweet, salty, bitter, or sour). Subsequent work by Mueller et al. (2005) purported to test this concept by genetically engineering mice in which taste bud cells expressing T1R2 sweet taste receptors were redirected to express T2R16 bitter receptors. The notion was that if T1R-expressing taste bud cells synapse with dedicated afferent sensory terminals and form a "labeled line" for sweet, then a T2R16 ligand (phenyl-β, PTC-D-glucopyranoside, PTC) which normally is "bitter" should elicit "sweet" in the genetically engineered mice. Indeed, transgenic mice preferred the bitter compound PTC in marked contrast to wild type mice, reinforcing their premise. The authors concluded "Together, these results substantiate the coding of both sweet and bitter pathways by dedicated (that is, labeled) lines" (Mueller et al. 2005).

Yet, there is strong evidence that labeled line coding in gustatory sensory neurons is insufficient. Notably, ever since recordings were made from single afferent gustatory sensory fibers, researchers have noted that individual afferent nerve fibers often responded to multiple (different) taste stimuli (i.e., entropy, $H > 0$). This is antithetical to a dedicated labeled line coding for taste quality. Frank (1973) attempted to resolve the problem by classifying sensory afferent fiber responses as "taste-best." That is, a single gustatory afferent fiber may respond to multiple tastants, but robustly only to one taste compound and less strongly to other(s). For

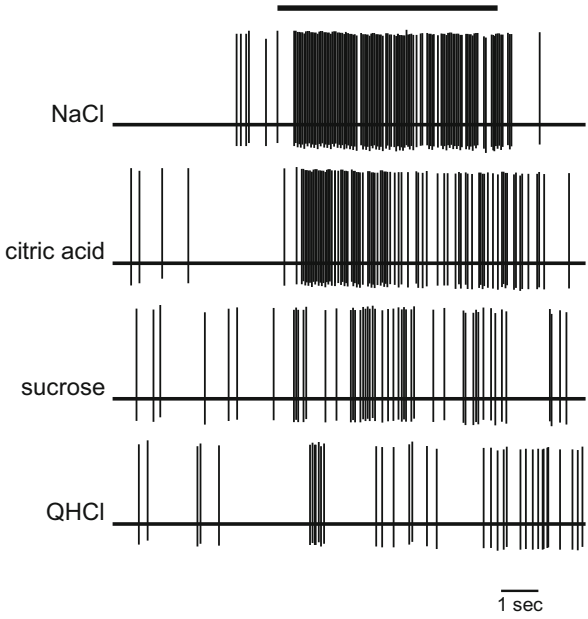

Fig. 12 Individual afferent fibers that innervate taste buds often show stronger responses to one tastant – the "taste best" response. Shown here are sequential recordings from a NaCl-best chorda tympani fiber from a rhesus monkey when the tongue was stimulated with NaCl, citric acid, sucrose, and quinine hydrochloride (QHCl). Bar at top indicates stimulation interval. Redrawn, from Hellekant et al. (1997a)

instance, there were "sucrose-best," "citric acid-best," or "NaCl-best" single fibers, and so forth (Fig. 12). Secondary, weaker responses were considered as "noise" or "side-band." Thus, a "sucrose-best" fiber would still be considered a dedicated (labeled) line for sweet.

Complicating the matter, however, is that the strength of responses, as stated above, is a function of the stimulus concentration. Thus, this modification of "labeled line" coding is unsatisfactory because activity in a single fiber, taken individually, could not be decoded to discriminate between two (or more) taste qualities, let alone between taste intensities. As an example, consider a "sucrose-best" fiber. Intense stimulation (high concentration) by a "side-band", non-sweet tastant could activate a "sucrose-best" fiber as strongly as a low concentration of sucrose. What information would this fiber, taken individually, thus convey? Importantly, in vivo functional imaging of geniculate ganglion neurons by Wu et al. (2015) conducted in parallel with and published shortly after Barretto et al. (2015), obtained results that contradicted Barretto et al. (2015) and provided further evidence for combinatorial/population coding of peripheral taste signals. Namely, Wu et al. (2015) (and replicated independently by Leijon et al. 2019) reported that about half the gustatory ganglion neurons were selectively responsive to a single quality; the remaining half responded to multiple tastants ($H > 0$) (Fig. 13). Most importantly, the proportion of selectively tuned ganglion neurons depended on the stimulus concentration. Wu et al. (2015) found that with increasing taste stimulus concentration, neurons became increasingly more broadly tuned, that is, responsive to multiple taste qualities. This echoed the findings obtained from single fiber recordings of hamster taste nerves obtained years earlier (Hanamori et al. 1988).

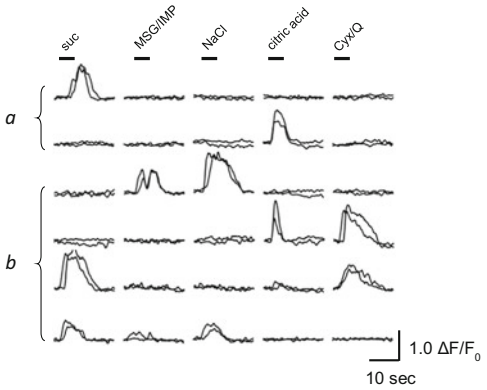

Fig. 13 Sensory ganglion neurons that innervate taste buds respond to single or multiple taste stimuli. Representative examples of Ca^{2+} imaging (GCaMP3) signals recorded from mouse geniculate ganglion neurons in response to prototypic sweet, umami, salty, sour, and bitter taste stimuli. Responses from six different neurons from two mice are shown. The panel of taste stimuli (top) was presented twice in succession. (**a**) these neurons responded only to one taste stimulus (sucrose, citric acid). (**b**) these neurons responded to two or more taste stimuli. Stimuli were sucrose (suc), 300 mM; MSG, 100 mM (with 1 mM IMP); NaCl, 250 mM; citric acid, 10 mM; cycloheximide (Cyx), 1 mM, plus quinine•HCl (Q), 0.3 mM. Reproduced here with permission from Wu et al. (2015)

To a lesser extent (although not studied in such detail), Hellekant et al. (1997b) reported the same finding – increased breadth of sensitivity – in chimpanzee single fiber chorda tympani responses when the tongue was presented with an increased concentration of NaCl.[7] These findings would not result if taste was encoded as a dedicated labeled line; the "label" would stay consistent throughout a range of stimulus concentrations. Further, if taste was encoded by labeled lines, these data would suggest that tastes would be more difficult to identify confidently (i.e., the signal would become "noisy") with increasing concentration, the opposite of what is observed. Instead, the data are more consistent with a combinatorial/population coding of signals generated by several individual ganglion neurons. In sum, the geniculate ganglion neurons that respond to multiple taste stimuli (32–51%, Barretto et al. 2015; Wu et al. 2015)[8] do not represent "noise" but instead are intrinsic to combinatorial/population coding for taste quality.

[7] To be fair, Hellekant et al. (1997b) reported only a minor increase in the breadth of tuning upon stimulation at a higher concentration NaCl (300 vs 70 mM), and saw little change in breadth of tuning with increased citric acid (200 vs 40 mM). They argued this showed constancy in tuning and argue for labeled line encoding of taste, particularly for sweet.

[8] The proportion of neurons that respond to multiple tastants depends on the concentration of taste stimuli, as detailed long ago by Hanamori et al. (1988) and more recently by Wu et al. (2015). The values cited above are for approximately similar concentrations of taste stimuli. A listing of studies reporting multi-responding geniculate ganglion neurons (or the equivalent, afferent sensory axons),

Additional, though indirect, support of combinatorial coding of the output from taste buds is the finding that there is some, yet ill-defined signal processing taking place between taste bud cells during taste excitation. Paracrine cross talk and autocrine feedback –both excitatory and inhibitory – take place among cells within the taste bud during taste stimulation, mentioned above vis-à-vis Type III taste bud cells and summarized in Roper and Chaudhari (2018). Cell–cell interactions in the taste bud are difficult to reconcile with a singular, dedicated, labeled line signal processing.

Detailed single cell RNAseq analyses on geniculate ganglia from mice may help resolve the question of "taste-labeled" sensory neurons (Anderson and Larson 2020; Dvoryanchikov et al. 2017; Zhang et al. 2019).[9] Transcriptomic profiling revealed three broad classes of sensory neurons that innervate taste buds, a population of neurons totally separate from those that innervate the ear (the geniculate ganglion receives sensory input from two totally separate regions – the oral cavity and the pinna). Intriguingly, Dvoryanchikov et al. (2017) found that one of the classes of gustatory neurons selectively expresses Piezo2, suggesting that these "taste" sensory neurons respond to tactile stimuli. Zhang et al. (2019) purported to identify specific sensory neurons ("labeled neurons") for each of the basic taste qualities (sweet, sour, salty, bitter, umami) (but see caveat in footnote 9). These reports clearly await functional confirmation.

Another possible factor for encoding taste identity by peripheral neurons is the element of impulse timing. Specifically, there may be significant information in the patterns of action potentials in individual gustatory sensory ganglion neurons. Early

including data from electrophysiological and Ca^{2+} imaging studies is as follows, in ascending order of multi-responsiveness:

	Geniculate ganglion neurons or CT fibers			stimulus concentrations				
	total neurons	multi-responding	% multi-responding	Suc	NaCl	Acid	Bitter	umami
Barretto et al (2015) Fig 4	904	244	27%	0.3 M	60 mM	50 mM CA	4 mM Q, 100-1000 μM cyx	49 mM MPK + 1 mM IMP
Wu et al (2015) Fig 6a (low conc)	101	28	28%	0.1M	60 mM	3 mM	0.1 mM Q + 0.6 μM cyx	60 mM MSG + 1 mM IMP
Barretto et al (2015) Extended data	971	310	32%	0.3 M	60 mM	50 mM CA	5 mM Q, 100-1000 μM cyx	50 mM MPK + 1 mM IMP
Sollars and Hill (2005) Fig 7	42	15	36%	0.5 M	100 mM	10 N HCl	10 mM Q	
Yoshida et al (2006) Fig 2b, Table 2	105	39	37%	(sacch, 20 mM)	300 mM	10 mM HCl	20 mM Q	
Wu et al (2015) Fig 6b (mid conc)	155	79	51%	0.3 M	250 mM	10 mM CA	0.3 mM Q+1 μM cyx	100 mM MSG + 1 mM IMP
Lundy and Contreras (1999) Fig 1	73	45	62%	0.5 M	100 mM	10 mM HCl	20 mM Q	
Breza et al (2006) Fig 2	50	35	70%	0.5 M	100 mM	10 mM CA	20 mM Q	

abbreviations: sacch, saccharin; CA, citric acid; cyx, cycloheximide; Q, quinine; MPK, monopotassium glutamate; MSG, monosodium glutamate; IMP, inosine-5′-monophosphate

[9]There are important discrepancies among and uncertainties in these reports that remain to be resolved. For example, many low-copy mRNAs reported in gustatory sensory ganglion neurons in Zhang et al. (2019) had previously been shown to be restricted to neurons that only innervate the ear (Dvoryanchikov et al. 2017). Or, genes used as selective markers for a single class of neurons dedicated to a specific taste (e.g., *Cdh13* for bitter) were, in fact, present across several different clusters of neurons (Dvoryanchikov et al. 2017; Zhang et al. 2019). Most importantly, assigning specific tastes to particular sensory ganglion neurons was based on behavioral analyses of *global* knockout mice. That is, the targeted genes were expressed in the hindbrain, gustatory insula, pyriform cortex, and elsewhere in the CNS, making it impossible to pinpoint a knockout phenotype to the geniculate ganglion.

recordings from single chorda tympani fibers in the hamster revealed different patterns of rhythmic firing in response to different taste stimuli, but the authors did not emphasize or discuss this observation at length (Fishman 1957). Others have made similar observations in rats (Hallock and Di Lorenzo 2006; Ninomiya and Funakoshi 1981; Ogawa et al. 1973; Ogawa et al. 1974) and even attempted to mimic gustatory coding by stimulating the chorda tympani nerve with taste-specific patterns of excitation (Covey and Erickson 1980). Interestingly, in experiments on human subjects, changing the frequency of electrical stimulation applied to individual taste buds did not affect taste quality sensation (Von Bekesy 1964). Unfortunately, these early observations on taste-evoked response patterns in peripheral gustatory neurons and their implication for temporal coding in the CNS have not been systematically followed up.

Parenthetically, the above discussion of stimulus identification and taste coding in sensory ganglion neurons does not take into consideration that gustatory sensory neurons in the different cranial ganglia (nodose, petrosal, geniculate) and that innervate different regions of the tongue (posterior, anterior) may convey different taste quality information, at least in rodents, reviewed in Spector and Travers (2005). This is not to say, however, that there is a "taste map" for the different regions on the tongue. This lingual taste map was derived from misinterpretations of original psychophysical measurements. The notion of a taste map on the tongue has long since been discarded (Bartoshuk and Pangborn 1993; Lindemann 1999).

3.7 Stimulus Intensity Coding in Afferent Fibers and Gustatory Sensory Ganglion Neurons

In his classic study on salt taste, Beidler (1953) examined the relationship between responses versus concentration for a number of salts for intensity. The resulting monotonically increasing curve (as well as certain other factors) led him to propose the ground-breaking concept at that time that there is a membrane-bound taste receptor, especially for salt (Beidler 1954). Unsurprisingly, others have since showed similar concentration/response relations for other basic tastes (e.g., Arai et al. 2010; Damak et al. 2003; Danilova and Hellekant 2003; Ganchrow and Erickson 1970) (Fig. 14). In addition to increasing response amplitude with increasing taste stimulus concentration in peripheral neurons, sensory ganglion neurons in mice, studied in vivo with confocal Ca^{2+} imaging, responded to increasing numbers of (different) taste qualities, that is, entropy (H) increases (Wu et al. 2015). As mentioned above, this was also observed in electrophysiological recordings from hamster taste nerve fibers (chorda tympani) (Hanamori et al. 1988) and to some extent in recordings from chimpanzee gustatory afferent fibers (Hellekant et al. 1997b).

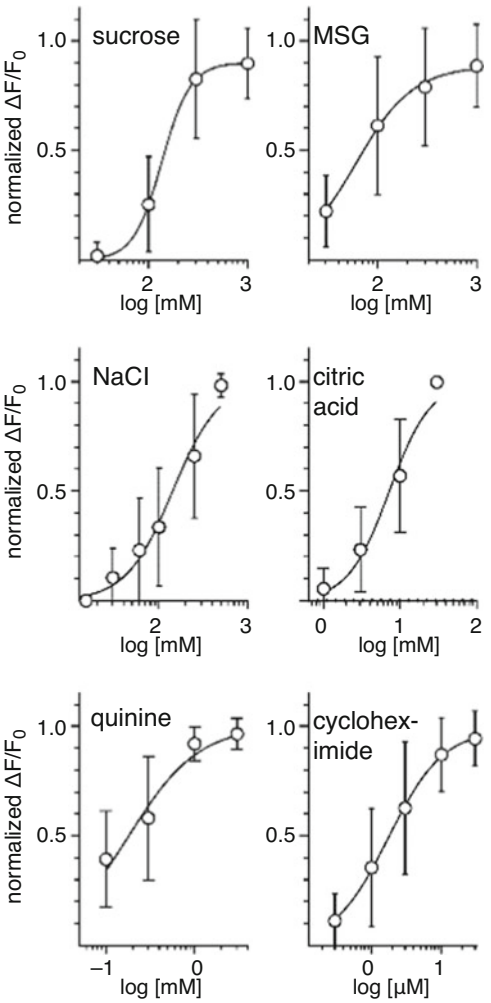

Fig. 14 Geniculate ganglion neurons respond to oral taste stimulation in a concentration-dependent manner. Stimuli were presented at increasing concentrations for each of six test compounds representing prototypic sweet, umami, salty, sour, and bitter tastes. All responses ($\Delta F/F_0$) from a given neuron were normalized to the peak response for that neuron. Symbols show means±s.d. Lines are best-fit sigmoidal curves with variable slope. Reproduced here with permission from Wu et al. (2015)

3.8 Gustatory Sensory Ganglion Neurons: Adaptation and Coding Stimulus Duration

Quantifying the precise duration of tastant is complicated by the nature of the tissue; determining the exact moment a stimulus arrives/disappears at taste buds distributed throughout the oral cavity is an inexact science. Nonetheless, a common observation in recordings from the chorda tympani and glossopharyngeal nerves (i.e., the peripheral processes of gustatory sensory ganglion neurons) is that taste-evoked responses decline during prolonged stimulation (adaptation). Little attention has been paid to adaptation of taste signals in the gustatory nerves. Smith et al. (1978) derived a quantitative model for the decline of responses during NaCl stimulation in the rat chorda tympani nerve but could not explain the cellular/molecular events

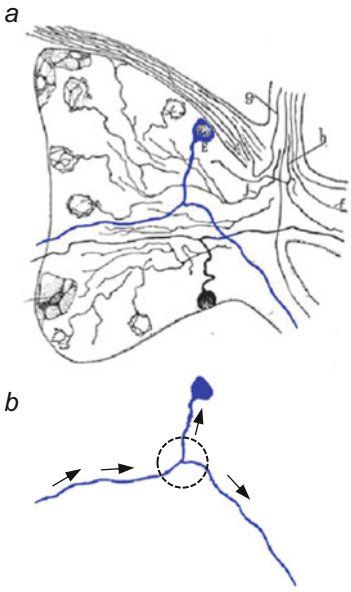

Fig. 15 The "T junction" of sensory ganglion cells is a site where action potentials can be filtered *en route* to the CNS. (**a**) drawing of histological section through a dorsal root ganglion (Cajal 1899). One ganglion neuron is highlighted for emphasis (blue). (**b**) highlighted neuron from above, illustrating the T-junction (dashed circle) and propagation of action potentials (arrows) from the periphery (left), into the soma, and to the CNS (right)

underlying this adaptation. Lyall et al. (2004) studied adaptation of rat chorda tympani nerve responses to sour taste stimuli. They concluded that the ability of the Na^+-H^+ exchanger NHE-1 to restore intracellular pH after acidic stimulation explained adaptation during sour taste. Conceivably, adaptation of taste signals transmitted to the CNS by gustatory sensory ganglion neurons might be mediated by some form of propagated impulse filtering at the T-junction that peripheral axons make with the short process that connects them to their parent neuronal soma (Gemes et al. 2013) (Fig. 15). This phenomenon would not, of course, explain adaptation of responses in the chorda tympani or glossopharyngeal nerves, recorded distal to that T-junction.

3.9 Gustatory Stimulus Discrimination in the CNS

There is a rich and complex literature surrounding how taste signals are processed in the CNS, from gustatory centers in the hindbrain, the Nucleus of the Solitary Tract, to higher centers in the primary gustatory cortex and in secondary, associated cortical areas (amygdala, orbitofrontal cortex, etc.). It is beyond this chapter to present a detailed analysis of taste coding in these areas. However, certain generalities are important to understand.

First, it must be recognized that until recently, many studies were based on recording CNS neuronal activity in deeply anesthetized animals. This is a major caveat insofar as it is widely accepted that anesthetics significantly affect neuronal

firing patterns in the CNS (Sorrenti et al. 2021) and to a lesser degree, also in the PNS (depending on the anesthetic, see Larson et al. 2015).

Second, as mentioned at the outset, the analysis of *neural circuits*, not of individual neurons, becomes paramount in information processing in the CNS. Although there is some indication that cell–cell interactions influence signal processing and information coding at the level of the gustatory end organs in the periphery (i.e., taste buds, *vide supra*), there is no question that neural *circuits*, not neurons taken separately, encode taste signals in the CNS.

Third, there are several major approaches that have been used to measure neural activity in the CNS – (a) electrophysiological recordings with microelectrodes, or more recently, microelectrode arrays; (b) functional imaging using Ca-sensitive probes; (c) functional imaging using magnetic resonance imaging and blood oxygen level dependent (BOLD) imaging, (d) electroencephalogram (EEG) recordings, and magnetoencephalography (MEG). These approaches can be and have been carried out on live, awake animals, and certain of them on human subjects, circumventing the problems of anesthesia.

The advantage of microelectrode studies is that they provide a msec by msec record of neuronal activity, and if carried out with multiple microelectrodes, of neural circuit behavior. As will be discussed below, detailed rhythmic activity in groups of neurons (circuits) appears to encode key aspects of taste. Thus, microelectrode studies are critically important in decoding taste signals in the CNS.

A powerful advantage of Ca^{2+} imaging is the ability to monitor the activity in large ensembles of neurons that respond to taste stimulation. This spatial localization helps guide microelectrode placement, among other things. It also provides information about the extent of activation by a given stimulus. With recent developments, fast-responding Ca-sensitive probes are now able to resolve action potentials in individual neurons, adding temporal to spatial resolution (Chen et al. 2013). Moreover, development of voltage-sensitive probes promises to yield even higher temporal and spatial resolution to functional imaging.

Finally, functional magnetic resonance imaging (fMRI), EEG, and MEG allow taste coding in human subjects to be studied and correlated with data from experimental animals. These methods lack the spatial (and for fMRI, temporal) resolution of microelectrode recordings and Ca^{2+} imaging, but they are non-invasive procedures and can be applied to human subjects. The ability to obtain a window into how human brains process taste is a powerful advantage of these methods.

Given these brief descriptions of methodologies, it might be possible to understand and explain the ongoing heated controversy regarding how the CNS encodes taste signals. This controversy pits "labeled line" (also referred to as a "topographic" or "spatial" coding) versus some form of combinatorial or distributed coding in neural circuits.

As explained above, labeled line taste coding originated from findings describing how peripheral receptor cells and gustatory sensory ganglion cells respond to taste stimuli. It has already been mentioned that some investigators were drawn to how many receptor cells and ganglion neurons appeared to respond to a single taste quality (were highly "tuned"), e.g., sweet compounds. The notion of labeled line

taste coding in the CNS was broadly promoted by an influential report published in 2011 (Chen et al. 2011). That publication described results from Ca^{2+} imaging studies on deeply anesthetized mice wherein distinct and different "hot spots" of activity in gustatory cortex were found to be associated with sweet, or bitter, or salty or umami tastes. Parenthetically, no sour-selective hot spot was identified. Because neurons activated by a given taste stimulus formed a localized cluster, this is also referred to as *spatial* or *topographical coding*.

The report of Chen et al. (2011) was followed by a study where channelrhodopsin was expressed in neurons belonging to either the sweet or the bitter "hot spots" in cortices of mice. These neurons were then stimulated optogenetically in unanesthetized, freely behaving animals while monitoring taste behavior (Peng et al. 2015). When the "bitter spot" was excited, mice exhibited aversive taste responses (stopping licking from a water spout, began gagging and attempted to clean their mouth). In contrast, when the "sweet spot" was optogenetically excited, mice avidly licked at the water spout even if it delivered a dilute quinine solution that mice normally avoid. In short, mice exhibited preferred taste behaviors when the "sweet spot" was optogenetically excited and conversely, showed aversive behaviors when the "bitter spot" was stimulated. The authors interpreted their findings to indicate "individual basic tastes are represented in the (brain) by finely tuned cells organized in a precise and spatially ordered gustotopic map" (Chen et al. 2011). That is, this report posits an extension of labeled line coding wherein neurons that are finely-tuned to one of the basic tastes (i.e., "labeled") are clustered into discrete hot spots. A recent report from the same group extended these earlier findings and purported to show that bitter or sweet cortical hotspots exert descending positive and negative feedback onto ascending brainstem taste signals (Jin et al. 2021). These reports have propelled labeled line/topographic coding of taste into the spotlight. Many modern textbooks cite these references and strongly promulgate labeled line/topographical coding as how the brain encodes taste signals.

Yet, historically, microelectrode studies in a wide variety of species have shown that CNS neurons recorded individually are *not* selectively tuned to a given taste quality and that there are *no focal regions* of selectivity for sweet, bitter, salty, etc. Indeed, gustatory cortical neurons respond broadly to different taste stimuli – their entropy (H) is high (Spector and Travers 2005; Yamamoto et al. 1984). This is not consistent with the presence of cortical "hot spots" where only one taste quality is represented. A possible "workaround" for this conundrum is that although cortical neurons may respond broadly to several tastes, they have a preferred ("best") taste stimulus to which they respond most robustly. Other tastes only weakly excite the neuron. That is, even though they are multiply-responsive (have high signal entropy), the ratio of "best taste" response to other taste responses (signal-to-noise ratio) (Spector and Travers 2005) is high. In such a scenario, tasting a sweet compound (e.g., sucrose) might robustly activate a specific patch of neurons in the cortex ("hot spot," labeled line/topographic coding) even though bitter, salty, umami, or sour taste compounds also activate those same neurons to a lesser extent. Calcium imaging, especially in anesthetized animals, might not faithfully report these differences and only reveal the one, most robust signal.

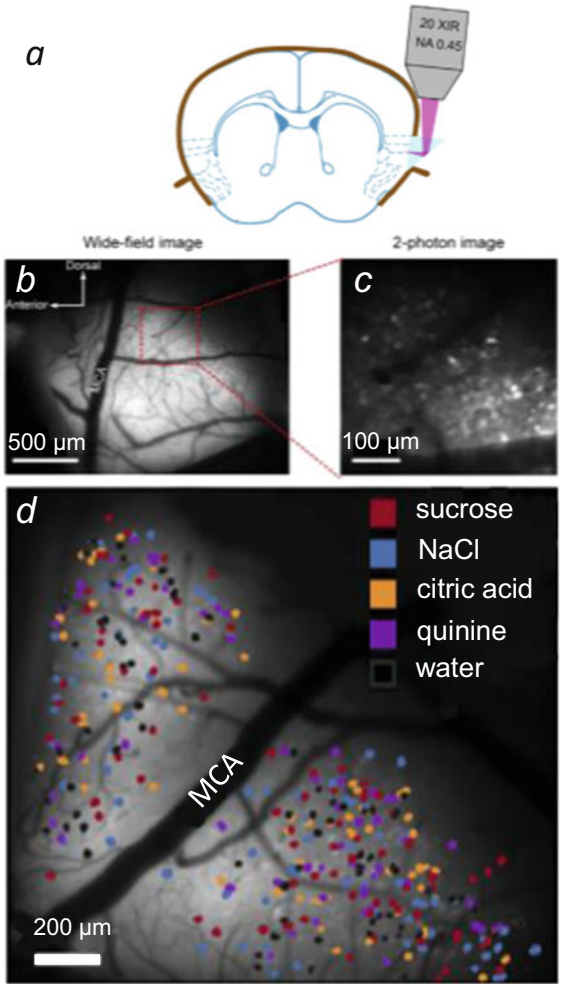

Fig. 16 The code for taste is sparsely distributed across the mouse gustatory cortex, with no apparent "hot spots."(**a**) schematic showing the prism positioned on the surface of the gustatory cortex with the microscope objective used for two-photon Ca^{2+} (GCaMP6f) imaging. (**b**) widefield image of the cortex, showing the expression of GCaMP6f (white) and the middle cerebral artery (MCA). (**c**) two-photon image from the field marked in (**b**). (**d**) representative map of neurons with best responses to sucrose (red), NaCl (blue), citric acid (orange), quinine (purple), and water (black). *N.B.*, There is no obvious clustering ("hot spots") of taste responses. Reproduced here with permission from Chen et al. (2021)

But more challenging to the report of taste-specific gustatory cortical "hot spots" and spatial (topographic) coding of taste has been the failure of other laboratories to replicate the original calcium imaging findings. On the contrary, other studies which also used Ca^{2+} imaging have not found distinct taste-specific regions in the gustatory cortex or rats or mice, anesthetized or behaving (Accolla et al. 2007; Carleton et al. 2010; Fletcher et al. 2017). Indeed, the most recent study – an exceptionally careful and detailed study using sophisticated two photon calcium imaging on awake, behaving mice – revealed a sparse, distributed representation of taste responses – including finely- and broadly tuned neurons – with no evidence for localized and segregated "hot spots" (Chen et al. 2021) (Fig. 16). This discrepancy is striking and strongly supports the notion that taste coding in the CNS is more complex than a topographic, labeled line.

If the code for taste information in the gustatory cortex is not particularly a labeled line, spatial, representation, then what are the alternatives? The leading concept is that central coding of taste signals is based on distinctive rhythmic neuronal firing patterns in dispersed circuits of gustatory neurons. The concept that the pattern of impulses might encode taste was mentioned above vis-à-vis activity in peripheral afferent fibers and was postulated for the CNS decades ago (Erickson 1963; Erickson et al. 1994; Johnson and Covey 1980; Scott and Mark 1986). Momentum for a central gustatory temporal code gained strength with publications by Di Lorenzo's group (Di Lorenzo and Hecht 1993; Di Lorenzo and Victor 2003). These researchers reported that sweet, sour, salty and bitter stimuli could be discriminated by the temporal patterns of taste-evoked neuronal firing in the rat Nucleus of the Solitary Tract (NTS). This is similar to the finding that had been reported for the peripheral taste system some decades previously (Covey and Erickson 1980). Importantly, merely applying patterned electrical stimulation to the NTS that mimicked neural firing to bitter or sweet taste solutions elicited taste behaviors appropriate for those tastes (Di Lorenzo et al. 2009b; Di Lorenzo and Victor 2003).

Yet, although temporal coding of taste in the brain seemed promising, a major limitation was that for the most part, neuronal activity was measured only from one or a very few neurons at a time. Katz et al. (2001) made a seminal breakthrough by recording taste-evoked activity in *ensembles* of neurons in awake, behaving rats with multiple recording microelectrodes implanted in the gustatory cortex. Those investigators identified taste-specific temporal patterns, or rhythms, of responses *across ensembles of neurons* in the 2–3 s following taste stimulus presentation in the oral cavity. Subsequent publications refined the analyses by using sophisticated statistical analyses (Hidden Markov modeling) to demonstrate that taste identification in the gustatory cortex evolves rapidly with shifting rhythms of firing within neuronal ensembles (Miller and Katz 2010; Moran and Katz 2014; Sadacca et al. 2016). The Ca^{2+} imaging findings of Chen et al. (2021), described above – who demonstrated sparse coding of taste in the gustatory cortex of mice – are entirely consistent with this concept of rhythmic firing within neural circuits.

In sum, modern technical advances have allowed researchers to investigate taste-evoked activity in *ensembles* of gustatory cortical neurons with high spatial and temporal resolution in experimental animals. There is abundant evidence for temporal coding of taste in the gustatory cortex of experimental animals (Stapleton et al. 2006). Studies firmly show that taste identification in the cortex is encoded by changing patterns of rhythmic firing across sparse neural circuits. This is nearly the antithesis of labeled line coding via "hot spots."

Importantly, recent studies in human subjects tend to come to similar conclusions regarding taste coding in the brain – that taste identification involves temporal coding without well-defined, taste-specific "hot spots" (Avery et al. 2020; Canna et al. 2019; Wallroth and Ohla 2018); but see Porcu et al. (2020) (Fig. 17). Notably, Avery et al. (2020) utilized ultra-high resolution fMRI and reported that their results "suggest that taste quality is not represented topographically, but by a distributed population code." Excellent topical reviews of studies on how the brain encodes

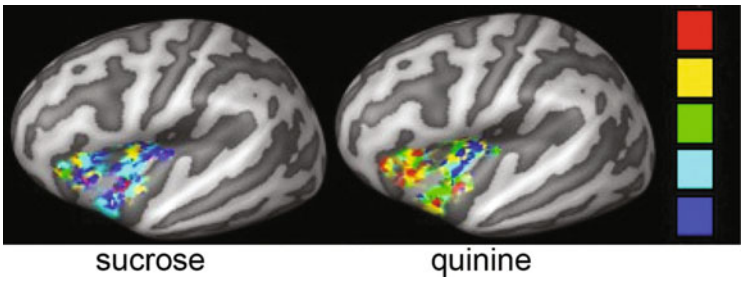

Fig. 17 The code for taste in the human gustatory cortex is not segregated into distinct, taste-specific "hot spots." Sucrose and quinine were presented orally to subjects at several different concentrations, ranging from high (red) to low (blue) concentration during functional magnetic resonance imaging (fMRI). At no stimulus intensity (tastant concentration) were distinctly separate cortical regions activated for sucrose versus quinine. Modified from Canna et al. (2019), with permission

taste identity in experimental animals and in humans have been published (Avery 2021; Boughter and Fletcher 2021; Di Lorenzo et al. 2009a; Hallock and Di Lorenzo 2006; Lin et al. 2021; Ohla 2021; Spector and Travers 2005).

3.10 Gustatory Stimulus Intensity Coding in the CNS

As discussed previously, taste intensity coding in the periphery appears to be rather straightforward. A stronger taste stimulus (i.e., more concentrated solution or lower pH for acid/sour) produces a larger signal in the receptor cells and innervating sensory neurons. However, as pointed out by Wu et al. (2015), in addition to the increased signal amplitude, a stronger taste stimulus increases the breadth of tuning, or "noise" in gustatory sensory ganglion neurons. In the CNS, encoding stimulus intensity becomes much more complex. There is some indication in the first central relay station (Nucleus of the Solitary tract), that in addition to a larger response amplitude, increasing the stimulus intensity (i.e., increasing the taste solution concentration) shows an altered temporal response (latency, time-to-peak, and decay; Schwartzbaum and DiLorenzo 1982). Increased stimulus intensity also produces a greater breadth of response in at least some NTS neurons (Geran and Travers 2009).

However, a more enigmatic coding of taste intensity occurs in higher brain centers, namely the insular cortex. Increasing stimulus concentration does not simply increase firing rates of neurons but has a more complex effect. Some neurons decrease firing rate with increased taste stimulus concentration, and conversely, other neurons show a monotonic increase in firing rate with increasing stimulus (MacDonald et al. 2012; Stapleton et al. 2006). Further, neuron action potential timing can convey information about stimulus intensity (Fonseca et al. 2018). Intriguingly, increasing taste intensity even appears to activate different ensembles

of neurons, as if different brain circuits are recruited with increasing stimulus intensity (Canna et al. 2019; Porcu et al. 2020).

4 Summary and Caveats

Regrettably, this overview overlooks certain aspects of a taste coding, especially with regard to taste signal processing in the CNS. For instance, the importance of convergent, *non-gustatory* sensory input (e.g., texture, temperature, astringency, olfaction) that contributes to taste identification is not discussed. Neither is there a mention of the critical importance of learning, attention, and expectation. The effect of active versus passive stimulus presentation on the decoding of taste information in the CNS is ignored. The role of hunger or satiety was not discussed, and how taste hedonics or valence (pleasurable versus aversive taste) might be encoded in the CNS was overlooked. These are all important factors that modulate, regulate, or change how neural ensembles in the CNS respond to a given taste stimulus at a given concentration, that is, how the brain encodes taste. Yet, such a global, comprehensive review is beyond the scope of this outline of taste coding. Excellent reviews that present the history of ideas concerning taste coding can be found in a special issue of *Physiology & Behavior* (2000), volume *69*, issue 1 (https://www.sciencedirect.com/journal/physiology-and-behavior/vol/69/issue/1), and Spector and Travers (2005). More recently, *Current Opinion in Physiology* (2021), (https://www.sciencedirect.com/journal/current-opinion-in-physiology/special-issue/10ND3QC7M6R) includes a collection of articles on taste coding in the brain that touches upon some of these important issues.

As a final word, it must be acknowledged that there remains today a heated controversy regarding how taste signals are encoded at all levels along the taste axis, from peripheral end organs to the highest levels of cortical processing. This controversy has existed since the first recordings of afferent taste nerve activity (Pfaffmann 1941; Zotterman 1936). Importantly, as noted in this review, a number of prominent reports in the more recent decades adamantly promote the concept that taste can be explained by "labeled line" coding and the existence of tastant-specific "hot spots" (spatial coding) in the CNS (Barretto et al. 2015; Lee et al. 2017; Mueller et al. 2005; Wang et al. 2018; Yarmolinsky et al. 2009; Zhao et al. 2003). Many modern textbooks have adopted these concepts in their chapters on taste (Kandel et al. 2013; Purves et al. 2017; however see Bear et al. 2016). Labeled line and spatial coding are simple and straightforward concepts, hence their attraction for the lay public, students, and scholars. It may be noteworthy that the aforementioned research reports promoting labeled line/spatial coding come mainly from the same group; the findings await validation by other laboratories. Moreover, as this chapter has attempted to show, the vast preponderance of data from researchers studying taste and using multiple different techniques strongly argues against any simple labeled line or spatial coding paradigm at any level of the taste axis. The huge dilemma is that if labeled lines and spatial codes cannot explain taste, how then does the nervous system deal with gustatory signal processing? These pages have argued

for some form of "combinatorial" or "population coding," or rhythmic activation of diffuse neural networks across time and space in the cortex encode taste. But these are vague concepts and difficult to pin down. It remains to be determined in detail how the nervous system analyzes and parses peripheral and central gustatory signals.

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The Role of ATP and Purinergic Receptors in Taste Signaling

Sue Kinnamon and Thomas Finger

Contents

Abstract

This review summarizes our understanding of ATP signaling in taste and describes new directions for research. ATP meets all requisite criteria to be considered a neurotransmitter: (1) presence in taste cells, as in all cells; (2) release upon appropriate taste stimulation; (3) binding to cognate purinergic receptors P2X2 and P2X3 on gustatory afferent neurons, and (4) after release, enzymatic degradation to adenosine and other nucleotides by the ectonucleotidase, NTPDase2, expressed on the Type I, glial-like cells in the taste bud. Importantly, double knockout of P2X2 and P2X3 or pharmacological inhibition of P2X3 abolishes transmission of all taste qualities. In Type II taste cells (those that respond to sweet, bitter, or umami stimuli), ATP is released non-vesicularly by a large conductance ion channel composed of CALHM1 and CALHM3, which form a so-called channel synapse at areas of contact with afferent taste nerve

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fibers. Although ATP release has been detected only from Type II cells, it is also required for the transmission of salty and sour stimuli, which are mediated primarily by the Type III taste cells. The source of the ATP required for Type III cell signaling to afferent fibers is still unclear and is a focus for future experiments. The ionotropic purinergic receptor, P2X3, is widely expressed on many sensory afferents and has been a therapeutic target for treating chronic cough and pain. However, its requirement for taste signaling has complicated efforts at treatment since patients given P2X3 antagonists report substantial disturbances of taste and become non-compliant.

Keywords

Adenosine triphosphate · Cough · Dysgeusia · EctoATPase · Geniculate ganglion · Ion channels · Purinergic receptors · Synapses · Taste buds

Taste buds, the sensory endorgans for the sense of taste are distributed widely throughout the oropharynx. Most taste buds reside in specific lingual papilla: fungiform papillae on the front of the tongue and foliate and vallate papillae on the sides and back of the tongue. In addition, taste buds occur on the arytenoid processes of the larynx. Lingual taste buds play an important role in appreciation of food quality while those on the larynx are more involved in reflex functions to protect the airways from erroneous entry of liquids and solids into the airways.

Regardless of location, taste buds comprise an assemblage of 35–100 specialized epithelial cells embedded in the oropharyngeal mucosa. About half of the cells in a taste bud, so-called Type I cells, serve a supportive function, much like astrocytes in the central nervous system (Kinnamon and Finger 2019). In this capacity, the Type I taste cells remove neurotransmitters (Bartel et al. 2006) and participate in ionic buffering (Dvoryanchikov et al. 2009) as well as separating the receptive elements of the taste buds one from another. The transducing cells of taste buds fall into two morphological classes: Type II and Type III.

These transducing cells of taste buds are not neurons, but rather are short, axonless receptor cells similar in this respect to hair cells of the inner ear. Similar to hair cells, taste cells release neurotransmitters from their basal aspects to activate the peripheral endings of primary sensory neurons of the system whose cell bodies reside in cranial ganglia of the facial, glossopharyngeal, and vagus nerves. These gustatory ganglion cells extend a central process into the primary gustatory nucleus in the medulla, nuc. solitary tract in mammals, where they release glutamate to activate the second-order neurons (Smeraski et al. 1996; Li and Smith 1997). Glutamate is also the peripheral neurotransmitter utilized by hair cells to activate the sensory nerve fibers (Usami et al. 2001), but is not the key neurotransmitter used by the taste transducing cells in taste buds.

The Type III cell is a neuron-like cell which responds to ionic taste stimuli including sour (H^+) and some salts. In the case of sour, the protons directly permeate Otop1 channels in the apical membrane to depolarize the cell ultimately activating

voltage-gated sodium channels to generate an action potential (Kinnamon and Finger 2019; Teng et al. 2019). The transduction mechanism for salty is less clear, in part because there is more than one mechanism involved – one for low concentrations of salt, which are appetitive and amiloride-sensitive, and others for high concentrations of salt, which are aversive and amiloride-insensitive (Oka et al. 2013). Both Type II and Type III cells likely transduce amiloride-insensitive salt, likely via different mechanisms. For Type II cells, recent data suggest chloride may play a role, although the precise mechanism that results in taste cell depolarization has not been identified (Roebber et al. 2019). At least 2 different mechanisms appear to contribute to amiloride-insensitive salt taste in Type III cells, but neither mechanism has been molecularly defined (Lewandowski et al. 2016). Whatever mechanism ultimately generates the action potential in Type III cells, as in neurons, the resulting strong depolarization opens voltage-gated Ca^{2+} channels which permit entry of Ca^{2+} into the basal compartment of the cell where typical synapses with synaptic vesicles reside. The Ca^{2+} influx permits fusion of the vesicles to the plasma membrane to release neurotransmitter (Vandenbeuch et al. 2010). The full panoply of neurotransmitters released at this synapse has yet to be delineated fully, but certainly includes serotonin and norepinephrine (Huang et al. 2005a, b, 2008, 2009, 2011a). But, as detailed below, neither serotonin nor norepinephrine appears to be necessary for transmission of taste information from taste buds to the taste nerves, but they may be involved in signaling between Type II and Type III cells (Roper 2021).

The Type II cells utilize a very different transduction cascade to effect release of neurotransmitter. These cells rely on G-protein coupled receptors to respond to sweet, umami, or bitter depending on the particular molecular receptor proteins expressed. Regardless of taste quality detected, the downstream signaling components are nearly identical involving PLCβ2, IP_3, the IP_3R3 receptor, TRPM5, and various voltage-gated Na^+ channels, ultimately culminating in an action potential (Kinnamon and Finger 2019). Unlike Type III cells, Type II cells lack voltage-gated Ca^{2+} channels and so do not require Ca^{2+} influx from extracellular space for synaptic transmission. Rather, the action potential directly gates a hexameric ATP release channel, described in more detail below, consisting of CALHM1 and CALHM3 subunits (Taruno et al. 2013, 2021; Ma et al. 2018).

In addition to being required for transmission of bitter, sweet, and umami stimuli, the CALHM1/3 channels also mediate appetitive salt taste, which is amiloride-sensitive (AS) and involves the epithelial channel ENaC. Although ENaC subunits are rather widely expressed in all types taste cells, including Type I cells, only those cells that also express CALHM1/3 signal salt taste to the nervous system via release of ATP (Nomura et al. 2020). The cell type responsible for this AS salt taste appears to be a unique type of Type II cell that lacks expression of TrpM5. Instead, influx of Na^+ via ENaC appears to be sufficient to depolarize the cell, activate the voltage-gated Na^+ channels, and trigger ATP release from the voltage-activated CALHM1/3 channels (Taruno et al. 2021).

1 Neurotransmitters in Taste Buds

The neurotransmitter(s) implicated in transmission of taste information from taste transducing cells to nerve fibers has only become clear in the last 15 years. The first candidate neurotransmitter, identified in 1975 on the basis of fluorescence histochemistry, was serotonin (Nada and Hirata 1975). In all vertebrates studied to date (Nada and Hirata 1977; Barreiro-Iglesias et al. 2008; Kirino et al. 2013), a subpopulation of taste cells accumulate and presumably release serotonin. Taste-dependent release of serotonin was described only 40 years after the seminal Nada & Hirata study (Huang et al. 2005a, b) but, as detailed below, is not crucial for activation of the gustatory nerve fibers, as will be described in the next section.

Other neurotransmitters and neuromodulators have been localized to taste cells, including acetylcholine, GABA, noradrenaline, and the peptide GLP-1 (Kusakabe et al. 1998; Dvoryanchikov et al. 2007; Roper 2007; Cao et al. 2009; Dando et al. 2012; Huang and Wu 2015, 2018), but their role in neurotransmission appears limited. These neuroactive substances may, however, play a substantial role in modulation of taste cell and nerve responses (Kataoka et al. 2012; Roper 2013). As documented below, only one transmitter, ATP clearly meets all of the criteria for being a taste transmitter.

2 ATP as the Key Neurotransmitter in Taste Buds

The first suggestion that ATP may be important in neurotransmission came for the description of the ionotropic purinergic receptors, P2X2 and P2X3 in the gustatory nerves of taste buds (Bo et al. 1999). P2X2 and P2X3 belong to a 7-member class of ATP-gated monovalent cation channels that exist functionally as trimers. In the case of P2X2 and P2X3 – both homotrimers and heterotrimers exist. The presence of specific receptors in the postsynaptic element is but one criterion for a neurotransmitter. The others are: (1) presence of the presumed neurotransmitter in the presynaptic cells, (2) release of the substance upon stimulation, (3) activation of the postsynaptic partner by the neurotransmitter, and (4) clearance of the transmitter after stimulation – either by re-uptake or degradation.

Since ATP occurs ubiquitously in cells as a source of energy, it trivially satisfies the first of these criteria to be a neurotransmitter, i.e. presence in the presynaptic cell. The functional importance of ATP acting on P2X receptors in taste transmission was demonstrated both by knockout and by pharmacology. Single knockouts of P2X2 and P2X3, as well as a double knockout of both subunits had been developed and used to demonstrate a role for ATP in bladder pain (Cockayne et al. 2000). To determine if P2X2 and P2X3 play a role in taste transmission, we obtained the single and double knockout mice and tested whether behavioral and gustatory nerve responses to tastants were affected (Finger et al. 2005). Remarkably, in the P2X2/P2X3 double knockout mice, responses to all taste qualities were absent in both the chorda tympani and glossopharyngeal nerves, while responses to tactile and temperature information were unaffected (Fig. 1). Further, behavioral responses to all

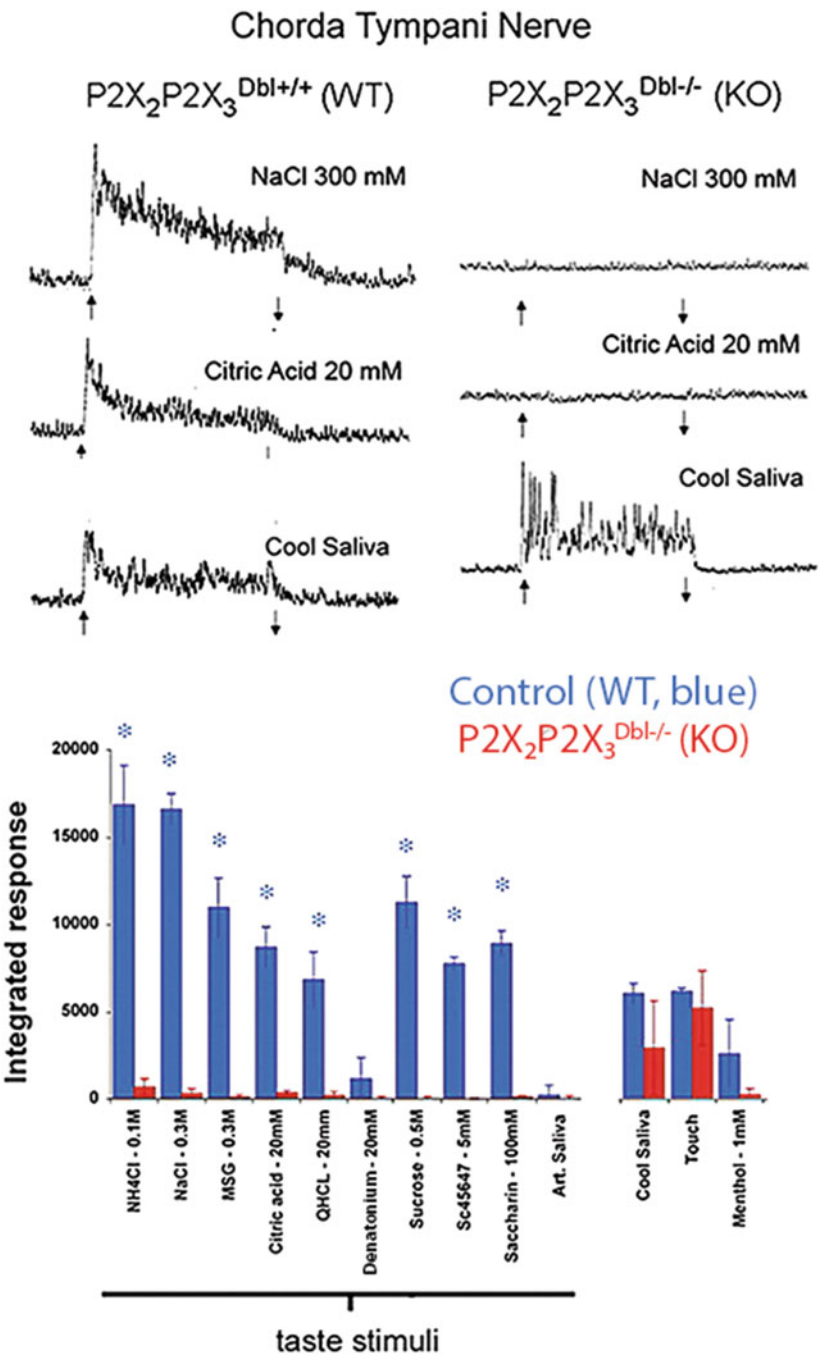

Fig. 1 Above: Representative recordings from the chorda tympani nerve of WT and P2X2/3 double-KO mice. Responses to tastants are eliminated while responses to cool temperature remain. Below: Bar graph comparing response magnitudes for WT (Blue) and KO (Red) animals to the array of taste and non-taste stimuli tested. Adapted from Finger et al. (2005)

tastants other than acids (sour) were also dramatically reduced documenting the effectiveness of the knockout on taste. The residual avoidance of sour is likely due to collateral activation of polymodal nociceptors rather than through taste buds (Hallock et al. 2009; Ohkuri et al. 2012; Yu et al. 2020). Neither P2X2 nor P2X3 single knockout mice exhibited such severe taste deficits although both knockouts were impaired relative to wild-type controls (Finger et al. 2005). These findings suggested that ATP released from taste cells activated the postsynaptic nerve fibers via P2X2 and P2X3 receptors co-expressed in most gustatory ganglion cells (Dvoryanchikov et al. 2017).

One problem with knockout mice is that pleotropic effects can occur, especially when the knockout is global and not tissue specific. In that regard, it was shown that P2X2, in addition to being expressed on taste nerve fibers, is expressed in Type II taste cells, where it is involved in potentiating the release of ATP (Huang et al. 2011a, b). Indeed, the P2X2/3 DKO mice have reduced release of ATP compared to wild-type mice. Because of this concern and the concern that ATP release had not been detected from the Type III taste cells (Huang et al. 2007), a search began for alternative means to further investigate the role of ATP in the transmission of all taste qualities. At that time, as is discussed in more detail below, pharmacologists had developed P2X antagonists as a therapeutic intervention for chronic pain and cough. One of the antagonists, AF-353, is a membrane permeant antagonist of all P2X3-containing receptors – whether they be homomers or heteromers. To determine if AF-353 would phenocopy the P2X2/3 DKO mice, we applied the drug to the tongue of mice along with various tastants during chorda tympani nerve recording. Application of AF-353 to the tongue completely abolished responses to all tastants, including sour, similar to the findings in the P2XdKO mice (Fig. 2). Further, i.p. injection of AF-353 not only abolished all taste nerve responses, but also abolished behavioral preference to a synthetic sweetener, SC45647 (Vandenbeuch et al. 2015).

To further demonstrate the role of ATP as a transmitter in the taste system, it was necessary to test whether ATP was released from taste buds with appropriate stimulation. Initial investigations showed that isolated taste epithelium released ATP when stimulated apically with bitter taste stimuli (Finger et al. 2005). Subsequently, two different teams showed measurable ATP release from individual Type II taste cells, i.e. those that transduce sweet, umami, or bitter taste qualities (Huang et al. 2007; Murata et al. 2010). Further, the amount of ATP released was directly related to the number of action potentials generated by the taste cell which is a measure of the magnitude of response of that cell (Romanov et al. 2007, 2008; Murata et al. 2010). Curiously, the Type II cells do not exhibit typical synaptic features complete with synaptic vesicles. Rather, at points of contact with the afferent nerves, Type II cells in mice show large mitochondria with tubular cristae closely apposed to the point of contact between taste cells and nerve fibers (Fig. 3) (Royer and Kinnamon 1988; Yang et al. 2020). These large mitochondria, termed "atypical" mitochondria appear to serve as a local reservoir for ATP (Romanov et al. 2018) which is released through large-pore, voltage-gated channels consisting of CALHM1 and CALHM3 heteromers (Taruno et al. 2013; Ma et al. 2018; Taruno

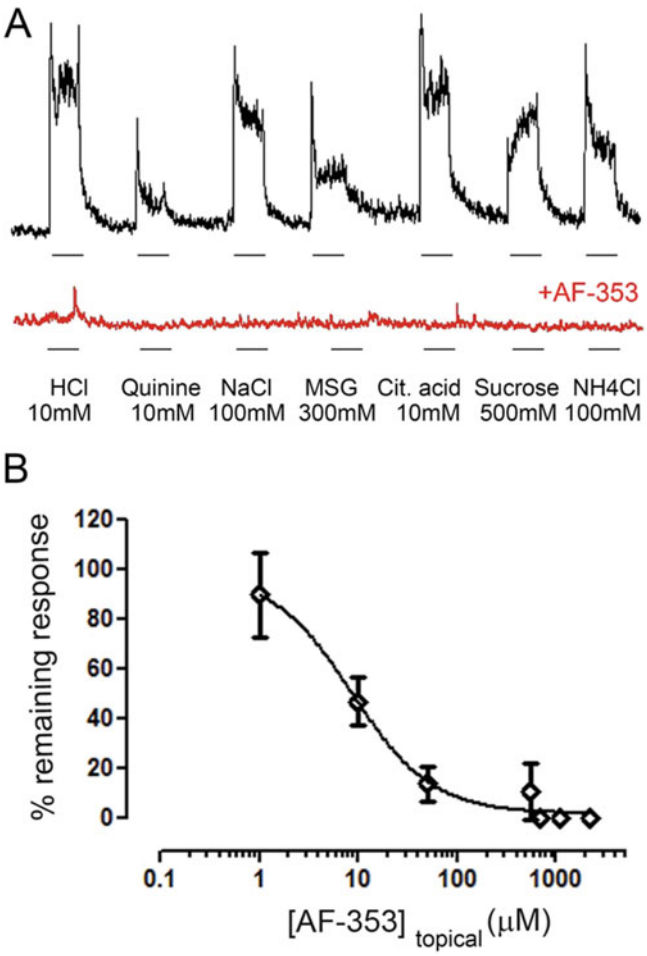

Fig. 2 Effect of topical application of AF-353 on chorda tympani nerve responses. A. Representative integrated chorda tympani nerve response to different tastants before and after (red) application of 1.1 mM AF-353. Responses to all tastants were totally abolished after a 10 min treatment with AF-353. Responses start recovering 30 min after a rinse with water, denoting a reversible effect of the antagonist (not shown). Taste stimuli were applied for 30 s (bar beneath recording) and rinsed for 50 s with water. B. Percentage of neural response remaining after application of AF-353 at various concentrations on the tongue for 10 min. As all qualities were similarly affected, responses to all qualities were averaged (means ± SD) for each concentration of AF-353 applied to the tongue. Increasing the concentration of AF-353 proportionally decreased taste responses to all qualities. Modified, with permission, from Vandenbeuch et al. (2015)

et al. 2021). The pore size of this channel, 15–18 nm is sufficient to accommodate passage of hydrated ATP (Taruno 2018). The CAHLM1/3 type of synapse, recently named a "Channel Synapse" is unusual in lacking synaptic vesicles. Since the CALHM1/3 channel synapses are gated by voltage, they offer a means for regulating

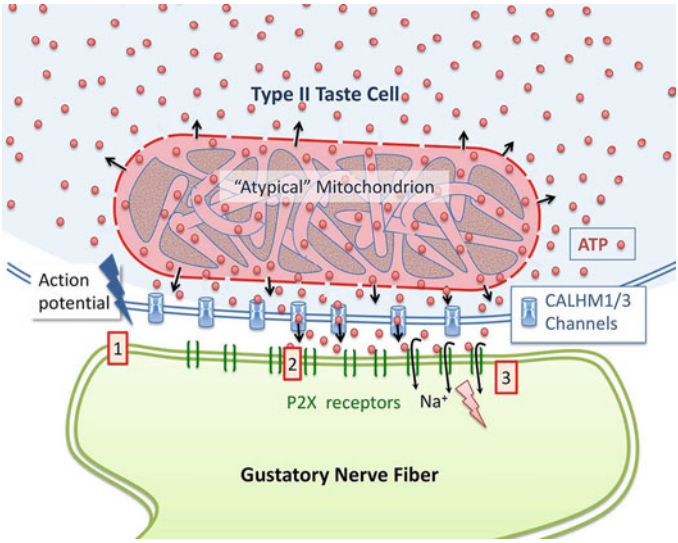

Fig. 3 Semi-schematic diagram of a channel synapse from a Type II taste cell (blue) onto a terminal of gustatory nerve fiber (green). CALHM1/3 channels are embedded in the taste cell membrane at the point of contact, closely apposed to the large, "atypical" mitochondrion with tubular cristae. When (1) the taste cell fires an action potential, the strong depolarization (2) gates open the CALHM1/3 channels to release ATP into the synaptic cleft where (3) it activates P2X receptors on the afferent nerve fiber to generate a neural action potential. Modified from Romanov et al. (2018)

release of ATP in proportion to the level of activity of the receptor cell (Murata et al. 2010).

Another criterion for demonstration of a neurotransmitter is a means for inactivating or removing the neurotransmitter once released. A potential means for elimination of extracellular ATP, an ectoATPase, was first noted in the mid-1960s (Iwayama and Nada 1967; Nada and Iwayama 1969) but the molecular identity of the highly specific enzyme, NTPDase2, was not elucidated until 2006 (Bartel et al. 2006). NTPDase2 is highly specific for ATP over other nucleotides and will rapidly convert ATP to ADP which is ineffective in activating the P2X receptors on the afferent nerves although it can activate the P2Y receptors on taste cells (Huang et al. 2009) (Dando et al. 2012; Kataoka et al. 2012). Further, NTPDase2 is expressed along the membranes of Type I taste cells which envelop the other cell types and nerve fibers. Accordingly, the ectoATPase is well-positioned to eliminate any ATP released by either Type II or Type III cells.

Taken collectively, these studies show that ATP meets all the criteria for being a crucial neurotransmitter in the peripheral taste system. While taste-related release is clearly shown for the Type II taste cells (sweet, bitter, umami), no one has yet detected ATP release from Type III taste cells that transduce sour (Huang et al. 2007). Since functional P2X3-containing channels are necessary for successful transmission of sour taste information (Vandenbeuch et al. 2015), the source of the

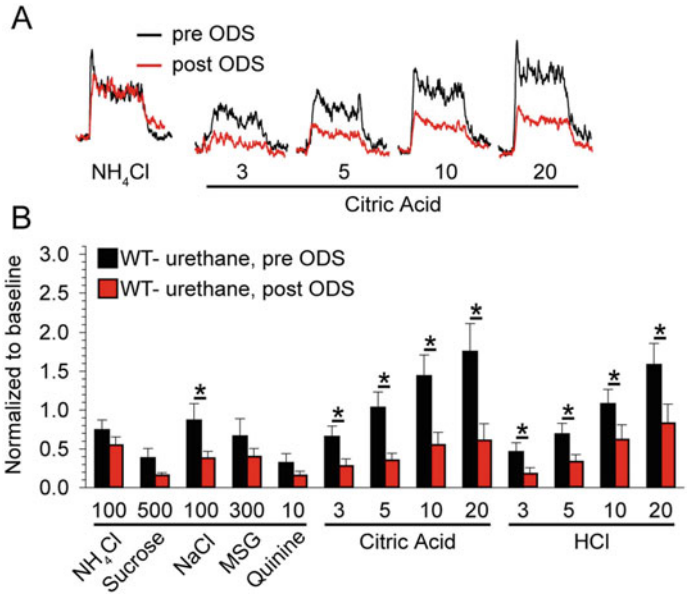

Fig. 4 The role of 5-HT_{3A} signaling in sour taste transmission. (**a**) Representative chorda tympani nerve recording to various concentrations of citric acid before and 15 min after (red) i.p. injection of the 5-HT3 antagonist ondansetron (ODS; 1 mg/kg). (**b**) Average chorda tympani responses in WT mice to various stimuli before and after injection of ODS. The responses to acids were, in general, significantly smaller after ODS treatment. Similar results were observed with 5-HT3A knockout mice (data not shown). Data are presented as mean ± SEM. Modified from Larson et al. (2015)

requisite ATP is unclear. As described above, activation of the Type III cells leads to release of serotonin via conventional vesicular type synapses. The released serotonin activates 5-HT3A receptors on the gustatory nerve fibers and contributes to the activation of these fibers (Fig. 4). However, serotonin alone is not sufficient to trigger the afferent fibers; co-activation of the P2X receptors is necessary (Larson et al. 2015, 2020). The source of the ATP for sour transmission is unclear, but it has not been detected from isolated Type III cells with biosensor cells expressing P2X receptors (Huang et al. 2007). Further, Type II cells are not the source of the ATP, since skn-1a knockout mice, which lack Type II taste cells, respond normally to sour stimuli (Larson et al. 2020). Other possible sources include the non-gustatory keratinocytes in the epithelium, but while keratinocytes are known to release ATP (Moehring et al. 2018) there is no evidence that they are stimulated by tastants. The source of the ATP required for Type III transmission remains one of the most important unanswered questions in the field.

3 Role of Purinergic Receptors in Intrabud Signaling

In addition to acting directly on nerve fibers via P2X receptors, ATP released from Type II cells stimulates purinergic receptors located on adjacent Type II and Type III cells to modulate ATP release from Type II cells (Roper 2013; Roper and Chaudhari 2017). ATP acts in an autocrine fashion to potentiate ATP release by stimulating P2X2 and P2Y1 receptors on Type II cells, causing an increase in intracellular calcium and an enhancement of ATP release in response to bitter, sweet, and umami stimuli. As mentioned above, knockout of P2X2 reduces ATP release as would be expected under these conditions (Huang et al. 2011b). However, ATP also acts in a paracrine fashion to reduce ATP release by acting on P2Y4 receptors on the Type III cells. Upon P2Y4 activation in response to ATP release from Type II cells, Type III cells release 5-HT, which subsequently binds to 5-HT1A receptors on Type II cells, causing an inhibition of further ATP release (Huang et al. 2009). One would expect that sour stimulation of Type III cells would also decrease responses to bitter, sweet, and umami stimuli since 5-HT would be released in response to sour stimuli. Since acids have non-specific effects on all taste cell types, this hypothesis was tested recently by expressing channelrhodopsin selectively in Type III cells and stimulating the tongue with blue light. Light stimulation decreased chorda tympani responses to bitter, sweet, and umami stimuli as well as to sour and salty stimuli, the latter presumably because the Type III cells were already activated by light and thus desensitized to further activation by sour and salty stimuli (Vandenbeuch et al. 2020).

4 Adenosine, a Product of ATP Hydrolysis, Modulates Sweet Taste via A2B Receptors

ATP is degraded first to ADP by NTPDase2 (Bartel et al. 2006) as well as other nucleosidases (Dando et al. 2012). The resulting ADP can be further degraded to adenosine by the action of ecto-5′-nucleotidase (CD73) expressed on Type III cells (Dando et al. 2012). The adenosine, in turn, can bind to adenosine receptors on gustatory neurons or taste cells to modulate taste responses. Two studies independently showed specific expression of the adenosine receptor Adora2b (A2B) on a subset of Type II cells in circumvallate taste buds (Dando et al. 2012; Kataoka et al. 2012). Both groups showed that A2B was expressed primarily in sweet-sensitive cells. Dando et al. (2012) used calcium imaging on a slice preparation of circumvallate taste buds to show that adenosine enhanced calcium responses and ATP release to sweeteners. Kataoka et al. (2012) showed that glossopharyngeal nerve responses to sweeteners were depressed in A2B knockout mice relative to controls. Further, they showed that the sweet receptors in posterior tongue that were modulated by adenosine were coupled to the G-protein Gα14 rather than gustducin, the main G-protein α subunit mediating sweet taste in anterior tongue as well as bitter taste throughout.

5 How Is Taste Function Altered in the Absence of NTPDase2?

As mentioned above, NTPDase2 is specifically expressed on the plasma membranes of the Type I glial-like taste cells, where it degrades the ATP released by the Type II cells to ADP. A specific knockout of NTPDase2 was developed by gene targeting methods and *Entpd2*-KO mice were characterized for changes in taste function (Vandenbeuch et al. 2013). All taste cell types were present in the knockout mice, including the Type I taste cells which normally express NTPDase2. Measurements of ATP release from the lingual taste epithelium of the knockout mice showed highly elevated levels of ATP in the epithelial tissues including the taste buds compared to their wild-type littermates. Chorda tympani and glossopharyngeal nerve recordings showed depressed responses to all taste stimuli, suggesting that the elevated ATP levels in the tissues desensitized the P2X receptors on the afferent nerve fibers resulting in decreased taste responses (Vandenbeuch et al. 2013). All taste qualities were affected, as would be expected since P2X3 is expressed on nearly all gustatory afferent nerve fibers including those that also express the 5-HT3 receptor (Larson et al. 2015).

6 Translational Implications

The P2X receptors, particularly P2X3, participate in widespread physiological activities including pain, cough, and urinary system functions. As a consequence, these receptors have become attractive pharmacological targets (Ford 2012). Several antagonists of P2X3 containing receptors (similar to AF-353) have been developed for the treatment of cough and have been very effective in diminishing chronic cough, although patient compliance has been an issue because, not surprisingly, these antagonists caused taste dysfunction (Abdulqawi et al. 2015; Smith et al. 2020). The P2X receptors on most cough and pain nerves are believed to be homotrimers of P2X3. In contrast, the geniculate ganglion neurons that innervate the tongue are likely heterotrimers of P2X3 and P2X2 since, in rodents, most ganglion cell neurons express both subunits (Dvoryanchikov et al. 2017). Calcium imaging of isolated geniculate ganglion neurons in mice showed that responses to ATP in the ganglion neurons differ in their sensitivity to the P2X3 antagonist AF-353 (Fig. 5) (Vandenbeuch et al. 2015). One population was completely blocked by I0 μM AF-353, while another population required 100 μM for a complete block of ATP responses, suggesting different composition of P2X subunits. Neurons isolated from P2X3 single knockout mice (presumably containing only P2X2 homotrimers) were completely unaffected by 100 μM AF-353. These data suggest that P2X receptors in all geniculate ganglion neurons contain at least one subunit of P2X3, since AF-353 applied to the tongue or injected i.p. blocks all taste responses. However, since the drug blocked some isolated neurons at lower concentrations than others, the P2X receptors in many ganglion neurons also contain at least one subunit of P2X2, which likely makes them less sensitive to the blockade by the

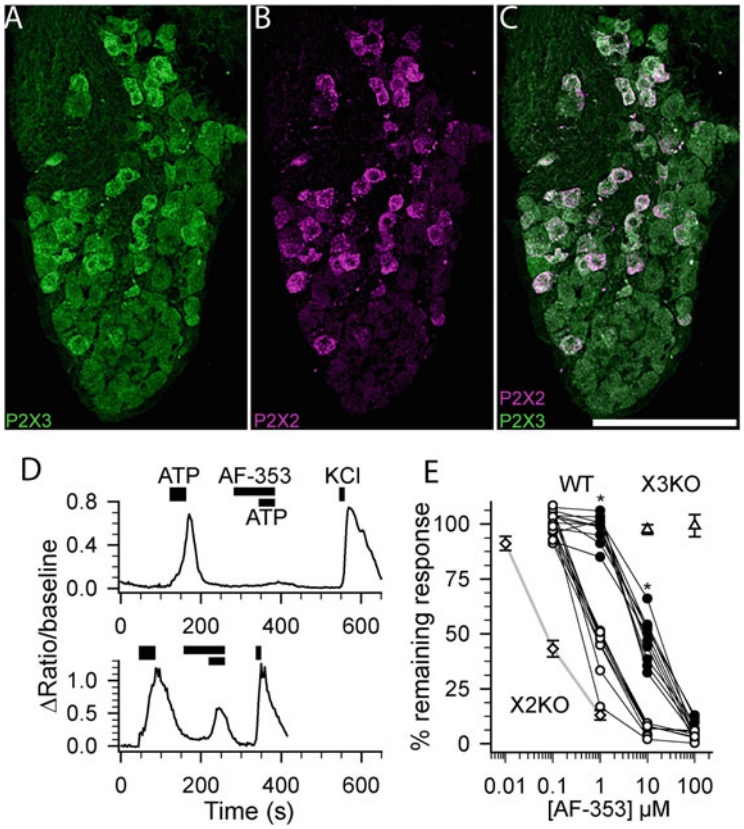

Fig. 5 A-C. Geniculate ganglion showing P2X2 (magenta) and P2X3 (green) immunoreactivity. Image is a maximum Z-projection of 12 optical sections through $a \sim 16$ μm tissue section. Scale bar = 100 μm. Brightness and contrast were adjusted linearly to preserve relative expression level information. D-E. Multiple populations of geniculate ganglion cells respond differently to AF-353. D. Change in fluorescence ratio of two ganglion cells in response to 10 μM ATP, 10 μM ATP with 10 μM AF-353 and 55 mM KCl. In the cell shown in the upper trace 10 μM AF-353 completely blocks the ATP response whereas it only blocks about 50% of the response in the cell shown in the lower trace. Drug application order was the same between top and bottom traces. E. Effect of various concentrations of AF-353 on ganglion cells of WT (circles; $n = 19$ cells), X2KO (diamonds; $n = 7$ cells), and X3KO; triangles; $n = 7$–9). WT cells were separated into two categories according to their response to ATP at 1 μM AF-353. Cells above the mean response were classified as "less sensitive" (closed circles) while cells below the mean response were classified as "more sensitive" (open circles). For WT, individual cells are represented as circles with straight lines connecting individual cells. For X2KO and X3KO symbols indicate means ± SEM. Asterisks indicate significance ($p < 0.001$ Mann–Whitney test between "more sensitive" and "less sensitive" cells). X2KO, P2X2KO; X3KO, P2X3KO; WT, wild-type. Adapted with permission from (Vandenbeuch et al. 2015)

AF-353 drug. Thus, since the majority of taste nerves in mice express P2X2/P2X3 heteromers, it seems reasonable that antagonists specific for P2X3 homotrimers might then spare taste function, since the heteromers would be less sensitive to the specific antagonist. Indeed, a new P2X3-selective antagonist, BLU-5937, does indeed ameliorate cough while sparing taste driven behaviors in rodents (Garceau and Chauret 2019). However, the rodents utilized all have dual expression of P2X2 and P2X3 in the taste nerves. For this approach to be effective in clinical situations, it is necessary that the taste neurons of humans be similar in stoichiometry to the rodents – i.e., contain at least one subunit of P2X2. Our preliminary data, however, suggest that taste nerves in most humans and primates do not express immunocytochemically-detectable P2X2 (Finger and High 2020). In keeping with this, more specific P2X3 antagonists may still show some taste disturbances (Friedrich et al. 2020). However, the full resolution of this issue requires further study.

7 Conclusions and Perspectives

Considerable evidence now exists that ATP is required for the transmission of all taste qualities to the nervous system. For the Type II taste cells, those that transduce sweet, bitter, or umami, ATP is released directly from the Type II cells via activation of large conductance CALHM1/3 channels. These channels in rodents are associated with a signaling complex that contains large atypical mitochondria in tight apposition to the neural membranes containing the P2X receptors. Action potentials in response to the taste stimuli activate the voltage-dependent CALHM1/3 channels to release ATP in a semi-quantal manner, causing activation of P2X2 and P2X3 on the sensory afferents and transmission of the taste information to the brainstem. For the Type III cells, the cells that transduce acids and some salts, the source of the ATP required for activation of sensory afferents is still unclear. Type III cells release serotonin in response to acid stimuli and the serotonin binds to 5-HT3a receptors on the sensory afferents, but serotonin alone is not sufficient to drive the afferents. ATP is also required since knockout or pharmacologic inhibition of 5-HT3a only partially reduces the nerve response, while P2X3 antagonists completely abolish the nerve response. The source of the ATP required is unclear – acid responses persist in mice that lack Type II taste cells and no one to date has measured ATP release from the Type III taste cells. This is clearly an important area of investigation for the future.

Another important unanswered question in the field is whether human taste nerves contain both P2X2 and P2X3 subunits. Antagonists selective for P2X3 homomers appear to be effective at reducing chronic cough in patients but cause minimal disturbance of taste. Yet preliminary data suggest that nerves innervating taste buds in humans express only P2X3 and not P2X2. Is this because human taste nerves contain only homomeric P2X3 receptors and if so, why do selective antagonists not block taste? Knowledge of the receptor stoichiometry in humans could address this question directly and help provide effective treatments for these conditions.

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Pharmacology of the Umami Taste Receptor

Guy Servant and Eric Frerot

Contents

Abstract

Umami, the fifth taste, has been recognized as a legitimate taste modality only recently relative to the other tastes. Dozens of compounds from vastly different chemical classes elicit a savory (also called umami) taste. The prototypical umami substance glutamic acid or its salt monosodium glutamate (MSG) is present in numerous savory food sources or ingredients such as kombu (edible kelp), beans, soy sauce, tomatoes, cheeses, mushrooms, and certain meats and fish. Derivatives of glutamate (Glu), other amino acids, nucleotides, and small peptides can also elicit or modulate umami taste. In addition, many potent umami tasting compounds structurally unrelated to amino acids, nucleotides, and MSG have

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been either synthesized or discovered as naturally occurring in plants and other substances. Over the last 20 years several receptors have been suggested to mediate umami taste, including members of the metabotropic and ionotropic Glu receptor families, and more recently, the heterodimeric G protein-coupled receptor, T1R1/T1R3. Careful assessment of representative umami tasting molecules from several different chemical classes shows activation of T1R1/T1R3 with the expected rank order of potency in cell-based assays. Moreover, 5′-ribonucleotides, molecules known to enhance the savory note of Glu, considerably enhance the effect of MSG on T1R1/T1R3 in vitro. Binding sites are found on at least 4 distinct locations on T1R1/T1R3, explaining the propensity of the receptor to being activated or modulated by many structurally distinct compounds and these binding sites allosterically interact to modulate receptor activity. Activation of T1R1/T1R3 by all known umami substances evaluated and the receptor's pharmacological properties are sufficient to explain the basic human sensory experience of savory taste and it is therefore unlikely that other receptors are involved.

Keywords

MSG · Receptor · T1Rs · Taste · Umami

1 Discovery of Umami Taste

The sense of taste is very important to drive our preference when we eat food. Our ability to taste sweetness or feel fattiness is a clue for consuming energy-rich food. Similarly, an aversively bitter or sour taste prevents us from consuming potentially poisonous plants or rotten food. Umami is now recognized as the fifth basic taste and it is intricately linked to protein-rich food. In 1908, professor Kikunae Ikeda demonstrated that the taste of dashi, a traditional broth used in Japanese cuisine, was attributed to Glu. Reading of the original paper, translated and published as an abridged version in Chemical Senses in 2002, is a delightful experience (Ikeda 2002). It is still fascinating today to learn how Ikeda fractionated the seaweed *Laminaria japonica* to get "a crystal the size of a rice corn." The isolated product clearly had the taste (brothy, meaty, savory) that Ikeda proposed to name UMAMI for convenience. As described in the isolation protocol nasty salts including barium chloride and lead nitrate were used . . . and probably ingested during tasting. Equally fascinating is the sensory aspect of Ikeda's work. He tasted various concentrations of NaCl and Glu and noticed that the salty taste disappeared before the umami taste upon dilution. This behavior, also noticed when diluting soy sauce, led him to the conclusion that the umami taste of MSG was due to the Glu component as opposed to the sodium cation. Ikeda also anticipated the industrial outcome of his discovery: "I hope that the production of seasonings will become a starting point for the development of a chemical industry in this field." He founded the company Ajinomoto in 1909.

2 Umami Compounds

As anticipated by Ikeda, MSG was the starting point of intense work on umami flavor, both from academic and industrial research teams, and it is still the case today. After the initial discovery of MSG, most of the research work toward new compounds was carried out following a taste guided fractionation approach. It consists of analyzing savory foods to identify the compounds responsible for their umami taste. The chemist first produces an extract which is separated into fractions that are taste evaluated and then further refined until the compounds of interest are isolated and their structure determined by spectroscopic methods. This approach has the advantage of providing a better understanding of the umami taste of food and proposing products that could eventually be registered as a natural ingredient for use in food. The analysis of natural products is tedious and has, most of the time, led to umami compounds including amino acids, peptide derivatives, or nucleotides.

2.1 Amino Acid and Derivatives

The umami taste of Glu (Fig. 1) has been extensively discussed in the literature (Kurihara 2015). It is widely considered as the main umami taste contributor in many foods since its content largely exceeds its taste threshold. It is generally found in many plants (tomato 150–250 mg/100 g), animals (scallop 140 mg/100 g), or fermented foods (soy sauce 410–1,260 mg/100 g) (Kurihara 2015). Besides Glu, aspartic acid (Fig. 1) is the only proteogenic amino acid to be considered as umami but it is less potent than MSG (Warendorf et al. 1992). Theanine (Fig. 1), an umami-tasting constituent of green tea, is structurally very close to Glu (Kaneko et al. 2006). Other Glu derivatives are also found in various foods (Fig. 1) such as acetyl-Glu in fermented tuna (Haseleu et al. 2013) or lactoyl-Glu and succinoyl-Glu in soy sauce or Parmesan cheese (Frerot and Chen 2013). They exhibit an umami-like taste but

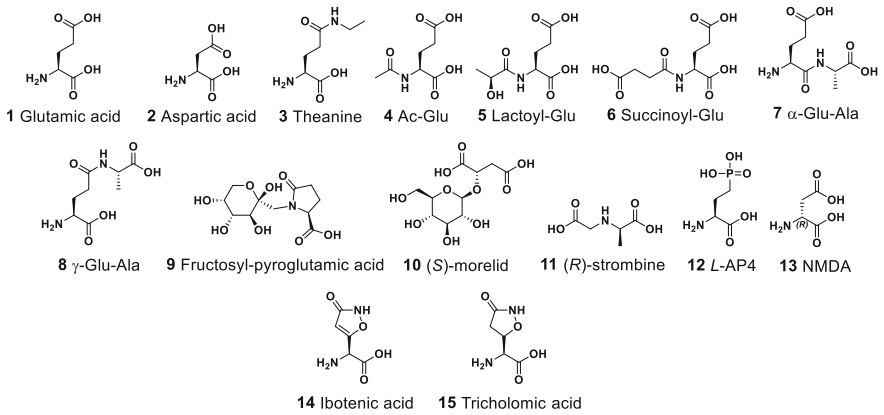

Fig. 1 L-glutamic acid and derivatives

not as strong as MSG. Small peptides, containing Glu residues present in savory foods may well contribute to the overall umami taste. Such peptides have also been described as contributing to a long-lasting savory effect, "increased continuity," "thickness," and "mouthfulness," a sensation described as kokumi (Zhang et al. 2017). It is still not fully understood how these peptides elicit this specific taste sensation, perhaps including the activation of both primary taste receptors expressed in taste receptor cells (TRCs) and chemesthetic receptors expressed in the somatosensory trigeminal (TG) afferents innervating the oral cavity (see Sect. 5 below). Glutamyl peptides present in Gouda cheese also impart a kokumi taste (Toelstede et al. 2009). Specifically, γ-glutamyl dipeptides provide a greater taste intensity than the corresponding α-glutamyl dipeptides. In agreement, α-Glu-Ala (Fig. 1) has a taste threshold ten times higher than γ-Glu-Ala (Fig. 1) (Toelstede et al. 2009). Conjugates of sugar and amino acids (such as compounds 9 and 10; Fig. 1) are described as umami compounds in soy sauce (Kaneko et al. 2011) and morel mushrooms (Rotzoll et al. 2005), respectively. The umami and sweet taste of scallop can be partially attributed to (R)-strombine (Starkenmann et al. 2009) (Fig. 1) which has a structural similarity with Glu. Other amino acid derivatives known to be agonists of ionotropic or metabotropic Glu receptors (iGluRs and mGluRs) in the central nervous system have also been studied for umami taste transduction (Oh et al. 2001). While the mGluR agonist L-AP4 exhibits an umami taste, the iGluR agonist NMDA does not (Fig. 1) (Delay et al. 2004). Ibotenic acid and its saturated derivative tricholomic acid (Fig. 1) elicit an umami taste stronger than MSG (Yamaguchi et al. 1971). However, ibotenic acid, originally found in Amanita muscaria, is also known as a neurotoxin (Eugster et al. 1965).

2.2 Nucleotides

One of the hallmarks of umami taste is a strong synergy with 5′-ribonucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′- monophosphate (GMP) (Fig. 2). This synergistic taste effect has been recognized and even quantified as early as in 1967 (Yamaguchi 1967) and it is now known that IMP, for example, can decrease the MSG taste detection threshold in humans by more than 100-fold (Yamaguchi 1991). The umami taste of theanine is also enhanced by 5'ribonucleotides (Narukawa et al. 2008). Surprisingly, IMP also enhances the umami taste of amino acids that are usually not typically described as umami such as the sweet tasting amino acids Ser and Gly (Kawai et al. 2002). GMP is 2.3 times more active than IMP, whereas AMP and XMP (Fig. 2) are less potent than IMP (0.18 and 0.61 times the activity of IMP, respectively) (Li et al. 2002). The purine residue is necessary for the umami taste enhancement effect of 5′-ribonucleotides, and it can be further improved by selected substitution (R group in Fig. 2). Thus, compounds 20–24 are all more potent than IMP (Mizuta et al. 1972; Cairoli et al. 2008; Morelli et al. 2010). Interestingly, compound 25 (Fig. 2) was discovered in a commercial yeast extract. The diastereoisomer (S)-25 is 7 times more active than IMP whereas its (R)-diastereoisomer is almost inactive (Festring and Hofmann

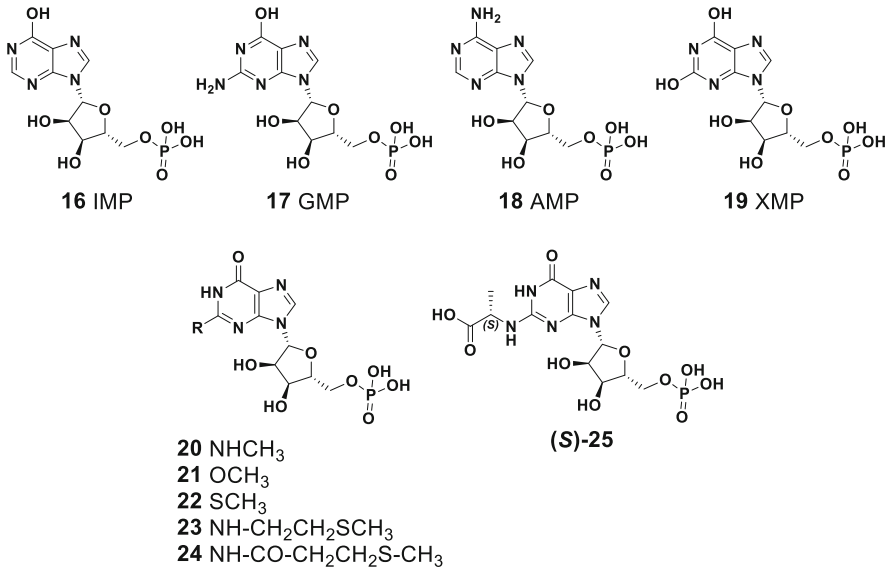

Fig. 2 IMP, GMP, and 5′-ribonucleotide derivatives

2010). Other analogs with similar activities can be formed by Maillard reactions similarly to what happens during cooking (Festring and Hofmann 2011). Yeast extracts contain high proportions of 5′-nucleotide monophosphates and of Glu which make them an ingredient of choice to be used by food manufacturers (Wang et al. 2019).

2.3 High Potency Natural and Synthetic Compounds

Natural products correspond to a great source of raw material to identify novel flavors and flavor modulators. Thus, scientists at International Flavor and Fragrances (IFF) were inspired by spilanthol (Fig. 3), the active hot and tingling component of *Spilanthes acmella*, to identify novel compounds providing different mouthfeel effects (Nakatani and Nagashima 1992). Whereas most of the amides of alkadienoic acid of various chain length are generally described as tingling, compounds 27 and 28 (Fig. 3), based on (E,E)-2,6-nonadienoic acid, unexpectedly exhibit an umami taste (Dewis et al. 2004). Contrary to compounds 26 to 28, the alkadiene chain, genarylamine, in compound 29 lies on the amine portion of the molecule (Fig. 3). Compound 29 also exhibits an umami taste at 45 μM (10 ppm) or below. Interestingly compound 29 is also described as a salty taste enhancer (Dewis et al. 2006, 2013). Many N-cinnamoyl phenethylamines known to occur in *Zanthoxylum rubescens* (Adesina and Reisch 1989), such as compounds 30 and 31 from the company Symrise (Backes et al. 2015) (Fig. 3), exhibit an intense umami taste (Fig. 3). Previously unknown in nature and also exhibiting an intense umami taste,

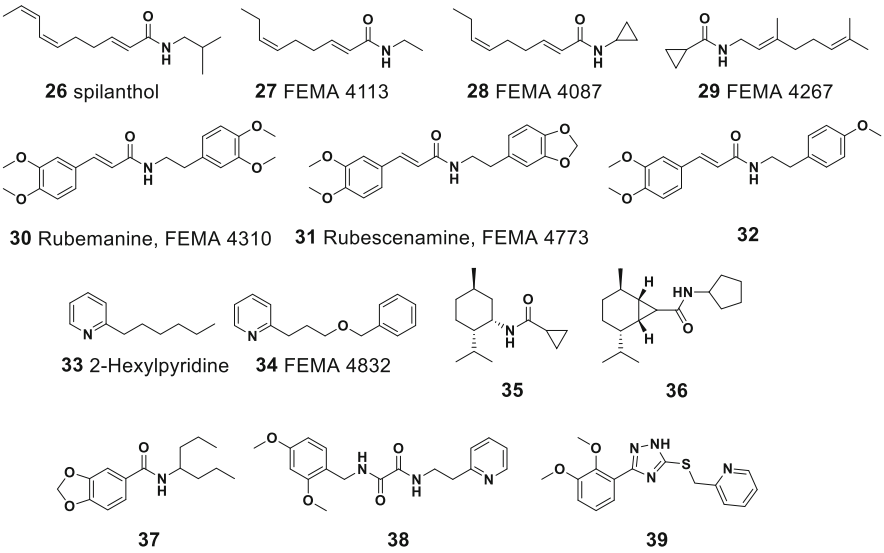

Fig. 3 Higher Potency Umami compounds

compound 32 was described in a structure-activity study with compound 30 and several analogues, and is found in the Japanese pepper *Zanthoxylum piperitum* (Frerot et al. 2015). During the analysis of volatile compounds in cooked chicken (Delort et al. 2011), Firmenich incidentally discovered that simple naturally occurring pyridines such as 2-hexylpyridine (Fig. 3) exhibit an umami taste (Delort et al. 2009). Unfortunately, alkylpyridines are very potent odorants that cannot be used in all food products. The artificial savory analog discovered by Hasegawa, FEMA 4832 (Fig. 3) is not as powerful an odorant and is therefore approved for use in foods (Miyazawa et al. 2017). The flavor company Symrise explored sterically hindered structures, including compounds 35 and 36, related to the cooling compound menthol which they also manufacture. These substituted cyclopropanecarboxylic acid (3-methyl-cyclohexyl)amides are reported to modify and enhance umami taste at low ppm levels (Backes et al. 2014; Looft et al. 2008).

3 Candidate Receptors for Umami Taste

Apart from being an essential component of proteins, physiologically the amino acid Glu is mostly recognized as a neuronal excitatory molecule and the most abundant neurotransmitter. Decades of research have been dedicated to the understanding of the physiology and molecular pharmacology of Glu receptors in the central nervous system (CNS) (Pin and Bettler 2016; Hansen et al. 2018), and as a result, earlier investigations on MSG taste detection and appetitive behavioral responses in rodents focused on these known and more obvious receptor targets. Glu binds to and activates two main families of cell surface receptors, the iGluRs and the mGluRs

present throughout the CNS (Pin and Bettler 2016; Hansen et al. 2018). iGluRs are ligand gated ion channels and orchestrate excitatory synaptic transmission. These are divided into 4 classes including the AMPA, Kainate, NMDA, and Delta receptors (Hansen et al. 2018). Contrary to their ionotropic counterparts, mGluRs consist of G protein-coupled receptors (GPCRs), which activity modulates the iGluRs responses in the CNS (Pin and Bettler 2016; Reiner and Levitz 2018). mGluRs include 8 different receptors that are divided into three main classes, class I, II, and III based on their sequence homology, pharmacology, coupling, and physiological activity (Pin and Bettler 2016). iGluRs and mGluRs are also expressed in taste tissues and these receptors have therefore attracted a lot of interest over the last ~20 years, mainly attempting to determine if they could serve as primary taste receptors for Glu (Beauchamp 2009; Chaudhari et al. 2009; Kinnamon and Vandenbeuch 2009).

Studies led by several different groups report expression of the brain forms of iGluRs and mGluR including Delta, Kainate, NMDA, mGluR1 through mGluR4, as well as specific taste splice variants in mouse and rat taste tissue (Chaudhari et al. 1996, 2000; Lin and Kinnamon 1999; Caicedo et al. 2000; Oh et al. 2001; Toyono et al. 2002, 2003, 2007; San Gabriel et al. 2005, 2009a; Vandenbeuch et al. 2010; Huang et al. 2012; Yasumatsu et al. 2015; Pal Choudhuri et al. 2016) and see the following reviews (Brand 2000; Yasuo et al. 2008; Beauchamp 2009; Chaudhari et al. 2009). Expression was assessed in taste tissue or isolated taste cells using RT-PCR analysis (Chaudhari et al. 1996, 2000; Toyono et al. 2003, 2007; Vandenbeuch et al. 2010), in situ hybridization (Chaudhari et al. 1996; Toyono et al. 2003, 2007), immunohistochemistry (Toyono et al. 2002, 2003, 2007; Vandenbeuch et al. 2010), molecular cloning (Chaudhari et al. 2000; San Gabriel et al. 2005), and functional studies using specific iGluR or mGluR agonists and antagonists (Lin and Kinnamon 1999; Caicedo et al. 2000; Oh et al. 2001; Vandenbeuch et al. 2010; Huang et al. 2012; Pal Choudhuri et al. 2016). While known functions of iGluRs and mGluRs as bona fide Glu receptors and their expression in taste tissue could be potentially indicative of an involvement as primary receptors in umami taste, their pharmacology is not as straightforward. Typical MSG affinity for iGluRs and mGluRs is in the low μM range (Caicedo et al. 2000; Chaudhari et al. 2000), or more than two orders of magnitude lower than the 1–3 mM taste detection threshold typically found in rodents (Yamamoto et al. 1991). Moreover, MSG exhibits dose-dependent taste nerve activation and appetitive behavior in rodents at levels from 10 mM to 600 mM (Zhao et al. 2003; Maruyama et al. 2006). So, if iGluRs and mGluRs were in fact the dominant primary receptors for umami taste, one would expect the Glu taste threshold to be in low μM Glu concentrations and that the Glu responses would reach a plateau at low mM Glu concentrations, or at receptor saturation. As observed in rodents, the MSG detection threshold in humans has been observed in the low mM range (0.7 mM to 3 mM) (Roper 2017). Compellingly, iGluR expression is not restricted to taste tissue on the tongue (Chaudhari et al. 1996) and isolated taste cell response to physiologically relevant Glu concentrations are mainly localized to basal processes, not the apical surface where other validated primary taste receptors are located (Caicedo et al.

2000). Finally, the taste of NMDA, Kainate, and AMPA does not generalize to the taste of MSG in conditioned taste aversion experiments (Chaudhari et al. 1996; Stapleton et al. 1999). Compared to other taste receptors, the current pharmacology, histological and behavior evidence for brain mGluRs as primary taste receptors remain lacking. It is possible these receptors simply function as mediators of taste signaling between taste cells as they do with the CNS (Huang et al. 2012; Vandenbeuch and Kinnamon 2016).

In addition to the receptors described above, two different "taste" variants of mGluR1 and mGluR4 have been reported in rat vallate and foliate papillae (Chaudhari et al. 2000, 2009; San Gabriel et al. 2005, 2009a). The discovery of these variants in taste tissue and results from preliminary functional characterization suggested that these receptors could be responsible, at least in part, for the detection of MSG by taste cells in rodents. Indeed, MSG concentrations closer to the taste detection threshold (Chaudhari et al. 2000) or characteristically higher than concentration required to activate the brain mGluRs (San Gabriel et al. 2005) could apparently trigger these receptors in cell-based assays or in electrophysiological recordings. L-AP4, an MSG analogue selective for group III mGluRs and known to elicit an umami taste in rodents and humans (Chaudhari et al. 1996; Kurihara and Kashiwayanagi 1998), activated taste-mGluR4 in cell-based assays (Chaudhari et al. 2000). Finally, high concentrations of selective mGluR1 and mGluR4 antagonists attenuated MSG-mediated taste, nerve recording, and isolated cell responses (Kusuhara et al. 2013; Pal Choudhuri et al. 2016). Although the pharmacology observed in cell-based assays is in better alignment than with the brain mGluRs, there are important experiments and questions which remain to undoubtedly link these specific variants to umami taste detection in rodents (Li et al. 2002; Zhao et al. 2003; Li 2009). First, behavioral studies using knock-out (K/O) animals required to confirm these receptors roles in umami taste detection have yet to be performed for mGluR1 and the preliminary results obtained using mGluR4 K/O animals actually point to an increase in MSG detection in the absence of the receptor (Roper et al. 1997). Even further, directed expression experiments of taste-mGluR1 or taste-mGluR4 in bitter TRCs, similar to those performed for other taste receptors (Zhao et al. 2003; Mueller et al. 2005), could be performed to show mGluRs are sufficient to drive umami behaviors. Second, taste-mGluR1 and taste-mGluR4 variants lack the first 409 and 308 amino acids, respectively. This region includes the whole upper lobe and part of the lower lobe of the Venus flytrap and importantly also includes every critical residue contacting Glu (Muto et al. 2007; Koehl et al. 2019). Though these observations leave open the possibility that secondary low affinity Glu binding sites may exist, either in the remaining Venus flytrap domain (VFD; if properly folded) of taste-mGluR1 or taste-mGluR4 or in their respective transmembrane domains (TMD), their locations have yet to be confirmed and these receptor variants still need a more thorough pharmacological and functional characterization. Lastly, and maybe most strikingly, activity of taste mGluR4 is not enhanced by 5′-ribonucleotides (Li 2009), molecules known to impart a strong umami synergy with MSG in taste studies (Yamaguchi 1991).

4 A Validated Receptor for Umami Taste: T1R1/T1R3

In the late 1990s and early 2000s two newer members of the family C GPCR gene family expressed in a subset of TRCs, TAS1R1 and TAS1R3, were identified (Hoon et al. 1999; Bachmanov et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001). These genes were later shown to encode for a heterodimeric receptor, called T1R1/T1R3 (Fig. 4). This receptor is likely the most relevant taste receptor for Glu and umami taste in humans, as demonstrated by its pharmacological properties, precisely matching psychophysical effects of MSG in humans (Li et al. 2002), and the receptor assay-guided development of entirely novel and targeted chemical classes of T1R1/T1R3 modulators, unrelated to MSG, that exhibit a strong umami taste (see below).

In rodents, the umami T1R1/T1R3 receptor behaves as a generalized L-amino acid receptor, responding primarily to cysteine, alanine, glutamine, serine, methionine, asparagine, glycine, and threonine (Nelson et al. 2002; Toda et al. 2013). On the other hand, the human T1R1/T1R3 receptor is significantly more selective,

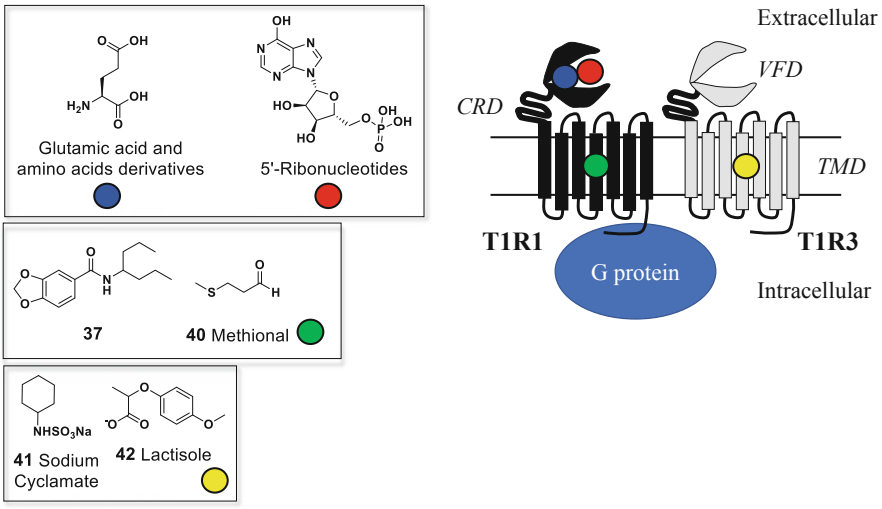

Fig. 4 The umami taste receptor composition, structure, and ligand binding sites. The umami taste receptor comprises two GPCR subunits, T1R1 and T1R3. Each subunit consists of a family C GPCR made up of an extracellular Venus flytrap domain (VFD), a cysteine rich domain (CRD) and a 7 transmembrane domain (TMD). MSG and probably other amino acids interact in the hinge region of T1R1-VFD, activating the receptor and leading to activation of the intracellular G protein through the CRD and TMD domain of T1R1. 5′-ribonucleotides are thought to interact anteriorly, in close proximity to the MSG binding pocket, and further stabilizing the closed and activated state of the T1R1-VFD. T1R1/T1R3 modulators identified by high throughput screening such as compound 37 interact within the T1R1-TMD, directly promote receptor activation, and enhance the activity of MSG. Another compound, methional, acts as a PAM. Sweeteners such as cyclamate, interacting with the T1R3-TMD, do not activate the umami receptor but rather behave as PAMs, enhancing the MSG activity on the receptor. Lactisole is an antagonist of the umami taste receptor and interacts at an overlapping site with cyclamate in the T1R3-TMD

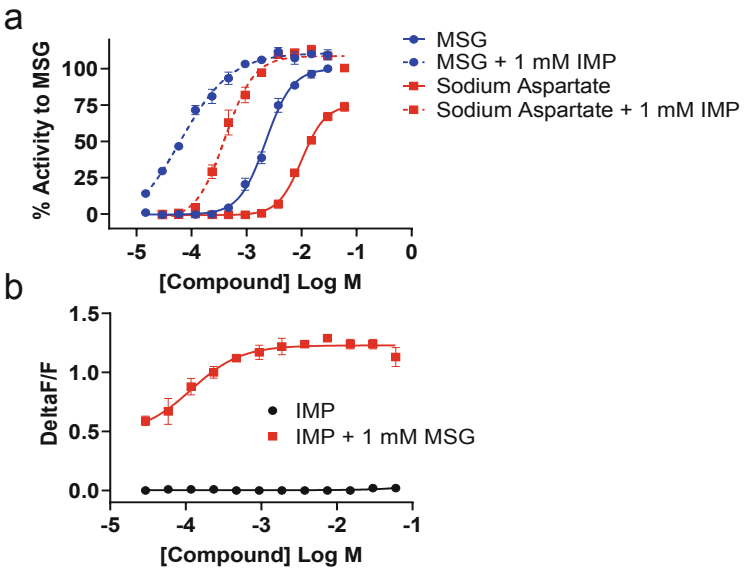

Fig. 5 IMP enhances the effect of MSG and sodium aspartate in a T1R1/T1R3 calcium mobilization cell-based assay conducted with a FLIPR. (**a**) Dose-response analysis of MSG and sodium aspartate performed in the presence and absence of 1 mM IMP. IMP causes a substantial leftward shift in the dose-response curves. (**b**) IMP does not exhibit agonist activity in a T1R1/T1R3 cell-based assay. Dose-response analysis of IMP performed in the presence and absence of 1 mM MSG. IMP only shows activity in the presence of MSG

responding mainly to Glu and to a lower extent L-aspartate (Li et al. 2002; Toda et al. 2013). In contrast to mGluRs, both mouse and human T1R1/T1R3 receptors show striking MSG activity enhancement in the presence of IMP. Under these conditions, the mouse T1R1/T1R3 taste receptor responds to virtually every amino acid (Nelson et al. 2002; Toda et al. 2013). The human umami T1R1/T1R3 taste receptor responses to MSG, L-aspartate and L-AP4, a Glu receptor agonist, are also drastically enhanced by IMP in cell-based assays, in agreement with psychophysical data, as shown in Fig. 5a (Li et al. 2002; Zhang et al. 2008; Toda et al. 2013). Furthermore, unlike mGluRs, the MSG potency (EC_{50}) in human T1R1/T1R3 cell-based assays (Table 1) is in line with its taste detection threshold range of 0.7 mM to 3 mM reported in humans (Roper 2017). The expression profile of this receptor in a unique TRC population, supporting a labeled line model similar to the other tastes (Chandrashekar et al. 2006), along with its pharmacological properties was compelling enough to initiate, close to 20 years ago, discovery programs leading to the identification of novel and potent savory agents, described further below.

Overall, the ensemble of T1R1/T1R3 K/O studies has confirmed a role of these proteins for the detection of MSG and/or MSG + IMP in mice (Damak et al. 2003; Zhao et al. 2003; Maruyama et al. 2006; Yasuo et al. 2008; Kusuhara et al. 2013; Blonde and Spector 2017; Blonde et al. 2018). Still, observations of residual

Table 1 Potency (EC50) summary from dose-response analysis depicted in Fig. 6

Compound	EC50 (μM), mean ± SD	*n*	EC50 (μM), mean ± SD with 1 mM IMP (0.034%)	*n*
Compound 38	0.07 ± 0.04	5	0.07 ± 0.05	3
FEMA 4267	0.37 ± 0.12	5	0.30 ± 0.16	3
Compound 39	0.39 ± 0.24	5	0.54 ± 0.31	3
Compound 37	0.66 ± 0.25	5	1.1 ± 0.16	3
Compound 32	10 ± 2	6	8.3 ± 5.9	3
FEMA 4832	14 ± 4	3	19 ± 2	3
2-Hexylpyridine	18 ± 11	3	25 ± 22	3
Ibotenic acid	525	1		
MSG	**2,315 ± 350**	**15**	**93 ± 34**	**5**
Sodium aspartate	9,742 ± 1809	6	451 ± 59	3
Glutathione	11,279 ± 1900	5	11,437 ± 2,156	3
γ-Glu-Ala	16,412 ± 1,482	5	10,352 ± 1,029	3
Theanine	24,887 ± 11,139	6	17,500 ± 5,935	3
Succinoyl-Glu	Inactive	4	3,423 ± 96	3
Glu-Glu-Leu	Inactive	5	7,646 ± 1,459	3
Lactoyl-Glu	Inactive	4	9,356 ± 7,240	3
α-Glu-Ala	Inactive	5	16,977 ± 2,748	3

behavioral, nerve, or signaling responses in T1R1 and T1R3 K/O strains, made in several independent studies over the years (Damak et al. 2003; Maruyama et al. 2006; Yasuo et al. 2008; Kusuhara et al. 2013), either point to the presence of additional MSG detection mechanisms in rodents or an inherent variability depending on readouts, strains, and other conditions affecting the outcome of experiments. This could possibly be due to the fact that mice are less responsive to MSG as a stimulus relative to other taste modalities. As noted by Spector in a relatively recent study (Smith and Spector 2014) "Wild-type and K/O mice, regardless of the missing T1R protein(s), have difficulty reliably detecting a relatively high concentration of MSG when the contribution of sodium is minimized by amiloride, and animals are forced to make immediate decisions on the presence or absence of a taste stimulus after a few licks," suggesting that most of the attractive or appetitive behavior of MSG in behavioral studies comes from the sodium ion. In any case, the predominant current working hypothesis in the field is that, when detected, the residual MSG responses in T1R1 or T1R3 K/O animals either involve brain or taste-mGluR1 and mGluR4 expressed in TRCs (but see Sect. 3 above) or other unknown receptor mechanisms (Yasuo et al. 2008; Kusuhara et al. 2013; Yasumatsu et al. 2015; Pal Choudhuri et al. 2016). It is still not clear if redundant umami detection systems could also potentially exist in humans. Perhaps the only data available to indirectly support the presence of such a system are included in a preliminary study describing a single nucleotide polymorphisms (SNPs) in

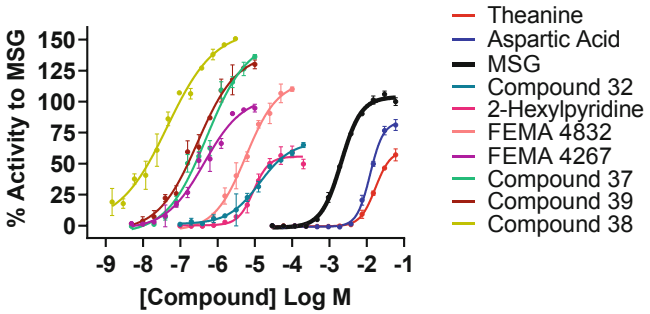

Fig. 6 Dose-response analysis of umami compounds in a T1R1/T1R3 cell-based assay. Depicted umami compounds activate the T1R1/T1R3 receptor with the expected rank order of potency. None of these compounds activate the cell line in the absence of T1R1/T1R3 (not shown)

mGluR1 which is linked to an MSG non-taster phenotype in humans (Raliou et al. 2009b). Still, it is currently impossible to pinpoint the mechanism of action of such mutations in mGluRs, as variants could either influence the MSG effect at a putative primary mGluR-MSG taste receptor or affect neurotransmitter taste bud signaling as described above.

Similarly, data supporting the effect of specific T1R1 and T1R3 SNPs on MSG or MSG + IMP detection in humans have been reported (Kim et al. 2006; Chen et al. 2009; Raliou et al. 2009a, 2009b, 2011; Shigemura et al. 2009; Chamoun et al. 2018). Three SNPs, A110V, A372T and R507Q, with allele frequencies across the population varying between 2% and 29%, affect umami taste detection in humans. The A110V and R507Q SNPs, located in the VFD of T1R1, are associated with a non-taster and hypo-taster phenotypes (specific ageusia to MSG) where individuals cannot easily discriminate between 29 mM NaCl and 29 mM MSG solutions (Raliou et al. 2009b). Accordingly, evaluation of these variants in a cell-based assays shows a reduced response to MSG, albeit not a full inhibition of the receptor activity (Raliou et al. 2011). The A372T variant, also located in the VFD of T1R1, is associated with an increased sensitivity to MSG and an increase in MSG potency in a cell-based assay (Shigemura et al. 2009). However, an independent evaluation of this variant in cell-based assays could not detect a difference with the wild-type receptor, probably due to the high variability of the assay (Raliou et al. 2011). It will be interesting to find out how or if these specific residues directly participate in MSG binding, once a crystal structure is available. SNPs are also located in T1R3, the other subunit of the umami receptor (Fig. 4) (Li et al. 2002; Nelson et al. 2002). The R757C variant with an allele frequency varying between 1% and 9% and located in the third intracellular loop of the TMD of T1R3, is associated with a reduced sensitivity to MSG and MSG + IMP and a lower MSG potency in a cell-based assay (Kim et al. 2006; Chen et al. 2009; Raliou et al. 2009a, 2011; Shigemura et al. 2009). The localization of this residue suggests a role in signaling or coupling/transmission of the signal to the G protein (Bourne 1997). Hence, one could envision that the sweet taste receptor, made of T1R2 and T1R3 (Nelson et al. 2001; Li et al.

2002) could also be affected by this mutation. However, it seemingly does not affect the taste recognition threshold of sucrose in humans (Shigemura et al. 2009).

In vitro and vivo, the human T1R1/T1R3 taste receptor couples to the G protein gustducin and also probably some members of the $G\alpha_{i/o}$ proteins that are abundantly expressed in TRCs (Ruiz et al. 2003; He et al. 2004; Ozeck et al. 2004; Sainz et al. 2007). Binding of gustducin occurs at the T1R1-TMD as suggested by G protein reconstitution assays with cell membranes containing either T1R1 or T1R3 (Sainz et al. 2007; Zhang et al. 2008) (Fig. 4). In TRCs, activation of gustducin releases the associated β3γ13 G protein subunits which in turn activate phospholipase β2 (PLCβ2) resulting in an increase in intracellular calcium (Ca^{2+}) concentration (Huang et al. 1999; Zhang et al. 2003; Palmer 2007). TRPM4 and TRPM5, TRP channels expressed basolaterally in TRCs, are then directly activated by the increased intracellular Ca^{2+} and allow the flow of sodium into the cells, ultimately leading to activation of other channels, elicitation of action potentials and neurotransmitter release through CALHM1/3 (Zhang et al. 2003; Taruno et al. 2013; Dutta Banik et al. 2018; Ma et al. 2018). Although this taste signaling pathway has yet to be reconstituted in heterologous cells, robust cell-based assays for the umami taste receptor were nevertheless developed using forced coupling to promiscuous G proteins such as $G\alpha_{15}$ and $G\alpha_{16/gustducin25}$, activation of endogenous PLCs and Ca^{2+} release from internal stores (Li et al. 2002; Nelson et al. 2002; Festring et al. 2011). Such assays have allowed not only the pharmacological characterization of known umami compounds but also the discovery of new very potent chemical classes of savory molecules (see below). Every single known umami compound evaluated in our cell-based assay activates the umami T1R1/T1R3 taste receptor (Fig. 6 and Table 1). Umami-tasting amino acids and derivatives such as MSG, sodium aspartate, theanine, succinoyl-Glu, and lactoyl-Glu have the lowest potency in the assay. Just like MSG, these molecules probably interact within the VFD of T1R1 (Fig. 4, Table 1) (Zhang et al. 2008). Indeed, IMP, a known positive allosteric modulator of T1R1/T1R3 interacts within the T1R1-VFD, further stabilizes its closed state and, as a result, increases the potency of VFD-bound agonists (Fig. 5a and Table 1) (Zhang et al. 2008; Toda et al. 2013). Similarly, IMP increases the potency of every amino acid derivative evaluated (Table 1). MSG is the most potent and efficacious agonist among amino acid derivatives, some of them producing only ~50% of the maximal MSG activity (see the theanine dose-response in Fig. 6). It is therefore possible that these could behave as competitive antagonists and limit the overall savory output if their concentration far exceeds that of MSG in certain consumer products or formulations. The highest potency umami compounds evaluated have EC_{50}s in the μM to nM range and are for the most part more efficacious than MSG (Fig. 6 and Table 1). These include molecules such as compound 32 and 2-Hexylpyridine, found respectively in the Japanese pepper *Zanthoxylum piperitum* and in cooked chicken. Very potent umami taste receptor agonists also include FEMA 4267, a derivative of spilanthol as well as three novel classes of molecules identified by high throughput screening using an umami receptor cell-based assay: compounds 37, 38, and 39 (structure are depicted in

Fig. 3). Unlike the low potency amino acid derivative and peptides interacting in the T1R1-VFD, the receptor activity of these high potency agonists is not enhanced by IMP (Table 1) action. Since IMP only synergizes with molecules interacting with the T1R1-VFD, it is likely that these molecules act elsewhere on the receptor. In fact, the binding site of compound 37 has been mapped to the T1R1-TMD (Fig. 4) (Zhang et al. 2008). Remarkably, the rank order of potency of different classes of T1R1/T1R3 agonists in the assay mirrors their relative umami potency in taste tests (Table 2 and Fig. 7), solidifying the notion that T1R1/T1R3 plays a dominant role, if not the sole role, for the detection of umami compounds in humans.

Intriguingly, IMP has also been suggested to impart an umami taste on its own (Yamaguchi 1991) and biophysical studies using purified and isolated human T1R1 (Huang et al. 2019) and human T1R1-VFD (Ahn et al. 2018) or cat T1R1-VFD (Belloir et al. 2017) suggest direct binding of IMP to T1R1 in the absence of MSG. However, in a validated cell-based assay, IMP does not activate the human T1R1/T1R3 receptor (Li et al. 2002; Zhang et al. 2008) at concentrations up to 60 mM (Fig. 5b). It is likely therefore that the umami taste observed at high concentrations of IMP (300 μM to 1.25 mM; (Yamaguchi 1991)) is due to enhancement of low Glu concentrations (18 to 49 μM) present in human saliva (Scinska-Bienkowska et al. 2006). In support of this hypothesis, in conditioned taste aversion experiments in mice, the taste of high levels of IMP generalizes to the taste of MSG (Murata et al. 2009). In addition, in cell-based assays, 1 mM IMP enhances the umami T1R1/T1R3 receptor's response in such a way that MSG, even at low μM concentrations, induces a significant receptor activation (Fig. 5a). In reality, binding of IMP (in the absence of MSG) to T1R1-VFD seems unlikely unless the receptor displays constitutive activity and it's T1R1-VFD is in a closed state, at least part of the time. Indeed, modeling and mutagenesis studies (Zhang et al. 2008; Mouritsen and Khandelia 2012) suggest that IMP probably does not have a binding pocket in the T1R1-VFD open state as it binds preferably the closed state. Evidently, more work is necessary to link the binding of different ligands to their specific sites to structural modification and receptor activation at the molecular level.

Other molecules have been shown to modulate the activity of MSG on T1R1/T1R3. Compound 37 not only activates the receptor, as described above, but also enhances the effect of MSG when used at lower concentrations indicating that these types of molecules (including compound 38) are in fact ago-potentiators of the umami taste receptor (Fig. 8a). These results suggest a rather tight functional coupling between the TMD and VFD of T1R1, where the stabilization of an active conformation in the TMD probably stabilizes a VFD conformation with a higher affinity for MSG. Similarly, methional (Fig. 4) and its derivatives, also interacting within the T1R1-TMD, are reported to behave as positive allosteric modulators (PAMs) on the human T1R1/T1R3 while behaving as negative allosteric modulators on the mouse T1R1/T1R3 (Toda et al. 2018). Remarkably, sweeteners (and therefore agonists) interacting at the T1R3-TMD in the sweet taste receptor act as pure PAMs (devoid of agonist activity) in the context of the umami taste receptor. Accordingly, cyclamate actually enhances the activity of MSG on the umami taste receptor,

Table 2 Summary of T1R1/T1R3 assay potency and umami taste potency for depicted compounds

	T1R1/R3 cell-based assay			Taste evaluation		
Compound	EC50 (μM), mean ± SD	Assay EC50 ratio (A)	Log A	Concentration (μM) eliciting umami taste equivalent to 0.05% (2,958 μM) MSG	Taste concentration ratio (B)	Log B
Compound 37	0.66 ± 0.25	3,507	3.545	3	986	2.994
Compound 32	10 ± 2	232	2.365	44	67	1.826
FEMA 4832	14 ± 4	165	2.217	88	34	1.531
MSG	2,315 ± 350	1	0	2,958	1	0
Glutathione	11,279 ± 1,900	0.21	−0.678	3,257	0.91	−0.041

EC_{50} values, taken from Table 1 were normalized to the EC_{50} of MSG in the assay to produce and EC_{50} ratio value (*A*). For the taste tests, 5 trained panelists evaluated the umami intensity of the compounds, diluted in 20 ml of Henniez bleu mineral water, in a blind and random order using a sip and spit procedure. Panelists then scored the umami intensity on a linear scale from "no taste" to "very strong umami taste" (from 0 to 10). The average value was calculated for each product and compared to the umami intensity of MSG at the concentration of 0.5 g/L, which was of "5 on a scale from 0 to 10," corresponding to moderate intensity in our tests. Concentrations of compound (in μM) eliciting a taste intensity equivalent to that of 0.5 g/L (~3 mM) MSG were then normalized to produce a taste concentration ratio (B). The $\log_{10}$ values of A and B were plotted in the graph depicted in Fig. 7

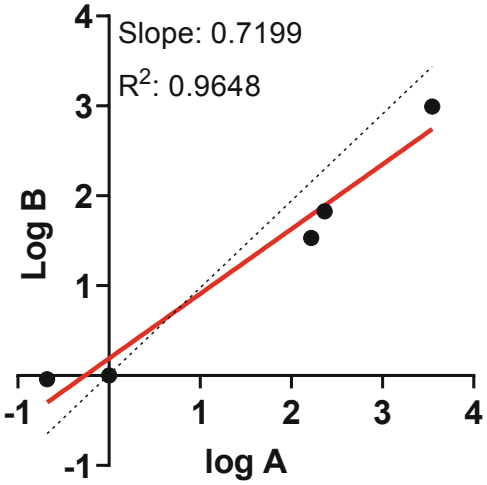

Fig. 7 Correlation analysis between the relative potency of umami compounds in the T1R1/T1R3 cell-based assay and the relative rank order of potency of umami compounds in taste tests. The Log A and Log B values from Table 2 were plotted and analyzed through linear regression. Analysis shows a strong correlation between the two data sets with a coefficient of 0.9648. The resulting slope (red plot) is less than unity (dashed black plot), showing that the cell-based assay is, in general, slightly more sensitive to umami compounds relative to their effect in taste tests

producing a leftward shift in the MSG dose-response (Fig. 8b) (Xu et al. 2004). This is in clear contrast to what is seen on the sweet taste receptor where TMD activation has only little influence on the EC_{50} of sweeteners binding at the VFD (Servant et al. 2020). Contrary to the numerous agonists and PAMs described above, relatively fewer human T1R1/T1R3 receptor antagonists have been identified and characterized. Molecules, previously described as sweet receptor antagonists, attenuating sweet taste and interacting within the T1R3-TMD, such as lactisole (Fig. 4) and clofibric acid, also inhibit the umami taste of MSG (Xu et al. 2004; Galindo-Cuspinera and Breslin 2006; Maillet et al. 2009; Kochem and Breslin 2017; Servant et al. 2020).

5 Kokumi Taste

Another flavor quality termed "kokumi" with somewhat similar properties as umami taste has gathered attention over the last three decades. Isolates from garlic (Ueda et al. 1990), beans (Dunkel et al. 2007), and cheese (Toelstede et al. 2009) contain γ-L-glutamyl peptides that confer "mouthfulness," "thickness," and "continuity" to flavors while also providing general taste enhancement properties. Interestingly, these kokumi peptides are present in foods typically known to exhibit a savory/umami taste and savory molecules, such as MSG, have also been known to act as overall flavor enhancers (Yamaguchi and Kimizuka 1979). Consequently, the

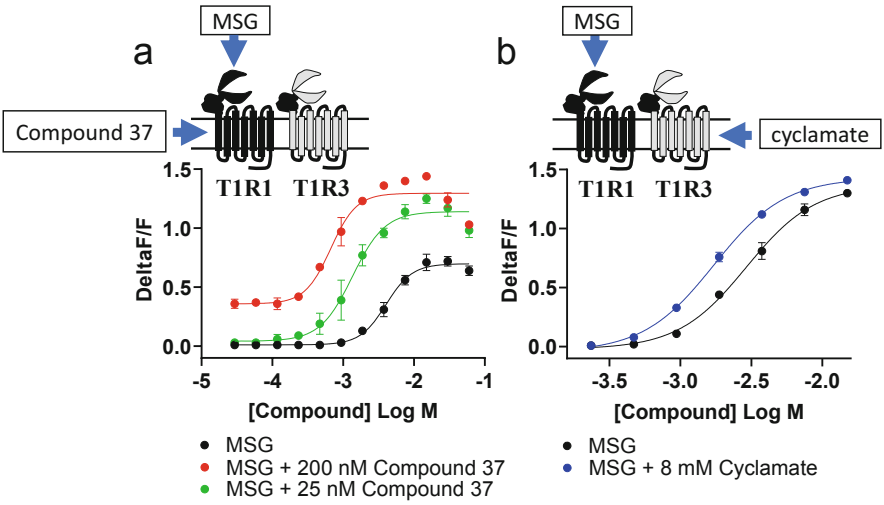

Fig. 8 Positive allosteric modulation of the umami T1R1/T1R3 receptor. (**a**) Agonists such as compound 37 and compound 38 (not shown) enhance the effect of MSG in a T1R1/T1R3 cell-based assay. Concentrations eliciting little or no agonist activity produce a significant leftward shift in the MSG dose-response. (**b**) The sweetener cyclamate binds to the TMD of T1R3, the subunit shared between the umami and sweet taste receptor. While binding of cyclamate to T1R3 causes receptor activation (and sweet taste) in the context of T1R2, cyclamate binding does not activate the umami receptor (in the context of T1R1) but rather synergizes with MSG, causing a leftward shift in the MSG dose-response

obvious question is whether these molecules acting more or less as general taste enhancers, are simply savory molecules with lingering or other TG properties, acting alone or in conjunction with MSG and interacting with the T1R1/T1R3 receptor, or do they function by recruiting other new primary taste receptors all together?

The calcium sensing receptor (CaSR), another class III GPCR working as an obligate homodimer (Brown et al. 1993), is a candidate receptor for kokumi taste. It is ubiquitously expressed, with its highest levels found in the parathyroid gland and the kidneys where it monitors the systemic levels of calcium (Brown et al. 1993; Riccardi et al. 1995). As its name would suggest, its natural agonist calcium binds to the VFD of the receptor to elicit intracellular signaling typically leading to a regulation of parathyroid hormone (PTH) secretion and calcium excretion (Vahe et al. 2017). The CaSR is also found in taste tissues, where it has been observed in rodent circumvallate and foliate papillae taste buds, including Type I, Type II TRCs and Type III presynaptic cells (San Gabriel et al. 2009b; Bystrova et al. 2010; Maruyama et al. 2012; Brennan et al. 2014). However, function of the CaSR is apparently mainly detected in Type III cells. CaSR agonist applications to isolated taste cells or lingual slice preparations lead to rapid calcium mobilization from internal stores through activation of PLC, while a CaSR antagonist, NPS 2143, attenuates the effect of kokumi peptides (Bystrova et al. 2010; Maruyama et al. 2012). The CaSR is also activated or modulated by numerous other molecules such

as L-amino acids, other divalent and trivalent cations, basic peptides such as protamine and poly-lysine, and the polyamine spermine (Brown et al. 1993; Conigrave et al. 2000; Vahe et al. 2017). In one study, these CaSR agonists such as calcium lactate, protamine, poly-lysine and a CaSR PAM, Cinacalcet, were reported to enhance the taste of a solution containing 0.1% MSG and 0.5% NaCl (Ohsu et al. 2010). Similarly, γ-glutamyl peptides have been reported to activate the CaSR in vitro with relative potencies (EC_{50}s) apparently correlating with their relative kokumi flavor intensity (Wang et al. 2006; Ohsu et al. 2010; Broadhead et al. 2011; Amino et al. 2016, 2018). Still, while there is an apparent correlation between the rank order of potency measured in the CaSR assays and the rank order in relative taste intensities for these molecules, there is a drastic discrepancy between the EC_{50} values and taste threshold concentrations. For example γ-Glu-Ala and GSH have low μM potencies in the CaSR assays (0.7 μM to 5 μM) (Broadhead et al. 2011; Amino et al. 2016) but their taste detection threshold in humans is about 3 orders of magnitude higher (900 μM to 3 mM) (Dunkel et al. 2007; Toelstede et al. 2009). Moreover, further studies revealed that kokumi peptides actually act as positive allosteric modulators (PAMs) of the CaSR rather than as agonists (Broadhead et al. 2011) indicating that in the absence of calcium these compounds are inactive. Indeed, at a calcium concentration less than 1 mM, potent γ-glutamyl peptides such as GHS, S-methyl GSH and γ-Glu-Ala fail to activate the CaSR in vitro (Broadhead et al. 2011). Therefore, since these molecules behave as PAMs, sensory experiments conducted to measure γ-glutamyl peptide's potential effect on kokumi taste should be done in the presence of calcium, a fact not necessarily clearly explained or indicated in published reports linking γ-glutamyl peptides to kokumi taste. In fact, tasting protocols typically specify the use of γ-glutamyl peptides, in the presence of high levels of MSG (0.02% to 0.1%), IMP (0.02% to 0.05%), and NaCl, without mentioning the use of calcium (Ohsu et al. 2010; Amino et al. 2016, 2018). Even calcium ions diluted in saliva are unlikely to contribute to the activity of these kokumi peptides. Average concentration of calcium ions in human saliva is 1.2 mM (Sewon et al. 1998) and sipping a taste solution of even a few mL most likely dilutes the saliva calcium concentration to levels significantly below 1 mM, as the average human saliva volume is around 1 mL (Lagerlöf and Dawes 1984) and taste solution sipping volumes for these studies typically range from 5 mL to 20 mL (Ohsu et al. 2010; Amino et al. 2018). Finally, the expression pattern of CaSR in taste buds is puzzling. It is found in too many cell types to accommodate a labeled line model of taste detection. Notably, activation of Type III cells by CaSR should be expected to lead to a sour or salty sensation, not a kokumi taste (Chandrashekar et al. 2006; Oka et al. 2013).

Additional mechanisms are likely at play to explain kokumi taste. We recently observed that typical kokumi compounds such as the di-peptide α-Glu-Ala, γ-Glu-Ala, or the tripeptides GSH and Glu-Glu-Leu (Toelstede et al. 2009; Ohsu et al. 2010; Broadhead et al. 2011; Kuroda and Miyamura 2015; Amino et al. 2016) activate the T1R1/T1R3 umami taste receptor with EC_{50}s (Fig. 9, Table 1) close to their reported taste detection thresholds in the mM range. Importantly, IMP is capable of modulating the activity of kokumi peptides such as α-Glu-Ala,

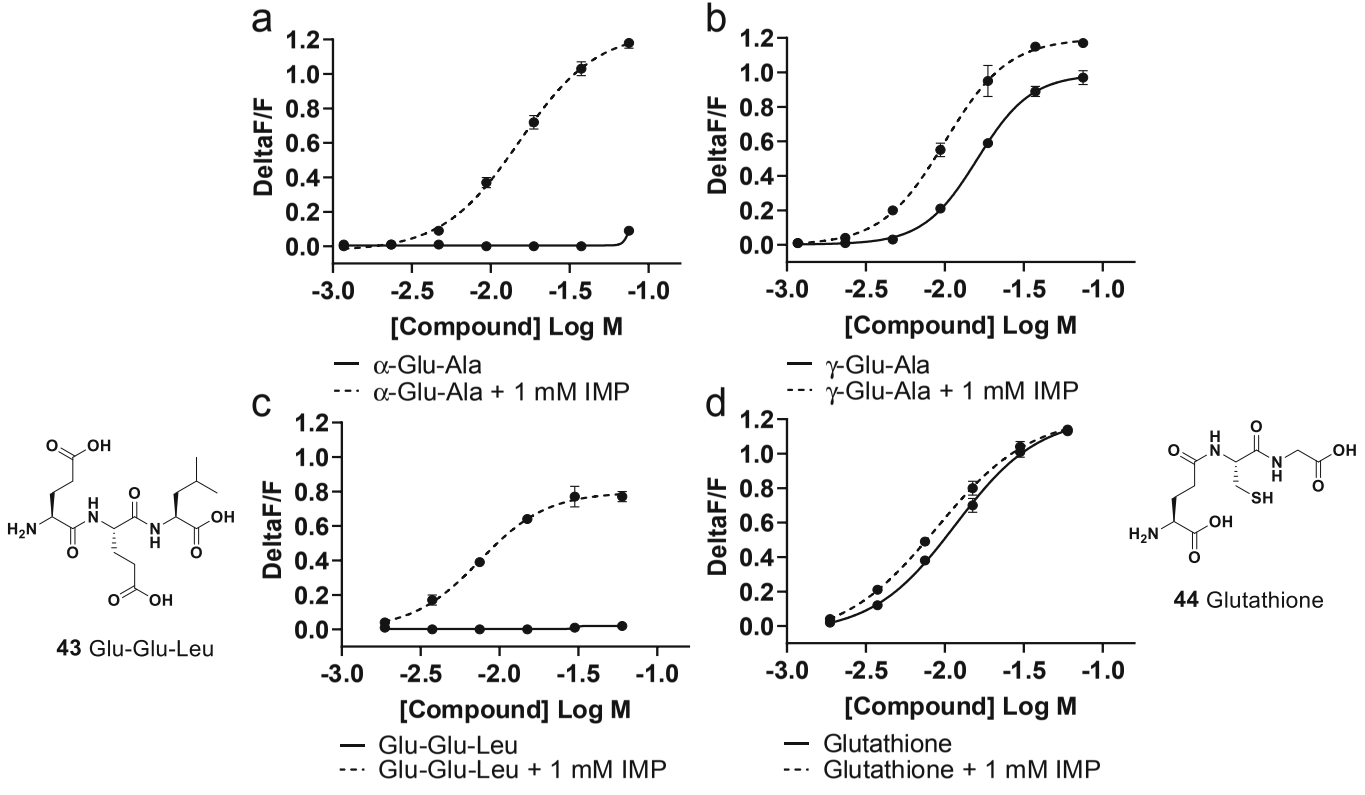

Fig. 9 Kokumi tasting peptides are active in a T1R1/T1R3 cell-based assay. Depicted peptides activate T1R1/T1R3 in a dose-dependent fashion. α-Glu-Ala and Glu-Glu-Leu activities can only be detected in the presence of IMP, indicating that these peptides are of low potency

γ-Glu-Ala, and Glu-Glu-Leu (Fig. 9, Table 1). This is also of importance since the kokumi taste analysis reported is characteristically performed in the presence of high concentrations of IMP (0.02% to 0.05%) (Ueda et al. 1997; Ohsu et al. 2010; Amino et al. 2018). Kokumi substances have recently been reported to activate TG neurons in mice, indicating that, in addition to a gustatory pathway, these molecules also recruit a somatosensory pathway (Leijon et al. 2019). This could explain the textural components of thickening and continuity usually attributed to these molecules. In short, kokumi taste is still a subject of intense debate. Whether kokumi is a new taste modality on its own, whether the CaSR is a genuine primary taste receptor or whether kokumi taste is just the result of umami taste receptor agonists also exhibiting some TG or other effects still needs to be elucidated.

6 Future Research on Umami Taste

As described earlier, it is still not clear if receptors other than T1R1/T1R3 are really involved in the detection of umami taste in rodents. The leading candidates, brain mGluR1 and brain mGluR4 lack the proper pharmacology while their taste variants have only been so far poorly functionally and physiologically characterized. Notably, it is not obvious that these variants *should* be able to bind MSG since most or all of the orthosteric binding domain is missing. Furthermore, K/O studies are a challenge since deletion of any one of these genes could affect not only primary detection of MSG but also neurotransmitter taste signaling. Gain of function studies, such as classic ectopic transgene expression of these targets in bitter TRCs could help in their validation as genuine taste receptors (Mueller et al. 2005).

Kokumi taste is getting more attention but the receptors, cells, and sensory pathways involved are not fully understood. The CaSR has remained more or less unchallenged since it was first proposed as a candidate primary receptor for kokumi taste (Ohsu et al. 2010). Indeed, the inherent properties of these molecules providing their reported taste enhancement effects mostly in the presence of IMP suggest that the T1R1/T1R3 receptor could actually be one of the key elements of the pathway. As shown in this chapter, four canonical kokumi γ-glutamyl peptides, α-Glu-Ala, γ-Glu-Ala, GSH, and Glu-Leu-Leu activate the umami taste receptor. Screening of additional kokumi peptides with different structure-activity relationships should help further our understanding on the role of T1R1/T1R3. Importantly, characteristics such as “mouthfulness,” “continuity,” and “thickness,” usually used to describe kokumi taste, would not be expected to be associated with activation of a primary taste receptor expressed in TRCs but rather receptors or channels expressed in TG neurons. So, it is possible that there is actually no kokumi primary taste receptor per se – other than T1R1/T1R3, but rather just a collection of savory peptides modulating both T1R1/T1R3 and TG receptors involved in mechanosensation or mouthfeel (Flegel et al. 2015). A tantalizing hypothesis is that the CaSR could even correspond to the actual umami peptide-activated somatosensory receptor expressed in TG neuron projections (Heyeraas et al. 2008) in the oral mucosa, perhaps explaining the apparent correlation between kokumi taste effect and activation of

the CaSR as well as the huge discrepancy between the EC_{50} values of cell-based assay potency and detection taste thresholds (since TG receptors are not readily accessible to ligands dissolved in saliva).

A structure for the heterodimeric T1R1/T1R3, in the presence or absence of selected modulators, is still missing. Importantly we still do not fully understand the role of the T1R3-VFD for binding or activation by agonists since the binding site for orthosteric agonists such as MSG (and probably derivatives and peptides) has been mapped to the T1R1-VFD (Fig. 4) (Zhang et al. 2008; Toda et al. 2013). Finally, taste receptors are also expressed outside the oral cavity and the functional significance of this expression pattern is still an important, relatively unexplored area of research (Zhuge et al. 2020).

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Conflict of Interest Statement Guy Servant and Eric Frerot are employed at Firmenich, a company involved in the development and commercialization of flavors and fragrances.

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The Application of In Silico Methods on Umami Taste Receptor

Giulia Spaggiari, Francesca Cavaliere, and Pietro Cozzini

Contents

Abstract

The umami taste receptor is a heterodimer composed of two members of the T1R taste receptor family: T1R1 (taste receptor type 1 member 1) and T1R3 (taste receptor type 1 member 3). Taste receptor T1R1-T1R3 can be activated, or modulated, by binding to several natural ligands, such as L-glutamate, inosine-5'-monophosphate (IMP), and guanosine-5'-monophosphate (GMP). Because no structure of the umami taste receptor has been solved until now, in silico techniques, such as homology modelling, molecular docking, and molecular dynamics (MD) simulations, are used to generate a 3D structure model of this receptor and to understand its molecular mechanisms. The purpose of this chapter is to highlight how computational methods can provide a better deciphering of the mechanisms of action of umami ligands in activating the umami taste receptors leading to advancements in the taste research field.

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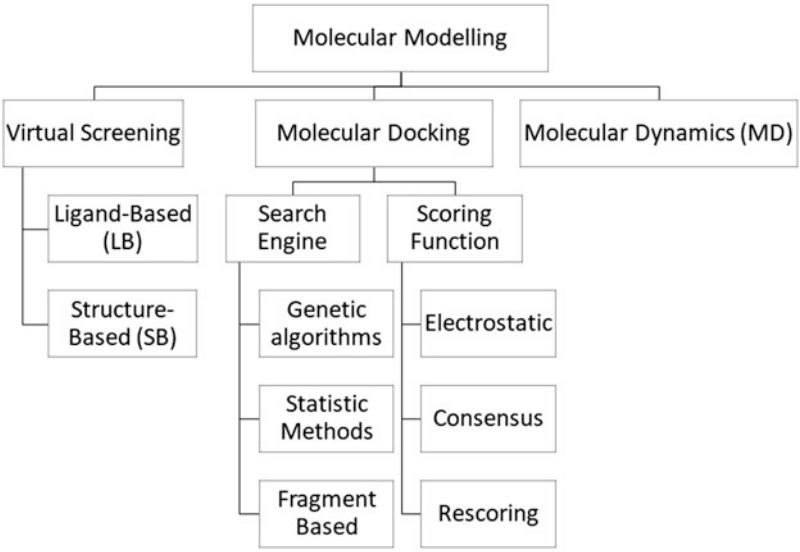

Fig. 1 Flowchart of molecular modelling approaches. Molecular modelling and simulation imply a multi-step process ad combine different analysis methods

Keywords

Homology modelling · In silico techniques · Inosine-5′-monophosphate · L-glutamate · Molecular dynamics · Umami taste receptor

1 Introduction

In silico techniques are used to study molecular systems and to predict the physical-chemical proprieties of molecules in the fields of computational chemistry and biology and material sciences. Molecular modelling includes techniques, such as homology modelling, molecular docking, and molecular dynamics, which are used to represent and/or simulate the behavior of molecules. The use of computers and information technologies allows us to predict the behavior of biological molecules quickly and at a low cost. In this chapter, we focus our attention on molecular modelling approaches, such as virtual screening, molecular docking, and molecular dynamics (Fig. 1).

Several structural and non-structural molecule databases are currently available for virtual screening tools: (1) ZINC, a free database of commercially available compounds (Irwin and Shoichet 2005), (2) ChEMBL, a database of bioactive molecules (Gaulton et al. 2017), (3) HMDB, a database of human metabolites (Wishart et al. 2018), (4) FooDB (http://foodb.ca/), a database of food-derived compounds, (5) FADB, a food additive database (Ginex et al. 2014), and (6) BitterDB, a database of bitter compounds (Dagan-Wiener et al. 2019). Protein structures used for structural analyses may come from experimental determinations,

such as X-ray, nuclear magnetic resonance (NMR) spectroscopy, cryogenic electron microscopy (cryo-EM), or computational structure prediction. These proteins are stored in various structural databases, such as the Protein Data Bank (PDB) (https://www.rcsb.org/). In PDB, protein 3D structures are represented as a set of coordinate triplets (x, y, and z) that define the position of protein atoms. The quality of the PDB structure is defined by two parameters: (1) the resolution value that is influenced by how well the crystal diffracts and by the amount of time needed to collect resolution data, and (2) the B-factor value that monitors the oscillation amplitudes of the protein atoms around their equilibrium positions, or it can be defined as a probability density function for the location of each atom in the protein (Cozzini et al. 2008). However, not all protein structures are crystallized. If a molecule cannot adopt sufficient compact and rigid structures to pack and form a crystal, the X-ray crystallography technique cannot be used. This gap can be filled in by homology modelling, the most common computational technique used for 3D structure protein prediction. The accuracy of 3D structures obtained by homology modelling is highly dependent on the sequence identity of the reference structural models. Several methods have been developed to check the quality of protein structures, such as PROCHECK (Laskowski et al. 1993) which evaluates the stereochemistry of the structures, and ProSA-web (Wiederstein and Sippl 2007) which evaluates the energy distribution of the models.

Virtual screening (VS) is a powerful tool used to predict the activity of a huge number of compounds in a reasonable time. Virtual screening approaches can be divided into ligand-based (LB) when information of known ligands is used or structure-based (SB) when the information of the targeted protein binding site is used. Structure-based virtual screening mostly uses molecular docking simulations to predict the most favorable orientation or binding mode of a compound into the protein target. Molecular docking is a computational technique that aims to find the most favorable binding mode of a ligand to the target protein. The identification of the most likely binding conformations requires two steps: (1) the different ligand conformations predicted in the protein active site, (2) the interaction energy (scoring function) associated with each of the predicted binding conformations. Numerous sampling algorithms are developed to calculate low-energy binding modes for the ligands and they characterize different molecular docking programs: (1) DOCK (http://dock.compbio.ucsf.edu/) algorithm addresses rigid-body docking using a geometric matching algorithm (MA) to superimpose the ligand within the image of binding pocket, (2) SLIDE (Zavodsky et al. 2002) (Zavodszky et al. 2009) is characterized by an incremental construction (IC) algorithm that fragments the ligand from rotatable bonds into various segments, (3) AutoDock (Goodsell et al. 1996) is defined by a Monte Carlo (MC) technique that gradually modifies the ligand using bond rotation and translation or rotation of the entire ligand, and iv) GOLD (http://www.ccdc.cam.ac.uk/products/life_sciences/gold/) is characterized by a genetic algorithm (GA) that is similar to the MC method but is used to find the global minima of the function. These molecular docking approaches can be divided into rigid docking if bond angles, bond lengths, and torsion angles of the ligand and the protein are not modified, and flexible docking that permits conformation

changes. Molecular docking programs use scoring functions to estimate the binding affinity of the predicted ligand-receptor complexes, to delineate the correct poses, defined as a number of possible conformations/orientations of the ligand within the protein binding site, from incorrect poses. Scoring functions can be divided into (1) force-field-based that usually considers the interaction energies of the protein-ligand complex (non-bonded terms) and the internal ligand energy (bonded and non-bonded terms), (2) empirical that approximates the binding energies of the complex by the sum of individual energy components (hydrogen bond, ionic interaction, hydrophobic bond, and binding entropy), and (3) knowledge-based scoring functions that obtain the interatomic contact frequencies and/or a distance between the two components used in a statistical analysis of the ligand-protein complex (Ferreira et al. 2015).

The increasing power of supercomputers allows us to carry out microsecond-scale molecular dynamics (MD) simulations in a few days or a week depending on the architecture of the system. The atoms in a biomolecule are in constant motion and both the molecular functions and the intermolecular interactions depend on the dynamics of the molecules involved. MD is a computational technique used to analyze the physical movements of atoms and molecules and to investigate the structure, dynamics, and thermodynamics of a biological system using high performance computers. Molecular dynamics simulation is based on Newton's second law or the equation of motion, F = ma, where F is the force exerted on the particle, m is the mass and a is the acceleration. From the knowledge of the force on each atom, it is possible to determine the acceleration of each atom in the system. Integration of the equation of motion then yields a trajectory that describes the positions, the velocities, and the accelerations of the particles as they vary with time. From the trajectory, average values of properties can be determined. MD trajectories provide a view of the motion of a molecular system in time-space, allowing determinations of the macro flexibility and the influence of the solvent. Water molecules solvate the protein but can also enter the cavity binding site and influence its shape or mediate the ligand-receptor binding. There are different approaches to treat water molecules during the simulations. When water molecules play an important stabilizing effect, explicit water treatment should be used. Some kinds of parameters can be exploited to analyze the MD simulation results. The most common is the use of (1) root-mean-square-deviation (RMSD),[1] which evaluates the general movements of the protein

[1]Root-mean-square-deviation (RMSD): standard measure of structural distance between coordinates. It measures the average distance between a group of atoms. The RMSD of certain atoms in a molecule with respect to a reference structure, r^{ref}, is calculated as

$$\text{RMSD}(t) = \left[\frac{1}{M}\sum_{i=1}^{N} m_i \left| r_i(t) - r_i^{\text{ref}} \right|^2\right]^{\frac{1}{2}}$$

where $M = \sum_{i=1}^{N} m_i$ and $r_i\,(t)$ is the position of atom i at time t after least square fitting the structure to the reference structure.

during the simulation time, (2) root-mean-square-fluctuation (RMSF),[2] which is used to evaluate the average motion of the residues during the MD. Additionally, the hydrogen bonds network between protein and ligand and/or protein and coactivator/corepressor can be monitored during the simulation time to explore in more detail how ligand interacts with the protein.

2 Case Study: The Umami Taste Receptor

The umami taste receptor belongs to the G protein-coupled receptors (GPCRs), one of the largest families of proteins in the mammalian genome (Schiöth and Fredriksson 2005; Venter et al. 2001). These receptors are activated by a wide variety of ligands, including peptide and non-peptide neurotransmitters, hormones, growth factors, and odorant molecules. GPCRs bind agonists (ligands) and activate specific heterotrimeric G proteins, leading to the modulation of downstream effector proteins (Rosenbaum et al. 2014). The umami taste receptor is a member of the family C GPCRs, characterized by seven transmembrane α-helices linked to a large extracellular N-terminal ligand binding region with a "Venus flytrap" (VFT) fold, having two lobes connected by a hinge (Schiöth and Fredriksson 2005; López Cascales et al. 2010), and a C-terminal intracellular domain. The umami taste receptor is a forced heterodimer composed of two monomers, T1R1 and T1R3 which exist in an "open" conformation in their unliganded form (López Cascales et al. 2010). L-glutamate was discovered as an umami tastant in 1908 by Ikeda. Glutamate umami taste intensity can be strongly potentiated by purinic ribonucleotides, such as IMP and GMP (Dang et al. 2014; Zhang et al. 2008). These ligands are the most renowned modulators of the umami taste receptor, but many other substances, including peptides and free amino acids, can elicit and/or enhance the umami taste (Yu et al. 2017; Zhang et al. 2019). The umami taste receptor shares the subunit T1R3 with the sweet taste receptor (T1R2-T1R3) but they recognize different types of taste stimuli via the VFT domains of T1R1 and T1R2 (taste receptor type 1 member 2), which are probably responsible for the ligand binding (Zhang et al. 2008). Once these multiple interactions have stabilized the activated "closed" conformation, the receptor is able to activate the intracellular signalling. Within the binding pocket of T1R1, two groups of residues have been identified: those that bind the amino acid ligands, such as L-glutamate, and those that

[2]Root-mean-square-fluctuation (RMSF): average deviation of a particle over time from a reference position. It analyzes the portions of the structure that are fluctuating from their mean structure. The RMSF is a measure of the deviation between the position of particle i and some reference position:

$$\mathrm{RMSF}_i = \left[\frac{1}{T} \sum_{t_j=1}^{T} \left| r_i(t_j) - r_i^{\mathrm{ref}} \right|^2 \right]^{\frac{1}{2}}$$

where T is the time over which one wants to average and r_i^{ref} is the reference position of particle i.

bind the purinic ribonucleotides (Zhang et al. 2008). The first interaction is established between L-glutamate and the first group of residues that are inside binding pocket. Cascales and co-workers have identified seven fundamental residues that are hypothesized to be involved in the interaction with L-glutamate: Ser148, Thr149, Ser172, Ala170, Glu301, Arg277, and Arg151 (López Cascales et al. 2010). In addition, Zhang and co-workers, using mutagenesis analysis, have identified other residues that are probably involved in the same interaction: Asp192 and Tyr220 (Zhang et al. 2008). On the other side, four critical residues for the binding with the purinic ribonucleotides are placed near the opening of the pocket: His71, Arg277, Ser306, and His308 (Zhang et al. 2008). Considering the T1R3 monomer, two groups of key residues might also be involved in the ligand (L-glutamate) interactions: the first, Glu124, Ser146, Asp166, and Glu277, and the second, Ser147, Ser170, His145, and Gly168 (Dang et al. 2019; López Cascales et al. 2010).

Current understanding of the umami taste receptor is limited, both with regard to the structure and the mechanism of umami taste perception, which is mainly studied through panel tests. No three-dimensional (3D) structure of the receptor deriving from X-ray crystallography or NMR studies of the human umami receptor has been achieved to date. Therefore, the only way to obtain the most realistic possible structure for our studies is the application of homology modelling, which models the protein based on other similar protein with known 3D structures. The umami taste receptor shares chemical and physical similarity with other receptor classes, such as the metabotropic glutamate receptors (mGluRs) (Dang et al. 2014; Toda et al. 2017). They are characterized by a binding pocket consisting of amino acids (AA) residues with physical-chemical characteristics similar to the T1R1 binding pocket. Another similarity is found with T1R2 monomer of the sweet taste receptor (Li et al. 2002; Hoon et al. 1999). The crystallographic structure of the *Medaka fish* T1R2-T1R3 ortholog (PDB ID: 5X2P) is available in the PDB and has also been used to build T1R1 and the T1R3 homology models (Nuemket et al. 2017). The aim of this work is to provide a summary of the tools and approaches typically used in homology modelling and docking studies. In fact, in the following case study, T1R1 and T1R3 models were built and molecular docking was performed using a series of ligands (L-glutamate, IMP, and GMP) in order to predict and evaluate the structural–physical interactions with the receptor subunits. In addition, so far, no such molecular dynamics simulation has been done on the heterodimer T1R1-T1R3 in complex with L-glutamate and IMP bound to the monomer T1R1 and with L-glutamate bound to the monomer T1R3. Thus, in order to study the stability during the time of the system, two molecular dynamics simulations were computed: 500 ns of the heterodimer T1R1-T1R3 in complex with L-glutamate, and 500 ns of the heterodimer T1R1-T1R3 in complex with L-glutamate and IMP. This work allowed us to study the synergistic effect and the mechanism of action of nucleotides, in particular of inosine-5′-monophosphate, focusing our attention on conformational changes of the dimer structure after the binding of the L-glutamate and IMP.

Table 1 The first alignment set. The first alignment set between T1R1, T1R2, and T1R3 AA sequences of *Medaka fish* and *H. sapiens*. The "range" means a percentage of aligned AA sequences. The percentages of positives and the percentages of Identities between the AA sequences of three receptors are shown in the table

Alignment	Species	% Positives	% Identities
T1R3 T1R3	*Medaka fish* *Homo sapiens*	56%	37%
T1R3 T1R1	*Homo sapiens* *Homo sapiens*	Two ranges: 49% and 52%	Two ranges: 31% and 39%
T1R2 T1R1	*Homo sapiens* *Homo sapiens*	Three ranges: 52%, 50% and 63%	Three ranges: 34%, 36% and 55%
T1R2 T1R1	*Medaka fish* *Homo sapiens*	Three ranges: 53%, 60% and 66%	Three ranges: 38%, 36% and 44%
T1R3 T1R1	*Medaka fish* *Homo sapiens*	Three ranges: 50%, 52% and 68%	Three ranges: 34%, 25% and 47%

3 Alignment of Amino Acid Sequences and Molecular Model of T1R1 and T1R3

BLASTP (Boratyn et al. 2012; Altschul et al. 1990) was used as searching tool to find the sequences of the protein that are more close to T1R1. The first alignment set was performed by taking as a reference the AA sequences of T1R1, T1R2, and T1R3 receptors, achieving positives scores (a chemical-physical similarity between two AA of two aligned sequences) >50% and identity scores (a quantity that expresses the similarity of two sequences) <50% (Table 1).

Since it is well known that T1R1 binds L-glutamate and that the metabotropic glutamate receptors (mGluR) structurally resemble T1R1, we also analyzed the alignment between crystal structure of different subtypes of mGluR and T1R1. For this purpose, among the structures found using the BLASTP tool, the sequences and the three-dimensional structures of mGluRs (subtypes 2, 3, 5, and 8) bound to glutamate were retrieved by PDB. The second alignment set was performed between AA sequences of mGlu2, mGlu3, mGlu5, mGlu8 and T1R1 AA sequence of *H. sapiens* (Table 2). However, no significant differences in the percentages of similarities were obtained compared to first alignment.

Because one of the aims of the present study was to understand the interaction between umami ligands and T1R1-T1R3 dimer, we also focused our attention on the ligand binding pocket. A third alignment set was performed to show the chemical-physical similarities among the binding pocket residues of the receptors considered.

Table 2 The second alignment set. The second alignment set between T1R1 AA sequence of *H. sapiens* and mGluR (subtypes 2, 3, 5, and 8) AA sequences of *H. sapiens*

Alignment	% Positives	% Identities
T1R1 mGlu2	Two ranges: 41% and 49%	Two ranges: 29% and 38%
T1R1 mGlu3	Three ranges: 44%, 43%, and 72%	Three ranges: 32%, 24%, and 55%
T1R1 mGlu5	Four ranges: 43%, 48%, 66%, and 70%	Four ranges: 26%, 25%, 56%, and 50%
T1R1 mGlu8	Six ranges: 45%, 46%, 54%, 58%, 70%, and 38%	Six ranges: 30%, 29%, 33%, 41%, 40%, and 29%

Table 3 The third alignment set. The third alignment set of T1R1 AA sequence of *H. sapiens*, T1R2 AA sequence of *Medaka fish*, T1R3 AA sequence of *Medaka fish* and mGluR (subtypes 2, 3, 5, and 8) AA sequences of *H. sapiens*. The residue numbers refer to the mGlu2 AA sequence

AA	mGlu8	mGlu2	mGlu3	mGlu5	T1R1	T1R2	T1R3
Arg61	Arg	Arg	Arg	Arg	Leu	Arg	Arg
Ala166	Ala	Ala	Ala	Ser	Ala	Gly	Gly
Asp295	Asp	Asp	Asp	Asp	Glu	Asp	Ser
Thr168	Thr	Thr	Thr	Thr	Ser	Ser	Thr
Ser145	Ser	Ser	Ser	Ser	Thr	Ser	Ser
Lys377	Lys	Lys	Lys	Lys	Ser	Phe	Thr
Gly296	Ser	Gly	Gly	Gly	Ala	Gly	Ser
Tyr144	Ala	Tyr	Tyr	Ser	Ser	Thr	Ser
Tyr216	Tyr	Tyr	Tyr	Tyr	Tyr	Phe	Tyr

The AA residues are shown in Table 3 (residue numbers refer to mGlu2 AA sequence). From the comparison, we note that: (1) mGluRs are characterized by the same AA residues, with the exception of two residues (Gly296 and Tyr144); (2) T1R2 and T1R3 receptors are characterized by the same AA residues or by AA residues with similar chemical-physical properties (e.g., serine and tyrosine); (3) among T1R1 binding pocket residues, Ala166, Thr168, Ser145, and Tyr216 are similar with the corresponding mGluRs; (4) T1R1 receptor is characterized by AA residues similar to T1R2 and T1R3.

FLAP (Fingerprints for Ligands And Proteins) (Baroni et al. 2007) was used to evaluate the binding pockets of proteins found from the alignment analysis with the following PDB codes (Berman et al. 2000): mGlu2 (PDB ID: 5CNI), mGlu3 (PDB ID: 5CNK), mGlu5 (PDB ID: 3LMK), mGlu8 (PDB ID: 6BSZ), chain A of T1R2 (PDB ID: 5X2P), and chain B of T1R3 (PDB ID: 5X2P). FLAP provides a common framework for comparing molecules. Fingerprints derive from GRID Molecular Interaction Fields (MIF) and/or GRID atom type and are characterized as quadruplets of pharmacophoric characteristics. The MIFs produced by the GRID force field describe type, strength, and direction of interactions that a molecule is able to establish. Fingerprints can be used directly to compare two molecules, or they can be used to overlay multiple molecules, allowing the more detailed MIF similarity

to be calculated. The analysis showed that all the receptors considered were characterized by hydrogen-bond acceptors, hydrogen-bond donors, and hydrophobic interaction regions. In fact, we found that amino acids with similar chemical-physical properties are present in the same position of the receptor binding pockets (mGlu2, mGlu3, mGlu5, mGlu8, T1R2, and T1R3).

Since the structure of T1R1 and T1R3 monomers of *H. sapiens* is not available in PDB their structures were modelled using homology modelling techniques. The T1R1 receptor model was built based on T1R2, T1R3, and mGluRs (subtype 2, 3, 5, and 8) structures, while the T1R3 receptor model was built based on T1R3 *Medaka fish* and mGluRs (subtype 5) structures. Two different software programs, SWISS-MODEL and Phyre2, were used with the goal to obtain a structural consensus model. SWISS-MODEL builds a homology model through four steps: (1) identification of structural templates, (2) alignment of the target sequence and template structures, (3) model-building, and (4) model-quality evaluation. I-TASSER uses a method to identify different structural fragments that are similar to the query structures. The different fragments are then reassembled into full-length models which are refined based on the free-energy states and at an atomic level using fragment-guided molecular dynamics simulations. Finally, multiple model-quality assessment methods are used to select the best model. Firstly, we calculated the alpha carbon RMSD values of the two superimposed models, both in the case of T1R1 and T1R3 models. The global RMSD is a quantitative measure of the similarity between two superimposed atomic coordinates: (1) 1.359 Å from T1R1 SWISS-MODEL and Phyre2 models; (2) 0.539 Å from T1R3 SWISS-MODEL and Phyre2 models. The RMSD values obtained confirmed that no critical differences were present between the two models. Thus, with the aim to choose the more reliable model, we used ProCheck and ProSA-web programs. The ProSA-web score was used to evaluate the z-score of each model that indicates the overall model quality: a high z-score (greater than zero) may indicate problematic parts of a fold. The ProCheck program provides a detailed check on the stereochemistry of a protein structure. We have considered G-factor, a parameter that provides a measure of how unusual, or out-of-the-ordinary, a property is. If the model has a total score of −0.5 or lower, the structure is considered of high quality. The overall z-score and G-factor of the models generated by Phyre2 were, respectively, −5.2 and 0.11 for T1R1, and −6.83 and −0.19 for T1R3. Those of the models generated by SWISS-MODEL were, respectively, −8.72 and −0.10 for T1R1 and −8.32 and −0.14 for T1R3. We analyzed the z-score and G-factor scores obtained from T1R1 and T1R3 models and found that T1R1 and T1R3 models generated by SWISS-MODEL presented a z-score and a G-factor score better than models generated by Phyre2. For this reason we have chosen SWISS-MODEL structure for the following molecular docking simulations.

4 Molecular Docking and Rescoring Procedures

The GOLD software v5.2.2 (Jones et al. 1997) was applied to dock ligands into the binding site of the receptors. GOLD (Genetic Optimization for Ligand Docking) is a software developed through the collaboration between the University of Sheffield, GlaxoSmithKline, and CCDC (Cambridge Crystallographic Data Center; https://www.ccdc.cam.ac.uk/solutions/csddiscovery/components/Gold/). It is a software widely validated and used, capable of predicting the correct binding mode of the ligand. GOLD allows the performance of a semi-rigid docking where the flexibility of the ligand and some residues of active site is considered, up to a maximum number of ten residues. The software can use three different genetic algorithms, and different scoring functions (GoldScore, ChemScore, and ASP), to generate and evaluate different poses of the ligand in the receptor pocket. Moreover, we used another scoring function, HINT, specifically developed as docking post-processor that considers both entropic and enthalpic effects.[3] From the first molecular docking performed on T1R1 and T1R3 receptors against L-glutamate and from subsequent rescoring, all HintScore (HS) values were positive and mostly >1,000 HS: this means that ligand interacts with receptor (Table 4). The docking results among the T1R1 receptor, L-glutamate, and IMP/GMP confirm the synergism between receptor and purinic ribonucleotides. In fact, HS values were all positive, especially those resulting from interactions of IMP/GMP and fundamental receptor residues (Table 4).

We have analyzed the interactions between IMP/GMP and key residues (His71, Arg277, Ser306, and His308): purinic ribonucleotides establish positive high interactions with Ser306 and His308. Moreover, another important interaction having high HS value is found between the purinic ribonucleotide and residue Ser382, confirming the results obtained by Mouritsen and co-workers (Mouritsen and Khandelia 2012). Zhang and colleagues identified the residue Arg277 as one of the key residues for the interaction between IMP and T1R1 (Zhang et al. 2008). However, in our analysis Arg277 established positive high interaction with L-glutamate, remaining one of the fundamental residues of the complex T1R1–L-glutamate–IMP (Fig. 2).

Based on the first docking analysis of L-glutamate and T1R1, the HS value was 1475.67. After the binding of purinic acid, additional interactions were formed among IMP, L-glutamate, and T1R1 raising the total HS value to 4,210 (Kellogg and co-workers stated 515 HS corresponds to 1 Kcal/mol (Eugene Kellogg and Abraham 2000)). Thus, the binding of IMP increases the interaction of ~2 Kcal/mol and, in our opinion, this could stabilize the "closed" conformation.

[3]HINT is a scoring function developed by Donald Abraham and Glen Kellogg of the University of Virginia in 1991 with the collaboration of Pietro Cozzini and Andrea Mozzarelli. HINT (Hydropathic INTeraction) is a force field that allows to estimate the variation of the Gibbs free energy (ΔG^0), expressed in kcal/mol or kJ/mol, which is generated in the formation of the protein-ligand complex, starting from the calculation of the logPo/w.

Table 4 HintScore values of three different molecular docking. (1) HintScore values result from molecular docking between T1R1 and L-glutamate. (2) HintScore values result from molecular docking between T1R1, L-glutamate, and IMP. (3) HintScore values result from molecular docking between T1R1, L-glutamate, and GMP

T1R1-L-glutamate		T1R1-L-glutamate-IMP		T1R1-L-glutamate-GMP	
Pose	HintScore	Pose	HintScore	Pose	HintScore
20	3,365,781	25	4,040,00	1	3,500,00
18	2,352,414	2	3,550,00	27	2,520,00
12	2,284,568	6	3,540,00	7	2,300,00
21	2,126,909	23	3,370,00	16	2,240,00
3	2044,133	22	3,330,00	2	2,210,00
22	2038,79	17	3,010,00	6	2,120,00
6	1990,687	1	2,970,00	21	2020,00
10	1960,944	16	2,920,00	24	1830,00
14	1777,323	30	2,850,00	5	1750,00
25	1769,552	14	2,850,00	28	1,630,00
29	1769,117	26	2,720,00	8	1,560,00
19	1,691,37	7	2,710,00	30	1,470,00
7	1,589,412	20	2,680,00	14	1,450,00
15	1,492,888	24	2,200,00	17	1,240,00
30	1,482,829	29	2,190,00	20	1,030,00
11	1,445,919	18	2010,00	13	990,00
8	1,303,218	8	1970,00	12	958,00
4	1,295,431	15	1,600,00	22	908,00
26	1,292,983	3	1,140,00	9	821,00
27	1,289,814	13	660,00	11	693,00
16	1,247,753	9	289,00	18	692,00
9	1,231,38	27	−135,00	4	665,00
17	1,179,477	21	−188,00	10	362,00
23	1,148,498	19	−582,00	3	322,00
24	1,139,253	11	−715,00	26	257,00
13	997,2,164	10	−3,190,00	19	78,50
28	953,0471	4	−16,100,00	25	−402,00
1	523,0946	12	−40,200,00	15	−511,00
2	190,1,165	28	−46,300,00	23	−1,320,00
5	−131,948	5	−1,790,000,00	29	−11,300,00

5 Model of the Dimer and Analysis of the Interface Key Interactions

T1R1-T1R3 dimer was built for the following molecular dynamics simulation. Since the sweet taste receptor is structurally and functionally very close to umami taste receptor, T1R1 and T1R3 monomers were superimposed on T1R2 and T1R3 monomers of *Medaka fish* structure, respectively (Li et al. 2002; Zhao et al. 2003).

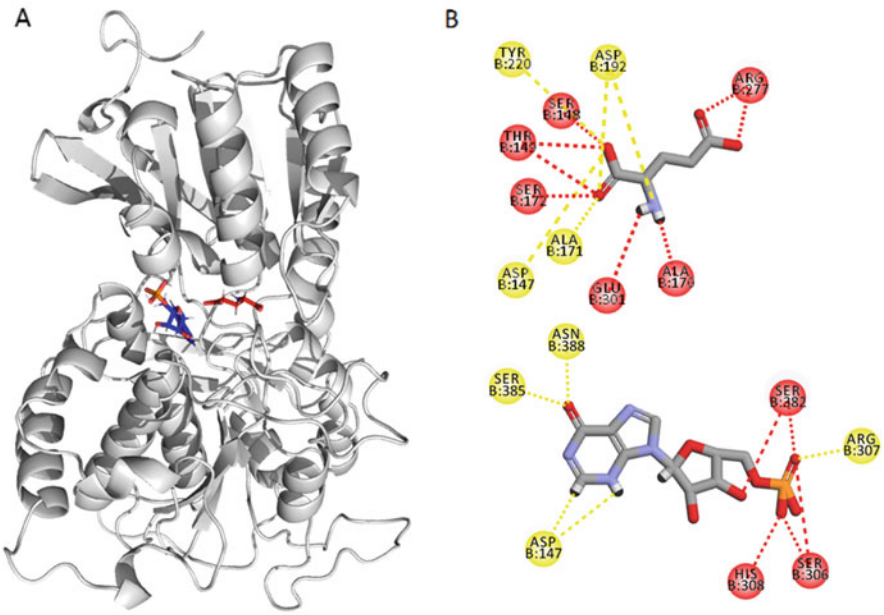

Fig. 2 (**a**) T1R1 in complex with L-glutamate and IMP. The complex T1R1 (*gray*)–L-glutamate (*red*)–IMP (*blue*) obtained from molecular docking. L-glutamate is located deep inside the ligand binding domain, whereas IMP is located near the cavity entrance, as described by Zhang et al. 2008. (**b**) Key residues. On the left, the interactions between L-glutamate and T1R1 residues; on the right, the interactions between IMP and T1R1 residues. Key residues are shown in *red*: Ser148, Thr149, Ala170, Ser172, Arg277, and Glu301 for L-glutamate, and Ser306, His308, and Ser382 for IMP (López Cascales et al. 2010; Zhang et al. 2008). In *yellow* secondary binding interactions between L-glutamate and IMP, and Asp147, Ala171, Asp192, and Tyr220, and Asp147, Arg307, Ser385, and Asn388, respectively (Liu et al. 2019)

To explore the importance of IMP in the dimer stability, two dimer structures, in complex with the respective ligands, were built: (1) one dimer was built taking the best pose of T1R1 in complex with L-glutamate and the best pose of T1R3 in complex with L-glutamate obtained from the respective molecular docking; (2) one was built taking the best pose of T1R1 in complex with L-glutamate and IMP and the best pose of T1R3 in complex with L-glutamate obtained from the respective molecular docking. Then, we analyzed the key interactions on the dimer at the VFT interface. As shown in Table 5, the interactions between T1R1 and T1R3 were stabilized by a combination of hydrophobic and hydrogen interactions of the two receptors.

Table 5 Interactions in the T1R1-T1R3 dimer interface. Residues involved in dimerization are listed along the respective atoms. The intermolecular interactions are grouped into hydrophobic, hydrogen, and electrostatic interactions

Hydrophobic Interactions		Hydrogen Interactions		Electrostatic Interactions	
T1R1	T1R3	T1R1	T1R3	T1R1	T1R3
Ile105	Ile127	Ile105 (H)	Ile127 (O)	Arg157 (NE)	Glu58 (OE1)
Val87	Pro161	Phe139 (O)	Tyr113 (HH)	Arg180 (NE)	Asp229 (OD1)
Ala107	Arg117	Ile105 (O)	Ile127 (H)		
Met90	Ala157	Pro161 (HA)	Glu58 (OE2)		
Val132	Ala157	Pro161 (HD3)	Glu58 (OE2)		
Met90	Leu158				
Phe136	Val112				
Phe136	Leu158				
Phe139	Ala110				
His126	His126				

6 Molecular Dynamics Simulations

To evaluate the stability and the mechanism of interaction of L-glutamate and IMP with T1R1-T1R3 dimers, 500 ns of molecular dynamics simulations were carried out for two different complexes: (1) T1R1-T1R3 both complexed with L-glutamate; (2) T1R1-T1R3 with L-glutamate both in the two receptors and IMP in T1R1. In the case of the molecular dynamics simulation of the T1R1-T1R3 receptor with L-glutamate, no relevant movement of the L-glutamate emerges in the binding pocket of the T1R1 monomer. On the contrary, L-glutamate in the binding pocket of T1R3 monomer goes out of the binding pocket in each three molecular dynamics simulations. This could confirm the hypothesis that L-glutamate interacts with the two monomers establishing more favorable interactions with the T1R1 monomer than T1R3 (López Cascales et al. 2010). In the case of the molecular dynamics simulation of the T1R1-T1R3 receptor with L-glutamate and IMP, no relevant movement of the L-glutamate and IMP emerges in the binding pocket of the T1R1 monomer. On the contrary, L-glutamate in the binding pocket of T1R3 monomer goes out of the binding pocket in each triplicate molecular dynamics simulations. L-glutamate comes out of the pocket before the molecular dynamics without IMP. This can confirm the synergistic effect of these two ligands in the umami taste receptor, in which IMP stabilizes the conformation of the T1R1 monomer (Yoshida et al. 2015; Zhang et al. 2008). The RMSD of the protein backbone was used to monitor conformational changes and, hence, the stability of each system during the total simulation run. From Fig. 3a, it can be seen that the RMSD value of the protein backbone (T1R1-T1R3) for the two systems ultimately reached the equilibrium after 100 ns (maximum 2 Å). Upon binding, the averaged RMSD for the complex of T1R1-T1R3 with L-glutamate, and L-glutamate and IMP was 6.45 and 5.56 Å, respectively. However, in both cases, the stability variations, which are noted up

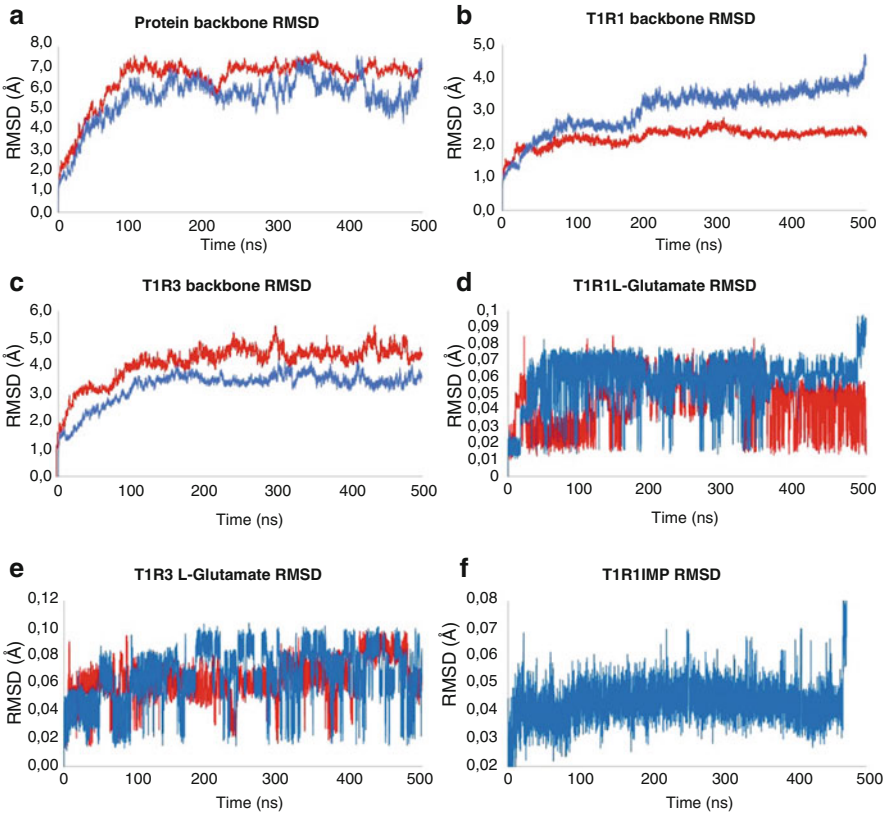

Fig. 3 RMSD results graphics of T1R1-T1R3. The molecular dynamics simulation of L-glutamate in complex with T1R1 and T1R3 is shown in *red*, while the molecular dynamics simulation of L-glutamate and IMP in complex with T1R1 and L-glutamate in complex with T1R3 is shown in *blue*. RMSD of protein backbone (**a**), RMSD of T1R1 (**b**) and T1R3 (**c**) monomer, and heavy atoms of the ligands of T1R1 (L-glutamate) (**d**), T1R3 (L-glutamate) (**e**), and T1R1 (IMP) (**f**)

to 500 ns, are due to the greater instability of the monomer T1R3 (Fig. 3c). To get insights into the stability of the systems, the RMSD value of the T1R1 and T1R3 monomers backbone was calculated. From Fig. 3b, it can be seen that T1R1 is more stable when in complex with L-glutamate and IMP than when in complex with only the L-glutamate. This is probably due to the fact that IMP establishes favorable bonds with protein residues reducing the conformational flexibility of T1R1 compared to the effect of L-glutamate on its own. Thus, the RMSD of each ligand was evaluated for each complex (Fig. 3d–f). During the simulation, there is no significant fluctuation in the L-glutamate molecules when they are present in T1R1. L-glutamate molecules have the same stability in T1R3, but in contrast to the L-glutamate in complex with T1R1, they leave the binding cavity.

In addition, the RMSF of the two complexes (L-glutamate in complex with T1R1 and T1R3, and L-glutamate and IMP in complex with T1R1 and L-glutamate in

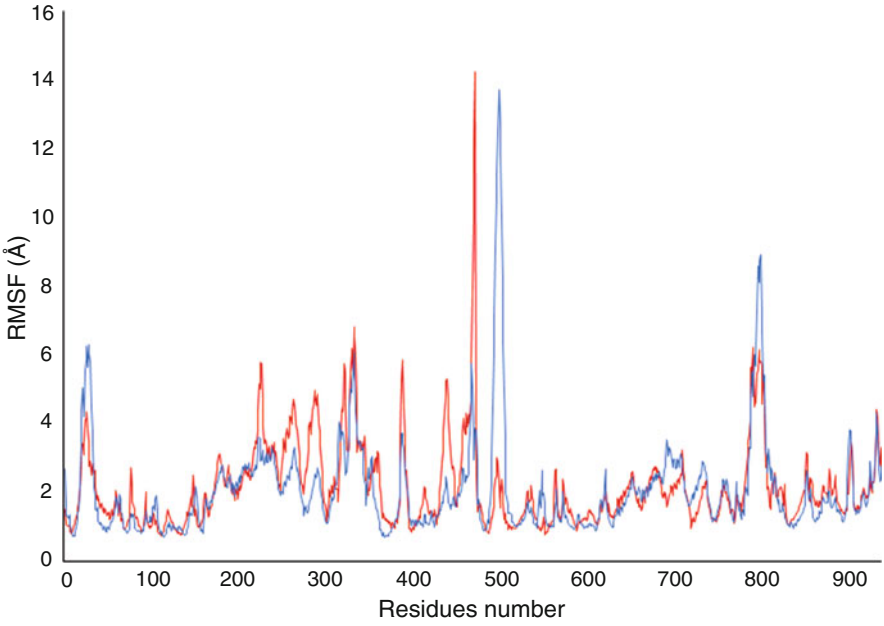

Fig. 4 RMSF of the two complexes, L-glutamate in complex with T1R1 and T1R3 (*red*), and L-glutamate and IMP in complex with T1R1 and L-glutamate in complex with T1R3 (*blue*), obtained by molecular dynamics simulations

complex with T1R3) was monitored to analyze the local mobility of protein residues. As shown in Fig. 4, the complexes had a similar trend. RMSF values >2 Å should correspond to the flexible residues of the protein.

In the same way for the two complexes, the residues (from residue 49 to residue 65, from 237 to 271, from 284 to 295, from 340 to 371, from 455 to 461, and from 483 to 494) in T1R1 monomer in complex with L-glutamate and the residues (from residue 49 to residue 65, from 232 to 295, from 340 to 374, from 455 to 461, and from 479 to 494) in T1R1 monomer in complex with L-glutamate and IMP correspond to loop regions and, therefore, are in constant motion and not present in the binding pocket. On the contrary, the residues (from residue 26 to residue 44, from 180 to 198, from 210 to 303, from 319 to 368, and from 436 to 468) in T1R3 monomer in complex with T1R1–L-glutamate and the residues (from residue 26 to residue 42, from 181 to 277, from 291 to 300, from 316 to 356, from 391 to 399, and from 460 to 476) in T1R3 monomer in complex with T1R1–L-glutamate–IMP correspond to loop regions, but also to regions close to the binding pocket, potentially involved in the interactions with ligands.

The RMSD and RMSF values demonstrate that L-glutamate binds more favorably and stably the T1R1 monomer than T1R3 monomer, to which it binds transiently and more weakly (López Cascales et al. 2010). Moreover, the molecular dynamics simulations results illustrate that IMP plays a synergistic role in the umami taste

receptor, increasing the stability of the monomer conformation to which it binds (Yoshida et al. 2015).

The information regarding the umami taste receptor is currently limited, both in terms of structure and taste perception (experimental data are generally coming from taste panels and cell-based assays), and no 3D structure of T1R1 of *H. sapiens* is presented. In this chapter we present data supporting computational methods, such as homology modelling, molecular docking, and molecular dynamics that can help to further understand ligand–receptor interactions at the molecular level and hopefully help the discovery and design of new umami compounds or enhancers.

As we stated in the recent review (Spaggiari et al. 2020) "*The recent computational advancements in the taste research field, and particularly the computation-driven investigations of the tastant-receptor binding, provided a better understanding of the molecular mechanisms underlying food tastants' sensing and could have an impressive contribution to the identification of new taste modulators in the future.*"

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Pharmacology of TAS1R2/TAS1R3 Receptors and Sweet Taste

Maik Behrens

Contents

Abstract

The detection of energy-rich sweet food items has been important for our survival during evolution, however, in light of the changing lifestyles in industrialized and developing countries our natural sweet preference is causing considerable problems. Hence, it is even more important to understand how our sense of sweetness works, and perhaps even, how we may deceive it for our own benefit. This chapter summarizes current knowledge about sweet tastants and sweet taste modulators on the compound side as well as insights into the structure and function of the sweet taste receptor and the transduction of sweet signals. Moreover, methods to assess the activity of sweet substances in vivo and in vitro are compared and discussed.

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Keywords

Binding site · Coupled receptor · G protein · Signal transduction · Sweet taste · Sweetener · TAS1R2 · TAS1R3

1 Introduction

Sweet taste is one of the five basic taste qualities and, next to umami taste, devoted to the assessment of the energy content of food items (Behrens et al. 2011). Sweetness is innately linked to feelings of pleasure upon consumption, a fact that supported survival during evolution, however, nowadays the striving for sweet food is seen rather differently as more and more cases of obesity and obesity-related syndromes are observed in the populations of industrialized and developing countries (Breslin 2013). Hence, numerous high-potency/low-energy sweeteners have been developed starting already in the nineteenth century and the search continues to date as the optimal sugar replacement has yet to be found (DuBois and Prakash 2012). The target of all these compounds is a highly interesting sensor expressed in sweet taste receptor cells in the oral cavity (Bachmanov et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001). This receptor, a heteromeric class C GPCR with the subunit composition TAS1R2/TAS1R3 (Li et al. 2002; Nelson et al. 2001), is activated by sweet compounds via interactions at both subunits and at various subdomains (Behrens et al. 2011). This chapter gives a brief overview about the main types of sweet tastants, before the sweet taste receptor is described in detail. Next, the transduction of sweet taste signals via the sweet taste receptor and proposed alternative signaling pathways are discussed. The locations of identified binding sites at the sweet taste receptor are summarized later in the chapter, before briefly some details about the evolutionary conservation of sweet taste receptor genes are presented. Then, experimental approaches for studying sweet taste move into the center of the chapter, first focusing on sensory experiments, second on the contribution of in vitro experiments for the elucidation of pharmacological features of the sweet taste receptor. Finally, the rather new discovery of sweet taste receptor modulators is discussed.

2 Sweet Tastants

The perception of sweet compounds allows assessment of the energy content of food. Hence, metabolizable mono- and disaccharides such as glucose and sucrose represent prototypical sweet stimuli, a fact that is underscored by the evolutionary conservation of their recognition by mammalian sweet taste receptors in contrast to a considerable variability in the detection of other types of synthetic as well as natural sweeteners (Jiang et al. 2004, 2005a, b; Li et al. 2002; Nelson et al. 2001; Winnig et al. 2005, 2007). Notably, detection of, e.g., sucrose does not occur with high sensitivity thus preventing that mammals consume energetically irrelevant concentrations of these molecules. Ironically, the elicitation of sweetness without providing metabolizable energy is exactly the purpose of the development of

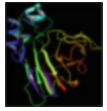
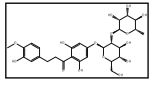
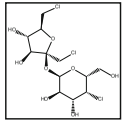
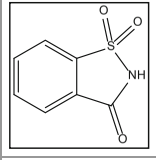
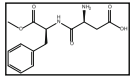
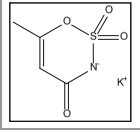
marketable high-potency low or no calories sweeteners to adjust the energy intake of humans in industrialized countries (DuBois and Prakash 2012). Among the best-known synthetic sweeteners on the market (see Table 1) are the sulfonyl amide sweeteners saccharin, acesulfame K as well as cyclamate and the chlorinated form of sucrose, sucralose (DuBois and Prakash 2012). Another group of synthetic sweeteners are based on amino acids as building blocks, aspartame, alitame, and neotame share a dipeptide backbone (DuBois and Prakash 2012). The origin of neohesperidin dihydrochalcone (NHDC) is somewhat peculiar as it is based on the bitter neohesperidin, which is subjected to hydrogenation resulting in the potent sweetener (DuBois and Prakash 2012). Another large group of sweeteners of mostly synthetic origin are sugar alcohols, which are commonly labeled as sugar substitutes (DuBois and Prakash 2012).

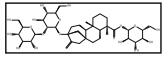
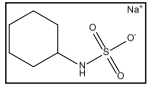
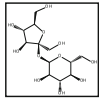
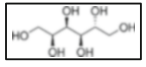
Natural high-potency sweeteners are a rapidly growing group of compounds due to the bias of consumers for non-synthetic food additives. Among the best-known natural high-potency sweeteners are the steviol glycosides from the leaves of the Stevia plant (*Stevia rebaudiana* (Bertoni)). Stevioside, rebaudioside A as well as mixtures of steviosides found in this plant have been on the market for quite some time now (DuBois and Prakash 2012). A variety of sweet proteins are currently already on, or on their way to the market. In general, these sweet proteins are marked by an extraordinary high sweet potency. Examples for sweet proteins are thaumatin, monellin, and brazzein (DuBois and Prakash 2012). For a list of these and further sweeteners, their structural formulas and sweetness potencies, see Table 1.

3 Sweet Taste Receptor Modulators

The fact that mono- and disaccharides, the prototypical sweet taste stimuli, may provoke negative health effects such as overweight-associated metabolic disorders or tooth decay for populations in industrial countries has raised considerable interest in the development of synthetic and natural high-potency sweeteners already decades ago (Servant et al. 2011). However, numerous problems associated with the application of high-potency sweeteners including thermal instabilities during cooking and baking, lack-of-volume in cooking recipes, lingering after-tastes and, most importantly, off-tastes, have prompted researchers to develop molecules with a different mode of action, namely sweet taste enhancers (DuBois 2016). Ideally, these molecules would not impart a taste on their own but would increase the potencies of sweeteners without altering their taste quality profiles. Indeed, the screening with cells expressing the human sweet taste receptor resulted in the discovery of such enhancers, which are, due to their course of action, also called positive allosteric modulators (PAMs). The first PAM was discovered by high-throughput screening of cells expressing human TAS1R2/TAS1R3 using the synthetic high-potency sweetener sucralose (Servant et al. 2010). This molecule, called SE-1, resulted in a pronounced enhancement of the sucralose potency without stimulating the sweet taste receptor on its own. Somewhat surprisingly, SE-1 was rather selective for sucralose. Further modifications of the chemical scaffold of SE-1 resulted in a

Table 1 List of selected sweet compounds

Compound	Formula	Sensory experiments			In vitro experiments	
		RS	MRS	References	EC_{50}	References
Thaumatin		14,200	912,659	Schiffman and Gatlin (1993)		
NHDC (Neohesperidin dihydrochalcone)		906	1,621	Schiffman and Gatlin (1993)	0.2 ± 0.1 mM	Winnig et al. (2007)
Sucralose		636	739	Schiffman and Gatlin (1993)	31–62 μM 80 ± 20 μM	Servant et al. (2010), Masuda et al. (2012)
Saccharin		444	266	Schiffman and Gatlin (1993)	42 ± 3 μM 43 ± 5 μM 190 ± 70 μM	Xu et al. (2004), Behrens et al. (2017), Masuda et al. (2012)
Aspartame		196	169	Schiffman and Gatlin (1993)		
Acesulfame K		140	82	Schiffman and Gatlin (1993)	0.12 ± 0.04 mM 0.54 ± 0.16 mM	Winnig et al. (2007), Masuda et al. (2012)

Stevioside		120	282	Schiffman and Gatlin (1993)	0.06 ± 0.01 mM	Behrens et al. (2006)
Cyclamate		32	19	Schiffman and Gatlin (1993)	2.2 ± 0.3 mM 0.69 ± 0.05 mM 2.56 ± 0.46 mM	Winnig et al. (2007), Behrens et al. (2017), Masuda et al. (2012)
Sucrose		1	1	Schiffman and Gatlin (1993)	19.4 ± 0.9 mM	Xu et al. (2004)
Sorbitol		0.82	0.44	Schiffman and Gatlin (1993)		

The potency of the sweeteners is reported as relative sweetness (RS) and molar relative sweetness (MRS), respectively, determined on the basis of equi-sweetness compared to a 5%-sucrose solution (P_{w5}) (DuBois et al. 1991). The EC_{50}-concentrations (=concentration leading to half-maximal activation of sweet taste receptor-expressing cells) were determined using cell-based calcium mobilization assays using the human TAS1R2/TAS1R3 heteromer. The substances are sorted based on their relative sweetness, starting with the most potent sweetener

small number of PAMs showing selective enhancement of other sweeteners including sucrose (for a review see Servant et al. (2020)). Another independent set of PAMs based on one of the initially described modulators was characterized by a common tripeptide core and permutated subsequently to assess the contribution of the substructures for the enhancement (Matsumoto et al. 2020; Yamada et al. 2019). One of these compounds, which showed a modification in the central region was found superior in sensory experiments (Yamada et al. 2019). For SE-1, the mode of action leading to the strong enhancement of sucralose was investigated as well. Interestingly, SE-1 binds close to the agonist sucralose in the venus-flytrap domain (VFD) of the TAS1R2 subunit (see Sect. 5). As SE-1 binds more anteriorly (=away from the hinge-region) from sucralose, it is believed that the agonist-induced closing of the VFD is stabilized by SE-1 thus explaining the increased potency of sucralose (Zhang et al. 2010). Moreover, it is assumed that the other currently identified PAMs exert the same mode of action, which could also explain the agonist-selectivity of the PAM enhancements (Servant et al. 2020). The striking similarity of these observations with the enhancement of the umami receptor by 5′-ribonucleotides (Zhang et al. 2008) strongly supports the proposed mechanism of PAMs on the sweet taste receptor and suggests the evolutionary conservation of basic pharmacological features among sweet and umami receptors.

In contrast to the previously described PAMs, the best-known and studied inhibitor of the human sweet taste receptor, lactisole, targets a different domain of the receptor, the heptahelical domain (HD) of TAS1R3 (see Sect. 5) (for a review see Sigoillot et al. (2012b)). Although in general, lactisole antagonizes the activation of the sweet taste receptor by all sweeteners investigated so far, its mechanism of action varies significantly and is strictly dependent on the location of the sweeteners binding site within the receptor. For example, sweeteners such as cyclamate or NHDC bind to an overlapping site with lactisole in the HD of the TAS1R3 subunit, and in this case lactisole behaves as a reversible competitive antagonist. Lactisole has also been shown to behave as an irreversible non-competitive antagonist with sweeteners interacting with the VFD of TAS1R2 such as sucrose, sucralose, and saccharin and as a negative allosteric modulator with sweeteners interacting with the HD of TAS1R2 (Servant et al. 2020; Winnig et al. 2007). Other, less well-investigated NAMs for the human sweet taste receptor exist (Sigoillot et al. 2012b). Of those, sweet-taste blockers isolated from the leaves of the plant *Gymnema sylvestre* (GS) received considerable attention. One of the active components present in GS, the polypeptide gurmarin (Imoto et al. 1991) exhibits long-lasting, sweet taste suppressing activity in rodents but not in humans. Another sweet taste suppressing active component is gymnemic acid, a triterpenoid glycoside (Hooper 1887) with effects that differ profoundly from those of GS's. While the sweet taste suppression is also long-lasting and species-specific, gymnemic acid inhibits sweet perception in humans and old-world monkeys but not that of a variety of other species including rodents (Diamant et al. 1965; Hellekant and Gopal 1976; Hellekant et al. 1985). Although the contact points between gymnemic acid and gurmarin and the sweet taste receptor are presently unknown, the differences in

species-specificities and the temporal profiles indicate that inhibition of the sweet taste receptor might be similarly complex as its activation.

4 Structure of the Sweet Taste Receptor

The functional human sweet taste receptor consists of two subunits (see Fig. 1), called TAS1R2 and TAS1R3, of which the TAS1R2 subunit is specific for the sweet taste receptor, whereas the TAS1R3 subunit is shared with the umami receptor consisting of TAS1R1 and TAS1R3 (Hoon et al. 1999; Li et al. 2002; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001, 2002). Distinctive features identify the sweet taste receptor as a typical member of the class C GPCR family, the formation of functional heteromers and the existence of long extracellular amino termini. Both subunits of the sweet taste receptor share the same overall architecture (from N-terminus to C-terminus): An ectodomain consisting of two lobes connected by a hinge-region resembling the leaf of a venus-flytrap plant (VFD), next to the VFD an extracellular cysteine-rich domain (CRD) is located, which is rooted in the

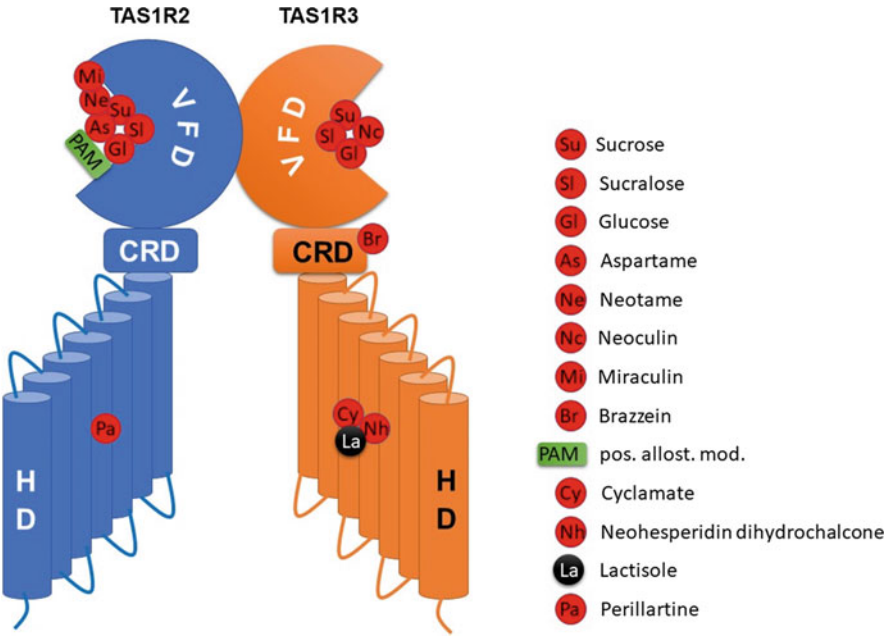


Fig. 1 *Schematic of the human sweet taste receptor with binding sites for the various sweet tastants and modulators.* The sweet taste receptor-specific TAS1R2 subunit (blue) dimerizes with the subunit TAS1R3 (orange) common to sweet and umami receptors. The venus-flytrap (VFD), cysteine-rich (CRD), and heptahelical domains (HD) are labeled. The red circles indicate the various agonist binding sites, the black circle highlights the binding site for the human sweet taste receptor-specific antagonist lactisole. The green box labels the site at which positive allosteric modulators (PAM) bind

cell membrane by a heptahelical domain typical for GPCRs. Experiments using recombinantly expressed ectodomains of the TAS1R2 and TAS1R3 species orthologs of the medaka fish, which share ~35% amino acid identity with human TAS1Rs, but in contrast to the mammalian sweet taste receptor responds to amino acids, demonstrated the formation of stable heterodimers. The heterodimers change conformation upon agonist binding (Nango et al. 2016), suggesting that dimerization is facilitated by the two HDs. This has further been corroborated by the first experimentally determined structure of the full-length medaka fish TAS1R2/TAS1R3 receptor heterodimer (Nuemket et al. 2017).

5 Binding Sites at the Sweet Taste Receptor Subunits

Over the past years a number of binding sites have been mapped within the sweet taste receptor of various vertebrate species (see Fig. 1). A particular fruitful approach originated from the observation that the sweet tasting abilities of humans and rodents differ considerably (Danilova et al. 1998). This, together with the possibility to functionally express the sweet taste receptor in mammalian cell lines (Nelson et al. 2001) and the confirmation of the observed differences between human and rodent sweet taste in vitro (Li et al. 2002), enabled the construction and testing of interspecies chimeric receptors. The first two reports used initially the entire TAS1R2 and TAS1R3 subunits of human and rodents to assign the agonist interaction site to one of the two moieties, then, after the identification of the interacting subunit, intramolecular chimeras were assembled to identify the corresponding subdomains (Jiang et al. 2004; Xu et al. 2004). This allowed the mapping of the interaction sites for the high-potency sweeteners aspartame and neotame to the VFD of human TAS1R2, whereas another high-potency sweetener, cyclamate, and the specific blocker of the human sweet taste receptor, lactisole, exert their functions at the HD of human TAS1R3 (Xu et al. 2004). The sweet protein brazzein exhibited interaction with yet another subdomain, the CRD of human TAS1R3 (Jiang et al. 2004). Subsequently, a considerable number of binding sites of additional sweet substances were mapped onto the human sweet taste receptor in a similar fashion, partially even adding more details about the architecture of the binding site by performing point mutagenesis and molecular modeling experiments. These studies assigned the interaction sites for NHDC (Winnig et al. 2007) and cyclamate (Jiang et al. 2005b) to human TAS1R3-HD and identified the residues critical for lactisole binding in the human TAS1R3-HD (Jiang et al. 2005a) and lactisole-insensitivity in the rodent Tas1r3-HD (Winnig et al. 2005). The binding site for the sweet protein neoculin was found to reside in human TAS1R3-VFD (Koizumi et al. 2007), whereas miraculin, a protein that becomes sweet under acidic conditions (Brouwer et al. 1968; Kurihara and Beidler 1968), binds human TAS1R2-VFD (Koizumi et al. 2011). The human TAS1R2-VFD has also been shown to interact with small molecule positive allosteric modulators (PAMs), which stabilize the ligand bound conformation of this domain by binding close to, but not overlapping with orthosteric agonists such as sucrose and sucralose (Servant et al. 2010; Zhang et al. 2010). Interestingly, the human

TAS1R2-HD alone is sufficient for cellular responses to the sweet compound perillartin in heterologous assays (Slack 2012; Zhao et al. 2018). Following a different approach Nie and colleagues were able to purify the recombinant mouse TAS1R2- and TAS1R3-VFDs and to analyze intrinsic tryptophan fluorescence intensities as indicator for conformational changes induced by ligand interaction in the absence and presence of glucose, sucrose, and sucralose (Nie et al. 2005). It was reported that the VFDs of both sweet taste receptor subunits interact with the 3 compounds albeit with different affinities demonstrating that mono- and disaccharides as well as some synthetic high-potency sweeteners are detected via the VFDs (Nie et al. 2005).

6 Canonical Sweet Taste Transduction

Although the sweet taste receptor occurs in a type II cell population largely separated from cells expressing the other GPCR-type taste receptors, namely the umami (TAS1R1/TAS1R3) and the bitter taste receptors (TAS2Rs), all three GPCRs share common signal transduction components (see Fig. 2). The first step in the sweet taste receptor signal transduction cascade is the activation of heterotrimeric G proteins. The composition of Gβγ-dimers is quite homogeneous, consisting of Gβ1 or Gβ3 and Gγ13 (Huang et al. 1999; Rossler et al. 2000), whereas the types of α-subunits found in various fields of the taste tissue are more numerous (for a review see Kinnamon (2016)). Indeed, α-gustducin knockout mice exhibit impaired, but not abolished, bitter and sweet taste perception (Wong et al. 1996) and Gα14, which is co-localized with the sweet taste receptor-specific subunit Tas1r2 in the posterior tongue (Tizzano et al. 2008), has been suggested to play an important role in sweet taste perception. Additional isoforms of Gα-proteins exist in taste tissue and contribute to sweet taste signaling (Kinnamon 2016; Ozeck et al. 2004). Upon activation of the sweet taste receptor, the heterotrimeric G protein dissociates and the β-γ-subunits activate phospholipase C β2 (PLCβ2) (Rossler et al. 1998), which results in the production of diacylglycerol (DAG) and inositol 1,4,5-trisphoshate (IP_3) from their precursor phosphatidylinositol 4,5-bisphosphate (PIP_2). The genetic ablation of PLCβ2 in mice results in the concomitant loss of sweet, umami, and bitter tasting abilities confirming its important role in taste signal transduction (Zhang et al. 2003). The IP_3 then mediates the subsequent release of calcium ions from intracellular stores via opening of the IP_3R3 channel (Clapp et al. 2001; Miyoshi et al. 2001). The rise in cytosolic calcium triggers the opening of the cation-channel TRPM5, leading to sodium ion influx causing the taste cell membrane to depolarize (Perez et al. 2002). The substantially impaired responsiveness of TRPM5-knockout mice to sweet, umami, and bitter stimuli confirmed that this channel is crucial for taste receptor-mediated taste transduction (Zhang et al. 2003). However, recently it was demonstrated that another TRP-channel, TRPM4, is equally involved in the transduction of these stimuli and that only the absence of both channels completely abolishes sweet, umami, and bitter reception in genetically modified mice (Dutta Banik et al. 2018). The membrane depolarization facilitates the activation of

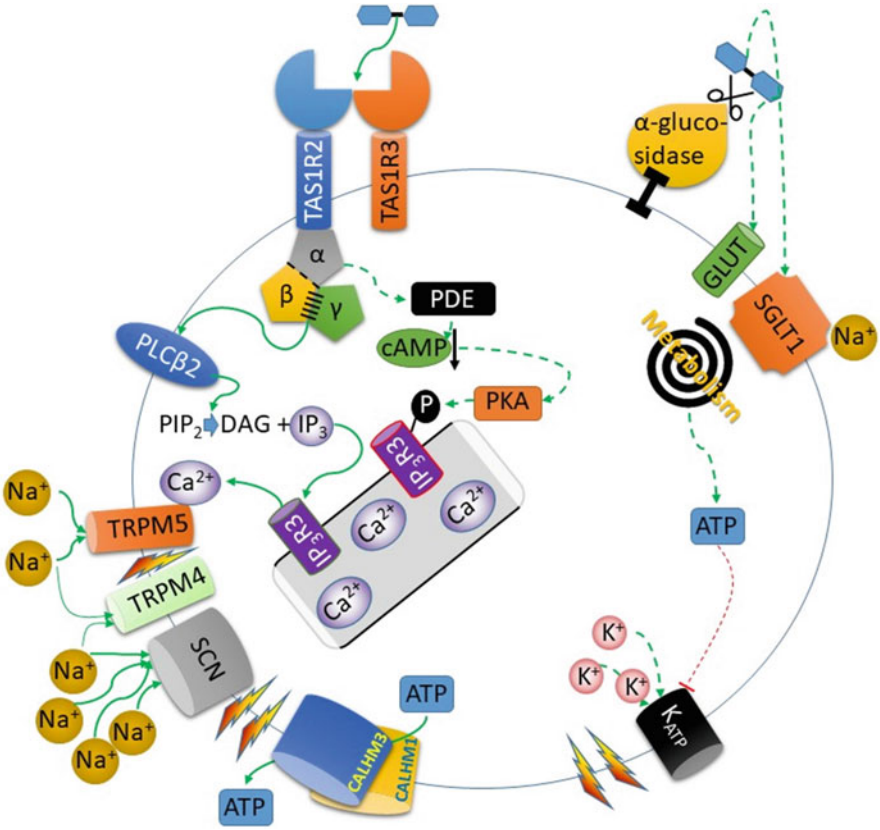


Fig. 2 *Sweet taste signal transduction.* The canonical sweet taste signal transduction pathway is depicted on the left side of the panel and highlighted with solid lines. Sweet substances such as disaccharides bind to the sweet taste receptor dimer, TAS1R2/TAS1R3. The heterotrimeric G protein (α, β, γ) dissociates and the βγ-subunits activate phospholipase Cβ2 resulting in the generation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) from phosphatidylinositol 4,5-bisphosphate (PIP_2). IP_3 triggers the release of calcium ions (Ca^{2+}) from the endoplasmic reticulum into the cytosol via activation of the inositol 1,4,5-trisphoshate receptor subtype 3 (IP_3R3). The calcium ions in turn facilitate opening of the cation channels, transient receptor potential channel subfamily M, members 4 (TRPM4) and 5 (TRPM5), allowing the influx of sodium ions (Na^+). The depolarization leads to the opening of voltage-gated sodium channels (SCN) and action potentials. Finally, the neurotransmitter ATP is released through the voltage-gated channel calcium homeostasis modulator 3 (CALHM3) forming functional heteromers with another subunit (CALHM1). The described pathway is suggested to be influenced by another second messenger system utilizing cyclic AMP (cAMP). This pathway is triggered by the α-subunit of the heterotrimeric G protein, α-gustducin, which increases the activity of the phosphodiesterase (PDE) present in taste cells leading to increased cAMP breakdown. The lower cAMP levels dampen the activity of protein kinase A (PKA), thus reducing phosphorylation of IP_3R3. The somewhat hypothetical nature of this pathway is emphasized by dotted lines. An additional sugar-sensing pathway proposed for sweet taste receptor cells is shown on the right side of the panel (again using dotted lines to indicate its hypothetical nature). Here, disaccharidases such as α-glucosidase at the plasma membrane of taste cells generate glucose, which can enter the cells via glucose transporters (GLUT) or sodium-glucose cotransporter 1 (SGLT1). Metabolization of glucose results in elevated intracellular ATP levels, which can block inwardly rectifying potassium channels (K_{ATP}) leading to cell depolarization

voltage-gated sodium channels (Gao et al. 2009) resulting in action potentials. Finally, adenosine triphosphate (ATP), the neurotransmitter of type II taste cells (Finger et al. 2005) is released through a voltage-gated ion channel, a hexameric channel composed of two subunits, the calcium homeostasis modulator 1 (CALHM1) (Taruno et al. 2013) and CALHM3 (Ma et al. 2018). α-gustducin itself seems to play a rather modulatory role in this signaling cascade by activating a phosphodiesterase (PDE) leading to a decrease in intracellular cyclic AMP (cAMP) levels. This in turn results in reduced activity of the cyclic AMP-dependent protein kinase A (PKA). As a consequence, activity-reducing phosphorylation of IP_3R3 is diminished and thus, the calcium signaling remains fully effective (Clapp et al. 2008).

7 Potential Additional Transduction Mechanisms for Sweet Compounds

If the sweet taste receptor would represent the sole sensor for all sweet tasting compounds in mammals, the mechanism for sweet sensation would be rather straightforward – whatever activates the sweet taste receptor must represent a sweet compound and vice versa. Initial reports about residual sweet tasting abilities in rodent knockout models suggested that sweet taste detection may not be that simple. Nevertheless, the observation that α-gustducin knockout mice showed residual recognition of GPCR-mediated tastants including sweet compounds (Wong et al. 1996) was believed to rely mainly on alternative Gα subunits expressed in the mammalian taste system (Tomonari et al. 2012). Moreover, the observation that knockout mice in which the signaling component of GPCR-mediated taste transduction, TRPM5, was genetically ablated, exhibited remaining sweet and bitter taste responsiveness (Damak et al. 2006) could be explained by the expression of TRPM4 in taste receptor cells and functional complementation of TRPM5 by TRPM4 (Dutta Banik et al. 2018). Also the report that Tas1r3-knockout mice still exhibit residual responses to sweet substances (Damak et al. 2003) could hint at post-ingestive preference learning that does not require sweet taste receptor signaling (Tan et al. 2020) rather than an alternative sweet taste transduction mechanisms. Still, such studies in Tas1r3-knoutout mice provided evidence for the existence of a potential alternative signaling pathway for detection of carbohydrate sweeteners (see Fig. 2). Intriguingly, Tas1r-expressing cells in the mouse taste system also express the components required for monosaccharide-sensing in pancreatic β-cells, namely glucose (hexose) transporters as well as ATP-gated inwardly rectifying potassium channels (Yee et al. 2011). More recently, enzymes capable of digesting di- and oligosaccharides were identified on the surface of taste cells (Sukumaran et al. 2016). These enzymes are typically found in intestinal brush-border membranes where they generate monosaccharides during digestion. In the taste system the generation of monosaccharides from their carbohydrate precursors could initiate the uptake and metabolization of monosaccharides and hence, may lead to the activation of the proposed alternative sweet sensing pathway (Sukumaran et al. 2016). Interestingly,

and somewhat surprisingly, despite the existence of disaccharidases on the surface of taste bud cells, which should rapidly deplete disaccharides in the vicinity of the taste cells, the sweet taste receptor is responsive to disaccharides such as sucrose. Future research may reveal why the recognition of disaccharides has remained biologically relevant.

8 Evolutionary Dynamics of the *TAS1R2/3* Genes

Despite its important function in the taste system and reported roles in non-gustatory tissues (Laffitte et al. 2014), the sweet taste receptor is frequently absent, pseudogenized or not functioning as sweet sensor (see Table 2). On the one hand, the importance of amino acid recognition in aquatic environments may provide the reason for the T1R2/T1R3 in fish to act as an additional amino acid receptor rather than as a receptor for the detection of sweet compounds (Oike et al. 2007). Moreover, the limited importance of sweet taste in the order of carnivores is easily understandable in light of their feeding habits (Jiang et al. 2012). On the other hand, the concomitant loss of the non-gustatory roles of sweet taste receptors, e.g., in pancreatic or brain tissue of these species should be difficult to compensate (for a list of vertebrates without functional sweet taste receptor, see Table 2). A possible way to circumvent serious metabolic consequences which may arise from the absence of sweet taste receptors in non-gustatory tissues in species lacking functional *T1R2* genes could be the existence of additional sensing mechanisms (cf. 2.8) also in other tissues. In fact, the sweet taste receptor independent glucose-sensing mechanism in, e.g., pancreatic β-cells (for a review see (Henquin 2009)) was firmly established long before *TAS1R* genes had been implicated in this mechanism (for a review see Kojima et al. (2017)). Recently, both glucose-sensing pathways were found to co-exist and to complement each other in pancreatic β-cells (Kyriazis et al. 2012). How and if the lack of functional sweet taste receptors in the various other organs of sweet taste receptor-devoid vertebrates is compensated is unknown and requires additional research in the future.

9 Sensory/Behavioral Testing of Sweet Stimuli

Long before the sweet taste receptor genes were identified (Bachmanov et al. 2001; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001) and thus functional heterologous expression assays became available, sweet compounds had been studied very successfully by sensory experiments. In order to assess the sweetening power of the various sweeteners sensory experiments using sucrose as reference substance were performed. The amount of a test compound on a weight basis necessary to elicit the same sweetness intensity as a sucrose solution of a given concentration is used to indicate its (relative) potency and labeled as P_w(%-sucrose) (DuBois and Prakash 2012). Owing to the long history of this comparative method for the assessment of the

Table 2 List of species which are devoid of functional sweet taste receptor genes

	Vernacular name	Latin name	References
Mammalian	African lion	*Panthera leo krugeri*	Li et al. (2009)
	Asian small-clawed otter	*Aonyx cinerea*	Jiang et al. (2012)
	Baleen whales	*Mysticei, five species*	Feng et al. (2014)
	Banded linsang	*Prionodon linsang*	Shi and Zhang (2006)
	Bottlenose dolphin	*Tursiops truncatus*	Feng et al. (2014), Jiang et al. (2012)
	California Sea lion	*Zalophus californianus*	Jiang et al. (2012)
	Cheetah	*Acinonyx jubatus*	Li et al. (2005)
	Domestic cat	*Felis catus*	Li et al. (2005)
	Fossa	*Cryptoprocta ferox*	Jiang et al. (2012)
	Fur seal	*Arctocephalus australis*	Jiang et al. (2012)
	Pacific harbor seal	*Phoca vitulina*	Shi and Zhang (2006)
	Spotted hyena	*Crocuta crocuta*	Shi and Zhang (2006)
	Tiger	*Panthera tigris*	Li et al. (2005)
	Toothed whales	*Odontoceti, 7 species*	Feng et al. (2014)
	Vampire bats	*Genus Desmodus*	Zhao et al. (2010)
Avian	Birds	*Ten species*	Baldwin et al. (2014)
	Chicken	*Gallus gallus domesticus*	Shi and Zhang (2006)
	Penguins	*Three species*	Zhao et al. (2015)
	Turkey	*Genus Meleagri*	Feng and Zhao (2013)
	Zebra finch	*Taeniopygia guttata*	Feng and Zhao (2013)
Amphibian	Western clawed frog	*Xenopus tropicalis*	Shi and Zhang (2006)
Teleostean	Zebrafish	*Danio rerio*	Oike et al. (2007)
	Medaka fish	*Oryzias latipes*	Oike et al. (2007)

sweetening powers of sweeteners, the Pw values of numerous substances have been established and can be found in the literature. Typically, sweetening powers are found in a range between Pw = 0.6 (D-glucose, derived from (DuBois et al. 1991)) to several thousands (Pw = 3,000, the sweet protein monellin, (Morris and Cagan 1972)). Currently, the most powerful sweetener is advantame with a P_w of ~20,000 (Bishay and Bursey 2012) (for a list of P_w-values (called relative sweetness (RS)-values), see Table 1). At present, it is unknown how the highly different sweetening powers of sweeteners can be explained on a molecular level. For the natural mono- and disaccharides the sweetness is well-balanced with their energy content, thus avoiding attraction to energetically irrelevant food sources (cf. Behrens and Meyerhof (2013)). Almost all high-potency sweeteners exhibit undesired side-tastes such as metallic taste, bitterness, or pungency at elevated concentrations. Moreover, their maximum sweetness intensity does not reach that of sucrose. Therefore, a lot of effort has been spent on the investigation of sweetener blends. When mixing two

different sweeteners it is anticipated that the combined activities at the sweet taste receptor may result in elevated sweetness and reduced off-tastes through synergistic activity, enabling the use of lower concentrations of both sweeteners. The outcome of these studies frequently has provided contrasting evidence on the existence of synergism. A large study conducted with binary mixtures of 14 natural and synthetic sweeteners revealed that rather few truly synergistic activations were evident and that most of the synergistic activities occurred at low concentrations, whereas at high concentrations not a single case of synergism has been observed (Schiffman et al. 1995). The discovery of the human sweet taste receptor subunits and the establishment of functional assays in mammalian cell lines has allowed testing of binary sweetener mixtures for synergistic effects in vitro. Testing sucrose and other sweeteners interacting within the TAS1R2-VFD with two sweeteners known to bind in the TAS1R3-HD, NHDC and cyclamate, suggested indeed apparent synergistic effects for all sweeteners when combined with either NHDC or cyclamate (Fujiwara et al. 2012). Careful analyses of similar experiments, however, revealed less obvious synergistic effects of NHDC and cyclamate on sucrose responses, which were dominantly affecting the efficacy rather than the potency (Servant et al. 2020). In order to improve the sweet taste of synergistic blends consisting of two sweeteners further, the addition of a third sweet substance was tested (Schiffman et al. 2000). Indeed, it was observed that most of these mixtures exhibited synergism, however, these experiments were done at even lower sweetness levels compared to binary mixtures and hence, synergy is restricted to low concentrations. Recently, an approach that is reminiscent of drug interaction studies in pharmaceutical industry, the isobole analysis, was applied to investigate synergistic effects of sweeteners (Reyes et al. 2019). It turned out that a number of sweetener pairs showed synergistic effects on sweetness perception, with NHDC being frequently included in synergistic mixtures, whereas thaumatin dominantly occurs in mixtures causing suppression or only additive effects (Reyes et al. 2019).

10 In Vitro Assessment of Sweet Compounds and Sweet Taste Receptor Modulators

Currently the currently most prominent approach used to characterize the sweet taste receptors of various species relies on the expression of the two subunits in heterologous cell lines (Bouysset et al. 2020; Hellfritsch et al. 2012; Hillmann et al. 2012; Jiang et al. 2004, 2005a, b; Koizumi et al. 2007, 2011; Li et al. 2002; Nakajima et al. 2008; Nelson et al. 2001; Poirier et al. 2012; Servant et al. 2010; Sigoillot et al. 2012a, 2018; Winnig et al. 2005, 2007; Xu et al. 2004; Zhang et al. 2010; Zhao et al. 2018). Others have used recombinant expression systems to overexpress and purify extracellular domains of the sweet taste receptor subunits in combination with biophysical ligand interaction studies to assess receptor binding (Maîtrepierre et al. 2012; Nie et al. 2005, 2006). These studies revealed that, in general, in vitro data correlated well with human psychophysical experiments (see Tables 1 and 3). Note that the EC_{50}-concentrations obtained by in vitro functional experiments are between

Table 3 Comparison of sweet taste receptor activating concentrations determined by sensory tests or cellular assays

Sweetener	Taste threshold	In vitro EC_{50}-concentr.
Acesulfame K	44.4 μM (Schiffman et al. 1981)	120–540 μM c.f. Table 1
Cyclamate	266 μM (Schiffman et al. 1981)	0.69–2.56 mM c.f. Table 1
Saccharin	21 μM (Dalton et al. 2000)	42–190 μM c.f. Table 1
Stevioside	11.1 μM (Hellfritsch et al. 2012)	60 μM c.f. Table 1
Sucrose	6.8 mM (Petty et al. 2020)	19.4 mM c.f. Table 1

As threshold concentrations observed in vitro are rarely provided in the literature, EC_{50}-concentrations were taken from Table 1 for comparisons

2.0-fold and 5.4-fold higher than the taste threshold concentrations determined in vitro, which is anticipated since one value reflects initial receptor activation (threshold concentration) and the other value indicates already half-maximal receptor responses (EC_{50}-concentration).

This suggests that the heteromeric sweet taste receptor alone can be considered as the critical receptive unit and that downstream effectors may not modify the receptor's sensitivity substantially, because the cell lines used for in vitro studies deviate with respect to the chosen Gα proteins/chimeras among each other and they do not utilize the canonical signal transduction components present in sweet taste receptor cells (see Sect. 6).

11 Conclusions/Outlook

Research on the molecular basis of sweet taste has made enormous progress since the discovery of the two genes coding for the functional sweet taste receptor at the beginning of this century. Unfortunately, an experimental structure of the entire human sweet taste receptor is still lacking, but data on the extracellular domains of the amino acid-detecting medaka fish sweet taste receptor offer clues on the receptor's molecular architecture. Having such a structure at hand will likely allow much more efficiently the design of alternative agonists and modulators in the future. This, or other approaches, may finally enable the development of improved alternative sweeteners. Another issue that needs to be tackled in the future is the role of the sweet taste receptor in non-gustatory tissues in light of the absence of a functional TAS1R2 gene in many species. Either there has to be a highly conserved back-up mechanism that compensates for the absence of a functional sweet taste receptor or individual solutions were found during evolution.

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Pharmacology of T2R Mediated Host–Microbe Interactions

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Abstract

Bitter taste receptors (T2Rs) belong to the G protein-coupled receptor superfamily. Humans express 25 T2Rs that are known to detect several bitter compounds including bacterial quorum sensing molecules (QSM). Primarily found to be key receptors for bitter sensation T2Rs are known to play an important role in mediating innate immune responses in oral and extraoral tissues. Several studies have led to identification of Gram-negative and Gram-positive bacterial QSMs as agonists for T2Rs in airway epithelial cells and immune cells. However, the pharmacological characterization for many of the QSM–T2R interactions remains poorly defined. In this chapter, we discuss the extraoral roles including localization of T2Rs in extracellular vesicles, molecular pharmacology of QSM–T2R interactions, role of T2Rs in mediating innate immune responses, and some of the challenges in understanding T2R pharmacology.

Keywords

Bitter taste receptor (T2R) · G protein-coupled receptor (GPCR) · Host–microbe interactions · Innate immunity · Quorum sensing (QS)

1 Introduction

1.1 G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) form the largest druggable receptor superfamily in the human genome. Chemosensory GPCRs that include both olfactory and taste receptors account for over half of the GPCR superfamily (Isberg et al. 2017). Humans are capable of sensing five basic tastes: salt, sour, umami, sweet, and bitter (Adler et al. 2000; Chandrashekar et al. 2000; Tu et al. 2018; Baumer-Harrison et al. 2020). Fat and kokumi (thickness of food) tastes are considered as possible additions (Laugerette et al. 2005; DiPatrizio 2014; Lewandowski et al. 2016). Sweet, umami, and bitter taste are mediated by GPCRs, whereas ion channels ENaC and OTOP1 mediate the salt and sour tastes (Chandrashekar et al. 2000; Heck et al. 1984; Gilbertson et al. 1992; Tu et al. 2018; Baumer-Harrison et al. 2020).

1.2 Taste Sensation

Taste sensation is initiated when a tastant molecule (taste inciting substance) comes in contact with taste proteins (receptors and ion channels) located on taste cells in the oral cavity. The perception of taste is complex involving both sensory and neural mechanisms. Further, taste perception varies between individuals and influences food preferences and hence nutritional choices (Bartoshuk 2000). While it is understood that the spectra of molecular detection via these taste proteins is quite broad, they also exhibit significant diversity in structure and functional roles, making them

important therapeutic targets. The pharmacology, physiology, and signaling of different taste proteins (receptors and ion channels) are extensively reviewed in different chapters of this book. In this chapter we will briefly discuss the extraoral activity of bitter taste receptors (T2Rs) and then focus on their pharmacology in host–microbe interactions.

1.3 T2R Classification

The human genome encodes 25 T2Rs localized as clusters on chromosome 5p15, 7q31, and 12p13 (Adler et al. 2000). T2Rs were previously classified under frizzled receptor family, however, recent classification by International Union of Basic and Clinical Pharmacology (IUPAC) has annotated T2Rs as a separate class that is denoted by "T" (Pándy-Szekeres et al. 2018). According to HUGO nomenclature the genes that code for T2Rs are denoted as TAS2R followed by the number of the receptor (Braschi et al. 2019). T2Rs are known to recognize diverse and a wide range of compounds. T2Rs are seven transmembrane proteins consisting of 3 extracellular, 3 intracellular loops, short N-terminus and a C-terminus. From an evolutionary standpoint, T2Rs have been hypothesized to have evolved as sentinels to detect noxious substances in food. Although T2Rs are primarily expressed on the tongue, recent studies have identified extraoral expression of these receptors with significant physiological roles.

1.4 T2R G Protein Selectivity and Signaling

Several studies have used co-expression systems where both T2R and the G proteins are co-expressed to analyze the receptor activation. The idea that bitter and sweet taste transduction occurs via the GTP-binding proteins was first conceptualized in 1992 (Kinnamon and Cummings 1992). Studies that followed have identified gustducin as a unique G protein α-subunit expressed primarily in taste receptor cells (TRCs), that is required for bitter taste signaling in vivo (Wong et al. 1996). In a classic study, Ueda et al. (2003) determined that the last 37 to 44 C-terminal amino acids of gustducin were critical for T2R coupling by creating a series of chimeric G proteins from Gα16 (which couples many GPCRs to calcium signaling) that incorporated different lengths of gustducin-specific C-terminal amino acid sequences. Resulting G16/gust44 and G16/gust37 chimeras effectively coupled T2Rs to calcium signaling, whereas chimeric G proteins with shorter terminal segments did not. Replacing the terminal 44 amino acids of Gα16 with the equivalent domains of Gαt (transducin) and Gαi also produced functional chimeras (Ueda et al. 2003). Besides gustducin and its close cousin transducin, several members of the Gi/o family have been demonstrated to functionally couple to T2Rs in vitro (Ozeck et al. 2004). Indeed, studies have shown selectivity of different G proteins to mT2R5, hT2R4, hT2R7, hT2R14, and hT2R43 and thus it is possible that binding of a ligand to a T2R can lead to biased downstream signaling in different tissues. All

these T2Rs were able to couple with Gαt, Gαi, Gαo but not with Gαs and Gαq (Sainz et al. 2007). Finally, gustducin knockout mice have been shown to recognize bitter compounds in various physiological and behavioral assays (He et al. 2002). Therefore, the idea of T2R coupling to only Gα gustducin is questionable.

Analyzing T2R activation also depends on the intracellular calcium detection methods employed (Freund et al. 2018; Jaggupilli et al. 2018). Two calcium-sensing dyes Fura-2 and Fluo-4 are routinely used in T2R assays. Fura-2 is a ratiometric dye with an excitation ratio of 340/380 nm and emission around 500 nm. The Fluo-4 is a single wave calcium probe with excitation maxima of 490 nm and emission at 520 nm. The emission intensity in the Fluo-4 system depends on the levels of calcium bound to the dye (i.e., higher calcium released by cell leads to brighter signal intensity). Fura-2 is a high affinity calcium probe (K_d: 140 nM) whereas Fluo-4 is a low affinity calcium probe (K_d: 345 nM). The Fura-2 dye would be useful in accurately measuring resting calcium and minute calcium increases with a rapid saturation. In contrast, Fluo-4 dye can measure higher calcium spikes with lower saturation. The Fura-2 dye is used to measure global Ca^{2+} levels as well as live single cell imaging that uses a fluorescent microscope (Freund et al. 2018; Gopallawa et al. 2020). The Fura-2 dye coupled with microfluorimetry is useful in analyzing calcium changes in individual cells or a group of cells. On the other hand, Fluo-4 is mainly used in FLIPR (fluorescent imaging plate reader) based assays. This approach has been mainly used in determining the efficacy of bitter agonists, inverse agonists and antagonists or when screening novel bitter compounds (Meyerhof et al. 2010; Pydi et al. 2014b, 2015). Apart from measuring the transient intracellular calcium mobilization several recent studies also measure the more stable and upstream secondary messengers such as inositol phosphate $(IP)_1$ and IP_3 along with calcium assays (Pydi et al. 2014b; Itoigawa et al. 2019). Interestingly, IP_3 assay has shown to be effective in measuring basal or constitutive activity of T2R4 mutants A90F and K270A, whereas the basal calcium levels in these mutant cells were comparable to that of wild type (WT) cells (Pydi et al. 2014b). GPCRs are known to activate multitude of downstream signaling such as activation of GPCR kinases (GRKs) and β-arrestin recruitment (Wei et al. 2003). The activation of GRK and β-arrestin signaling pathways leads to receptor desensitization and downstream activation of mitogen-activated protein kinase (MAPK) pathways (DeWire et al. 2007). T2R activation can also lead to the recruitment of arrestin (Woo et al. 2019) and the activation of MAPK (Ozeck et al. 2004).

2 Extraoral Expression of T2Rs

Studies in the recent years have highlighted the expression of T2Rs in several extraoral tissues including gut, brain, cancer cells, and endometrium, which are briefly reviewed in this section. For more comprehensive discussion on extraoral roles of T2Rs, readers are referred to one of our earlier reviews (Shaik et al. 2016) and accompanying chapters in this book.

2.1 T2Rs in Brain, Gut, and Cancers

The first study to suggest expression of TAS2Rs in brain cells was pursued in rats (Singh et al. 2011). This study showed by RT-PCR and immunohistochemistry analysis that TAS2R4, TAS2R107, and TAS2R38 were expressed in rat brain stem, cerebellum, cortex, and nucleus accumbens (Singh et al. 2011). After this study, there were reports of TAS2R expression in brain tissues of both animal models and humans but the functional role of these T2Rs in these tissues remains to be understood and characterized. The human fundic primary cells were shown to express T2R10 and its activation was shown to influence ghrelin secretion (Wang et al. 2019), TAS2R5 and TAS2R38 were detected in human duodenal sections and enteroendocrine cells (EEC) responsible for secreting anorexigenic peptides such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) (Park et al. 2015; Latorre et al. 2016). Studies have shown increased secretion of GLP-1 and PYY hormones in NCI-H716 cells upon treatment with T2R5 agonist 1,10-phenantroline (Park et al. 2015). The GLP-1 and PPY secretion was decreased upon downregulation of α-gustducin and multiple T2Rs (Kim et al. 2014). TAS2R3, TAS2R4, and TAS2R10 along with their downstream signal transducing partners are expressed in the human gastric smooth muscle cells (Avau et al. 2015). The presence of T2Rs in the brain and gut suggests their involvement in neuroendocrine systems; gut–brain feedback systems and the regulation of energy homeostasis. Recently, a study has shown that intra-gastric administration of quinine resulted in the enhanced brain activity in the hypothalamus and the hedonic regions (Iven et al. 2019). In contrast, decreased plasma levels of octanoylated ghrelin, total ghrelin, and motilin hormones were observed in subjects treated with quinine versus placebo leading to decreased hedonic food intake (Iven et al. 2019). Several questions have been raised about the role of T2Rs in obesity and fat tissue differentiation. T2R38 was shown to be expressed in human adipocytes and was found to be highly expressed in obese subjects as compared to the lean subjects (Cancello et al. 2020). The same study also showed T2R38 to be responsible for adipocyte differentiation and delipidation (Cancello et al. 2020).

Recent studies suggest the expression of T2Rs in many cancers including breast, pancreatic, neuroblastoma, prostate and ovarian cancers (Gaida et al. 2016b; Seo et al. 2017; Martin et al. 2019; Singh et al. 2020). However, the pathophysiological role of T2Rs in these cancers is varied and not fully understood. The tissue biopsies from pancreatic ductal adenocarcinoma patients and membrane preparations from Su8686 and HuH7 cell lines revealed co-localization of T2R38 with a lipid droplet marker perlin (Gaida et al. 2016b). In another study, T2R8 and T2R10 were shown to suppress cancer stem cell markers such as DLK1, CD133, NOTCH1, and Sox2 and neuroblastoma cell migration (Seo et al. 2017). TR24 and T2R14 are differentially expressed in breast cancer tissues and their activation induced anti-proliferative and anti-migratory responses in highly metastatic breast cancer cells (Singh et al. 2020). In ovarian and prostate cancer cell lines, T2R14 specific responses were shown to induce pro-apoptotic proteins Bcl-XL and caspase-3 leading to apoptosis as measured by increased annexin V on cell membranes (Martin et al. 2019). The

expression of 9 TAS2Rs genes was observed in post-meiotic spermatids of mouse testis (Xu et al. 2013).

2.2 T2Rs in Extracellular Vesicles

The extracellular vesicles (EVs) are nanocarriers released by many human cells. EVs are membranous vesicles ranging between 50 and 500 nm in size and are secreted by endocytosis of membranes ultimately leading to exocytosis of multivesicular bodies (MVBs) (Trams et al. 1981). EVs play an important role in intercellular communication and are involved in regulating several biological processes such as inflammation, cell proliferation, and neuronal function (Raposo et al. 1996; Simhadri et al. 2008).

EVs carry unique biological molecules from the parent cells they are released such as CD3 in T-cells, transferrin receptor (TFR) in reticulocytes and GPCRs like somatostatin receptor 2 (SSTR-2), chemokine receptors 4 and 7 (CXCR4, CCR7), and angiotensin II type 1 receptor (AT1R) (Blanchard et al. 2002; Delcayre et al. 2005; Estelles et al. 2007; Pironti et al. 2015). We have recently shown the localization of T2Rs in EVs isolated from HEK293 cells and in saliva using immunotransmission electron microscopy (TEM) and ELISA (Medapati et al. 2017). It was the first report to suggest expression of T2R4 and T2R38 in EVs isolated from saliva of healthy individuals. However, the specific role of T2Rs in EV life cycle and conversely, the contributions of EVs in T2R signaling remain to be determined.

The intertwined mechanism of intercellular communications between GPCRs and EVs was recently reviewed (Bebelman et al. 2020). Although the role of T2Rs in EVs is not yet known, the notion of extracellular localization of GPCRs is well established in the case of AT1R (Pironti et al. 2015), GPCR5B (Kwon et al. 2014), and kinin1B receptors (Kahn et al. 2017). The process of GPCR internalization and sorting into endosomal compartments is similar to that of MVB formation in EV life cycle. Hence there exists a mechanistic link between GPCR internalization via endosomal sorting leading to their EV secretion. Conventional knowledge suggests endosomal GPCR MVBs fuse with lysosomes leading to degradation. However, these GPCR MVBs can also fuse with plasma membrane leading to their extracellular release. The localization of T2R4 and T2R38 in EVs might offer renewed focus onto T2R recycling in cells via EV secretion pathways. Currently, few studies were pursued on T2R internalization or recycling upon activation by cognate ligands (Upadhyaya et al. 2016; Woo et al. 2019). Studies on T2R4 suggest that it does not internalize upon agonist treatment and the agonist quinine has a pharmacochaperone activity increasing the cell surface expression of T2R4 (Upadhyaya et al. 2016). In contrast, recent studies on T2R14 suggest all agonists except diphenhydramine cause T2R14 internalization (Woo et al. 2019). These studies suggest that internalization might be T2R and agonist specific.

3 T2R Host–Microbe Interactions

The sections that follow will discuss the role of T2Rs in sensing various bacterial quorum systems and the associated molecular pharmacology.

3.1 Bacterial Quorum Sensing Molecules as New Class of Ligands for T2Rs

Bacteria secrete quorum sensing molecules (QSMs) during colonization. Once colonized these bacteria are very difficult to eradicate and result in episodic exacerbations and eventual system failures. The QSMs as the name suggests are important for the bacteria to sense their numbers (hence, quorum) and then signal each other to mediate relevant responses.

Both Gram-negative and Gram-positive bacteria employ quorum sensing (QS) for communication, but they use different molecules. Gram-negative bacteria such as *Pseudomonas aeruginosa* secrete three molecules: N-acyl homoserine lactone (AHL) molecules (autoinducer-1, AI-1) forming its main QS signal, followed by Autoinducer-2 (4,5-dihydroxy-2,3-pentanedoine (DPD) and PQS (2-heptyl-3-hydroxy-4-quinolone) (Lee and Zhang 2015; Li et al. 2015). In contrast, Gram-positive bacteria use modified oligopeptides (autoinducer peptides, AIP) (Taga and Bassler 2003). These peptides possess a large structural diversity and frequently undergo posttranslational modifications (Sturme et al. 2002). Both Gram-positive and Gram-negative bacteria can produce and detect a third type of QSMs known as AI-2. These are boron-furan-derived signal molecules (Li and Nair 2012). Besides these 3 main groups, there is also a fourth group of miscellaneous QSMs (Barber et al. 1997; Flavier et al. 1997; Holden et al. 1999). In host–microbe interactions, these mechanisms become relevant when bacteria infect and colonize the host, thus understanding the effects the QSMs have is paramount.

It is well understood that eukaryotic host and colonizing prokaryotes have co-evolved (Lyte and Ernst 1993). For example, Enterohaemorrhagic *E. coli* (EHEC) is able to modify its motility and virulence expression as per the host's adrenaline and noradrenaline concentration (Pacheco and Sperandio 2009). The neurotransmitter γ-aminobutyric acid (GABA) produced by various Lactobacilli plays an important role in mammalian brains (Barrett et al. 2012). Thus it is no longer surprising that their communication systems evolved as well (Shiner et al. 2005; González and Venturi 2013). In a major finding, it was reported that nasal solitary chemosensory cells (SCCs) expressed T2Rs and responded to AHLs produced by bacteria (Tizzano et al. 2010) establishing QSMs as a new class of ligands for T2Rs.

3.2 AHLs

These are the most common class of autoinducers and structurally possess a homoserine lactone (HSL) moiety, acyl side chains with 4–18 carbons that determine the structure diversity and a substitution primarily belonging to one of the three: (a) simple acyl, (b) 3-hydroxyacyl, and (c) 3-oxoacyl groups. Though there are several subtypes of AHLs, most of the available studies so far have focused on C-12 AHL. Indeed, it is a key and a very well-studied molecule in the biofilm formation and regulation of virulence in *P. aeruginosa*. AHLs are also now recognized to be important for inter-species communication between bacteria (Bhargava et al. 2012), which highlights their importance in the development of multi-species chronic infections. The first clue that AHLs could play a role as signaling molecules in inter-kingdom communication was N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL or C12-AHL) secreted by *P. aeruginosa* (Telford et al. 1998). Given the high hydrophobicity of C12-AHL it was hypothesized that it could diffuse freely through lipid membranes and mediate responses in the host (Barrett et al. 2012; Barth et al. 2012).

Studies in the recent past have also shown the effects of C12-AHL on respiratory epithelial cells, such as enhanced ciliary movement and nitric oxide production (Lee et al. 2012), indicating that C12-AHL induces activation of defense-related functions in these cells. Cystic fibrosis (CF) is a life-threatening genetic disease characterized by pulmonary exacerbations mainly due to infections with *P. aeruginosa*. The concentrations of C12-AHL in CF sputum are believed to be in the nanomolar range (Shiner et al. 2006) but reach 5 μM in *P. aeruginosa* culture supernatants and may be greater than 100 μM in regions adjacent to biofilms (Kravchenko et al. 2006, 2008). A previous study suggested that C12-AHL stimulates CFTR-dependent Cl^- and fluid secretion in an intact CFTR expressing, CaLu3 airway epithelial cells by activating the inositol trisphosphate receptor and store-operated cAMP production (Schwarzer et al. 2010). C12-AHL treatment of myeloid or non-myeloid cells resulted in the morphological alteration of mitochondria and especially endoplasmic reticulum (ER) (Kravchenko et al. 2006). Further the study also showed that the N-(3-oxo-acyl) homoserine lactone ring moiety in AHLs is required for the induction of phosphorylated mitogen-activated protein kinase p38, and that of eukaryotic translation initiation factor 2α (eIF2α) (Kravchenko et al. 2006). Canonical host recognition molecules such as MyD88, TRIF, TLR2, TLR4, Nod1, and Nod2 were also found to be dispensable for the C12-AHL mediated signaling and it was hypothesized that C12-AHL could in fact function as a pathogen-associated molecular pattern (Kravchenko et al. 2006).

3.3 AHLs Interaction with T2Rs

Recent studies have shown expression of T2R38 on peripheral blood leukocytes, monocytes, and neutrophils as well as monocytic cell lines (Maurer et al. 2015; Gaida et al. 2016a). T2R38 has been found to associate with IQGAP1, a scaffolding

protein, which participates critically in the rearrangement of the cytoskeleton and in regulating cell morphogenesis (Gaida et al. 2016a). Among others, it is a key regulator of chemotaxis. C12-AHL has been found to induce chemotaxis (Karlsson et al. 2012) and thus, it is hypothesized that C12-AHL may cause chemotaxis via the T2R38-IQGAP pathway. There is a correlation between T2R38 receptor allotypes and susceptibility to airway infections (Shah et al. 2009; Tizzano et al. 2010; Lee et al. 2012), and T2R38 has been shown to respond to at least two other AHLs produced by *P. aeruginosa*: N-butyryl-L-homoserine lactone and N-hexanoyl-L-homoserine lactone (Lee et al. 2012).

Though the T2R-AHL relationship is being explored, the mechanism is poorly understood. For example, in heterologous systems T2R38 is not activated by AHLs (Lossow et al. 2016). However, co-immunoprecipitation, immunofluorescence, and antibody blocking have shown C12–AHL interaction with T2R38 on myeloid cells (Maurer et al. 2015; Gaida et al. 2016a). In another study it was suggested that the solvent (DMSO) for these AHLs caused activation of T2R38 (Verbeurgt et al. 2017). An extensive structure-function study was conducted to analyze the interaction between several T2Rs and AHLs (Jaggupilli et al. 2018). This study identified that C4-AHL could activate T2R14 while C8-AHL and C12-AHL activated T2R4, T2R14, and T2R20. Taken together, these results suggest that AHLs can bind to T2Rs and participate in local host defense.

3.4 Pharmacological Characterization of AHL–T2R Interactions

The binding sites of the QSMs in T2R4, T2R14, and T2R20 were recently characterized (Jaggupilli et al. 2018). In addition, the analysis of bitter sensory profile of C4-AHL, C8-AHL, 3-oxo-C12-AHL and T2R agonists quinine, caffeine, dextromethorphan (DXM), diphenhydramine (DPH), cromolyn using E-tongue (Alpha MOS) suggests that the bitterness score of the above AHLs (~10) was slightly higher compared to quinine (8.45). These E-tongue experiments provide the first sensory insights into the bitter intensity profiles of bacterial AHLs. Future studies involving human taste panels are warranted to analyze the sensory properties of AHLs and to support the notion that humans can taste bacterial biofilms.

The QSM mediated T2R activation was functionally characterized using HEK293T cells stably expressing T2R1, T2R4, T2R14, and T2R20 (Jaggupilli et al. 2017, 2018). The $(Ca^{2+})_i$ assays in HEK293T cells showed no activation of T2R1 by any of the AHLs tested. However, T2R4 was activated by 50 μM C8-AHL and 100 μM C12-AHL, while T2R14 was activated by C4-AHL, C8-AHL, and C12-AHL. Finally, T2R20 was activated by C8-AHL and C12-AHL. Moreover, C8-AHL and C12-AHL were able to activate T2R4 and T2R14 in a concentration dependent manner. Surprisingly, the AHLs were able to activate T2Rs in the lower micromolar ranges with higher potencies than conventional bitter taste receptor agonists. Though the maximum effect (E_{max}) caused by AHLs in terms of intracellular calcium mobilization is much lower as compared to that of conventional bitter agonists (Table 1). Unfortunately, some of these AHLs could not be used at a

Table 1 Pharmacological characterization of QSMs and bacterial metabolites activating T2Rs in heterologous and endogenous cell systems

QSMs and bacterial metabolites (conc.)[a]	Heterologous system (HEK293T cells)	Primary or immortalized cells	References
C4-AHL (50 μM)	T2R14 EC_{50} ND Emax ND		(Jaggupilli et al. 2018)
C8-AHL (3.15–200 μM)	T2R4 EC_{50} 30.13 ± 12 μM Emax 37.81 ± 5.0 RFU T2R14 EC_{50} 19.65 ± 10 μM Emax 20.33 ± 3.8 RFU		(Jaggupilli et al. 2018)
C12-AHL (3.15–200 μM)	T2R4 EC_{50} 40.9 ± 13 μM Emax 26.89 ± 3.2 RFU T2R14 EC_{50} 69.54 ± 40 μM Emax 29.59 ± 5.1 RFU T2R20 EC_{50} 58.31 ± 12 μM Emax 24.31 ± 4.1 RFU		(Jaggupilli et al. 2018)
PQS (100 μM)	T2R4, T2R16, T2R38, T2R39 EC_{50} ND Emax ND	Airway epithelial cells and macrophages	(Freund et al. 2018; Gopallawa et al. 2020)
HHQ (100 μM)	T2R14 EC_{50} ND Emax ND	Airway epithelial cells and macrophages	(Freund et al. 2018; Gopallawa et al. 2020)
Acetone[a] (3.5–185 mM)	T2R38 EC_{50} 60 mM		(Verbeurgt et al. 2017)
2-Butanone (0.3–80 mM)	T2R38 EC_{50} 11 mM		(Verbeurgt et al. 2017)
2-Pentanone (0.1–20 mM)	T2R38 EC_{50} 4 mM		(Verbeurgt et al. 2017)
2-Methylpropanal (0.39–25 mM)	T2R38 EC_{50} 11 mM		(Verbeurgt et al. 2017)
γ-Butyrolactone (1–100 mM)	T2R38 EC_{50} 12 mM		(Verbeurgt et al. 2017)
Dimethyldisulfide[a] (0.25–3 mM)	T2R38 EC_{50} 3 mM		(Verbeurgt et al. 2017)

(continued)

Table 1 (continued)

QSMs and bacterial metabolites (conc.)[a]	Heterologous system (HEK293T cells)	Primary or immortalized cells	References
Methylmercaptan[a] (0.36–10 mM)	T2R38 EC_{50} 8 mM		(Verbeurgt et al. 2017)

ND not determined
[a]Solvents used to solubilize the QSMs

concentration higher than 200 μM to reduce any unwanted cross effect caused by the solvent DMSO. Hence direct comparison between AHLs and bitter agonists remains elusive. Nevertheless, these results establish AHLs as new class of agonists in the T2R family.

3.5 T2R Amino Acids Interacting with QSMs

Since there is no crystal structure available for any of the T2Rs, thus far structure-function studies have utilized site-directed mutagenesis and computational based approaches (Jaggupilli et al. 2016). To understand the nature of interaction between AHLs and T2Rs, the binding sites of C8-AHL and C12-AHL in several T2Rs were characterized (Jaggupilli et al. 2018). The validated homology models of T2R4, T2R14, and T2R20 were docked with C8-AHL and C12-AHL using Libdock algorithm. The analysis of different ligand binding poses revealed several amino acids on extracellular side in T2Rs to be within 4 Å distance of the AHLs (Fig. 1). The amino acids identified to be interacting with homoserine lactone group in AHLs include N165, T166, K262 in T2R4; R160, K163, T182 in T2R14; and H65, W88, W164, Q265 in T2R20. The homoserine lactone group in AHLs was shown to interact predominantly with extracellular residues in T2Rs (Fig. 1). Both C8-AHL and C12-AHL activated the three T2Rs (T2R4, T2R14, and T2R20) with different intrinsic efficacies. This is due to the orientation of AHL molecules within the ligand binding site. Within the AHLs, it was suggested that the homoserine lactone group is essential in binding to the extracellular orthosteric site in T2Rs and the acyl chain is shown to interact with hydrophobic amino acid residues located in the TM regions of T2Rs. In T2R4, the acyl chain of C12-AHL is extended towards TM3-TM7, while in T2R14 and T2R20, the acyl chains were located deep within the TM core. The orientation of acyl chains of C8-AHL is observed to be opposite in the TM core of T2R4 and T2R14. These observations suggested that hydrophobic amino acids in the TM regions of T2Rs might play an important role in stabilizing AHL binding to receptor and/or facilitating T2R activation. In the absence of an actual ligand binding assay that measures Kd (ligand affinity), it is currently not possible to differentiate between T2R amino acids important for ligand binding and/or T2R activation. However, the above study reinforced the significance of amino acids in extracellular loop 2 in T2R ligand binding and activation.

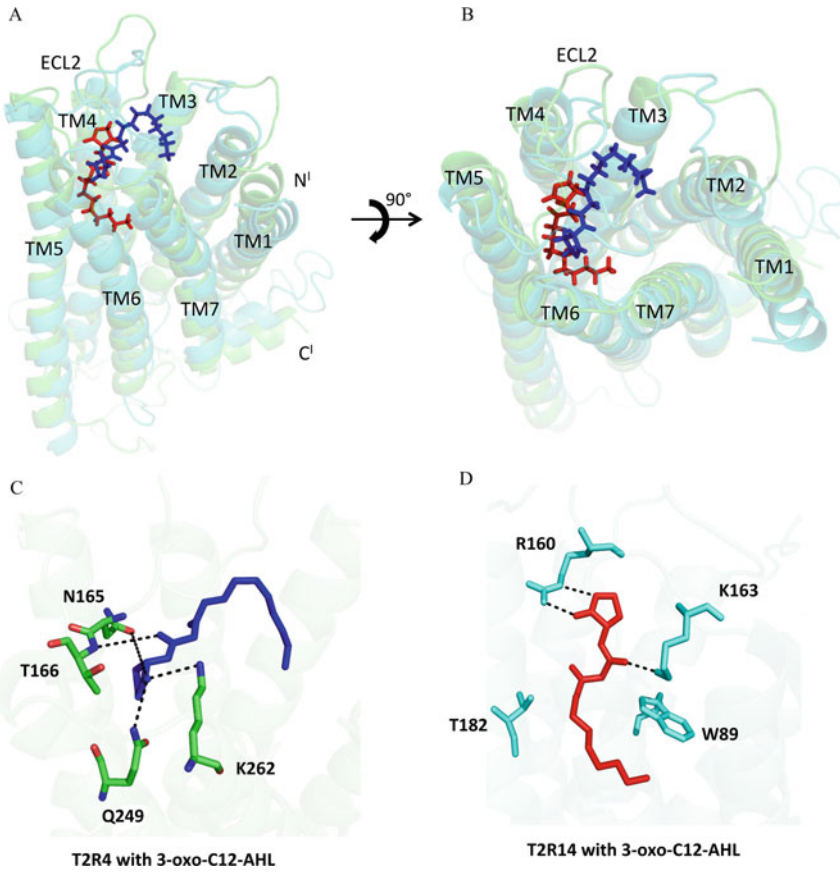

Fig. 1 The binding site of 3-oxo-C12-AHL in T2R4 and T2R14 based on site-directed mutagenesis and molecular modeling analysis (Jaggupilli et al. 2018). (**a**) Superimposed molecular models of T2R4 (Green) and T2R14 (Cyan) docked with the 3-oxo-C12-AHL (blue in T2R4 and red in T2R14). (**b**) Top view of the ligand binding pocket showing the arrangement of the cylindrical seven transmembrane (TM 1-7) helices, extracellular loop 2 (ECL2) and the 3-oxo-C12-AHL (blue in T2R4 and red in T2R14). (**c**, **d**) Amino acids interacting with 3-oxo-C12-AHL in T2R4 and T2R14, respectively

3.6 Quinolones Interaction with T2Rs

The second class of molecules for QS in *P. aeruginosa* are 4-hydroxy-2-alkylquinolines (HAQs). HAQs include derivatives of 4-hydroxy-2-heptylquinoline (HHQ) and the dihydroxylated derivatives, such as 2-heptyl-3, 4-dihydroxyquinoline (PQS, pseudomonas quinolone signal), and 2-nonyl-4(1H)-quinolone (NHQ) (Heeb et al. 2011). HHQ is synthesized from anthranilic acid and PQS is synthesized by hydroxylation of HHQ. Both HHQ and PQS act as co-inducing ligands for the transcriptional regulator controlling genes encoding for HAQs (Lepine et al. 2004; Bredenbruch et al. 2005; Wade et al. 2005; Diggle et al.

2006). The sensory profiles of these QSs are yet to be characterized by human taste panels. Though, bitter sensory profiles analyzed by emerging technologies such as the E-tongue suggest HHQ and NHQ with higher bitterness scores (>10) than AHLs, quinine, or caffeine (Jaggupilli et al. 2018).

In chronic infections such as in CF, the environment over time becomes hypoxic or oxygen deficient (Montgomery et al. 2017). Under anaerobic conditions, PQS is not produced due to the absence of oxygen. HHQ is the predominant signaling molecule under chronic hypoxic environments and PQS during early infections (Schertzer et al. 2010). Studies on both HHQ and PQS have shown that they can strongly suppress innate immune responses in vitro and ex vivo, mediated by the NF-κB signaling pathway (Kim et al. 2010). A recent study also showed that PQS stimulates neutrophil chemotaxis via activation of the MAPkinase p38 possibly employing the same pathway as C-12 AHL (Hansch et al. 2014) (Fig. 2). A recent study has shown that PQS activated T2R4, T2R16, and T2R38, whereas HHQ activated only T2R14 (Freund et al. 2018). PQS and HHQ also increased calcium responses but decreased both baseline and stimulated cAMP levels in cultured and primary airway cells (Freund et al. 2018). Further activation of T2Rs also increased nitric oxide generation, a robust innate immune response against *P. aeruginosa*, suggesting a role in innate immune responses (Freund et al. 2018).

3.7 Autoinducer Peptides (AIP) Interaction with T2Rs

The AIPs are oligopeptide QSMs used mainly by Gram-positive bacteria. These peptides are typically 2–20 amino acids long often with side chain modifications (Lazazzera and Grossman 1998). Classically, these peptides are synthesized as precursor peptides by the ribosomes and undergo posttranslational modification to form an active (leader) peptide. The secretion of the active peptide is usually facilitated by a membrane associated ATP-binding cassette (ABC) transporter (Kleerebezem et al. 1997). When the concentration of the AIP in the external environment reaches a threshold, the peptide binds to a membrane sensor. The activated sensor kinase phosphorylates the intracellular response regulator. The response regulator influences the transcription of downstream target genes to mediate a feedback loop (Verbeke et al. 2017). In contrast to the previous two categories (AHLs and Quinolones) AIPs cannot bypass the membrane easily and require dedicated transporters or receptors (Kleerebezem et al. 1997). AIPs are known to be involved in playing two important roles: one, to induce the production of class II antimicrobial peptides (bacteriocins) as seen in *Lactobacillus plantarum* (Milioni et al. 2015) and second, providing genetic competence as seen in *Streptococcus pneumoniae* (Salvadori et al. 2019). These are mostly linear peptides and are characterized by the double-glycine leader peptide. Autoinducing peptides from *Escherichia coli* and *Staphylococcus aureus* contain a cyclic thiolactone structure (Thoendel et al. 2011). In *Bacillus subtilis*, the 10 amino acid AIP is actually processed from a 55 amino acid precursor peptide and then modified at a tryptophan

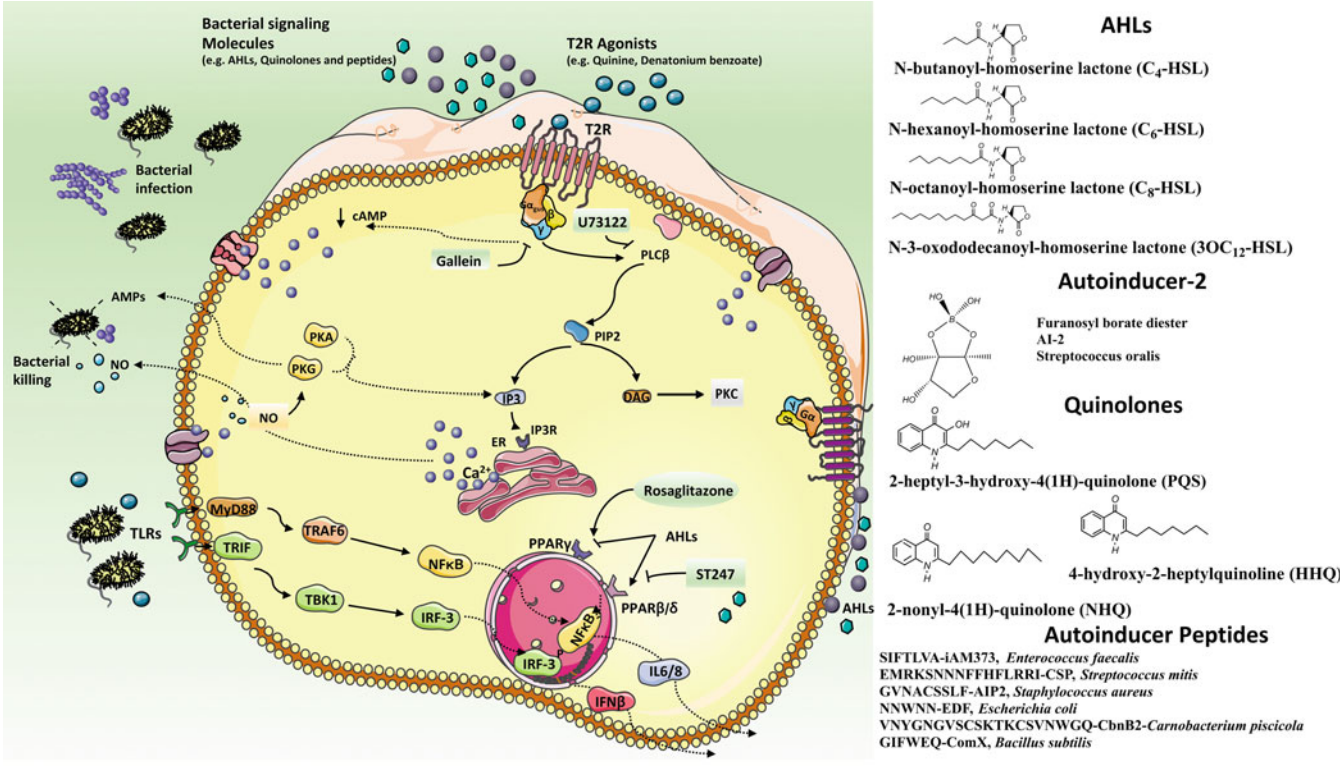

Fig. 2 Schematic of bacterial QSM mediated activation of canonical T2R signaling pathways. *P. aeruginosa* secreted QSMs (AHLs, quinolones, and AI-2), *S. aureus* Gram-positive bacterial peptides (AIPs) and bitter agonists activate Gβγ-PLCβ-IP3 pathway leading to intracellular calcium mobilization. The intracellular calcium mobilization leads to regulation of downstream signaling elements including activation of PKA, PKG pathways, and secretion of antimicrobial peptides (AMP) and NO, which facilitate bacterial killing. The AHLs are also shown to enter the cells and activate PPAR (γβδ) receptors expressed on nuclear membrane. The PPAR receptors then activate NF-kB signaling pathway and several transcriptional factors leading to secretion of pro-inflammatory cytokines

residue possibly by an isoprenoid group (Schneider et al. 2002). Crosstalk by AIPs is an important factor in shaping the microbial ecosystems (Miller et al. 2018).

The 17 amino acid AIP is referred to as the competence stimulating peptide (CSP) in *S. pneumoniae* (Håvarstein et al. 1995). A structure-activity analysis identified that the N-terminus region of the CSP and the hydrophobic residues at the central region of the peptides were most important for its recognition and activity (Yang et al. 2017). CSPs are now identified in various other streptococcal species including *S. gordonii*, *S. intermedius*, and *S. mutans* (Cvitkovitch 2001). *S. mutans* is a key organism in causing dental caries. It secretes a 21 amino acid competence stimulating peptide (CSP-1) to mediate biofilm formation and bacteriocin production (Hossain and Biswas 2012). Interestingly, AIP derived from *S. aureus* (Ard D1 thiolactone) and *S. pneumoniae* CSP-1 (EMRLSKFFRDFILQRKK) did not activate T2R38 (Verbeurgt et al. 2017). Preliminary work suggests that CSP-1 from cariogenic *S. mutans* activates T2R14 in gingival epithelial cells leading to innate immune response (Medapati et al. 2018). Therefore, the ability of CSPs from different streptococci to activate T2Rs remains to be investigated. Apart from AIPs, D-amino acids secreted by several Gram negative and positive bacteria (Lam et al. 2009; Brandenburg et al. 2013; Sarkar and Pires 2015) are known to activate T1Rs in upper airways (Lee et al. 2017). However, their interaction with T2Rs is not characterized so far.

4 T2Rs in Innate Immunity

The expression of T2Rs in extraoral tissues and interaction with bacterial QSMs suggests that T2Rs are involved in mediating some aspects of innate immunity, which are discussed below.

4.1 Role of T2R38 Genetics in Innate Immunity

T2R38 expressed in primary human sinonasal epithelial cells is probably activated by QSMs, mainly C4-AHL and C12-AHL (Lee et al. 2012). These QSMs were able to mobilize intracellular Ca^{2+} and nitric oxide (NO) secretion leading to mucociliary clearance (Lee et al. 2012). Moreover, the AHL mediated Ca^{2+} and NO was suggested to be TAS2R38 genotype dependent. The cells containing PAV/PAV (super-taster) genotype were able to induce better innate immune responses compared to PAV/AVI (intermediate-taster) and AVI/AVI (non-taster) genotypes. Therefore, the polymorphisms in T2Rs probably play an important role in ligand recognition and downstream signaling functions. In another recent study on 217 subjects from different ethnic backgrounds it was shown people expressing $T2R38^{PAV/PAV}$ genotype have less sinonasal symptoms as compared to both the intermediate-taster and non-taster genotypes (Farquhar et al. 2015). A study with a small sample size of 25 subjects with chronic rhinosinusitis (CRS) suggested increased bacterial populations isolated from sinuses in AVI/AVI and PAV/AVI

compared to PAV/PAV genotype (Rom et al. 2017). It was suggested that $TAS2R38^{PAV/PAV}$ genotype, as opposed to non-functional AVI/AVI genotypes, is associated with the absence of *P. aeruginosa* sinus infection (Lee et al. 2012). In contrast, the studies that followed could not establish this association (Gallo et al. 2016; Turnbull et al. 2018).

4.2 T2R Mediated Innate Immune Responses in Chemosensory and Immune Cells

The solitary chemosensory cells expressed in sinonasal and gingival tissues express T2Rs and taste signaling components such as Gnat3 (gustducin), TrpM5, and PLCβ2 (Barham et al. 2013; Saunders et al. 2014; Zheng et al. 2019). T2R agonist denatonium was able to induce Ca^{2+} signaling leading to robust antimicrobial peptide (AMP) β-defensin1 and 2 secretions in these SCCs (Lee et al. 2014; Carey et al. 2017a). The denatonium treated SCC supernatants were able to decrease growth of *P. aeruginosa* as compared to control cultures (Lee et al. 2014). A recent study has shown activation of T2R38 by several organic bacterial metabolites but not AHLs in heterologous cells systems (Table 1) (Verbeurgt et al. 2017). Surprisingly, T2Rs expressed in sinonasal epithelial cells were also activated by Gram-positive bacteria such as *Bacillus cereus* leading to increased intracellular calcium mobilization and NO production, however, these effects were observed to be independent of T2R38 leading to speculation that other T2Rs are also involved in recognizing bacteria and their molecules (Carey et al. 2017b).

The well-known T2R agonist quinine induced T2R signaling dependent NO generation and CBF (Ciliary beat frequency) in sinonasal cells (Workman et al. 2018). Quinolones, a class of bacterial QSMs that activate multiple T2Rs (Table 1), lead to intracellular calcium mobilization and decreased cAMP signaling in heterologous systems as well as in primary human lung epithelial cells (Freund et al. 2018). A recent study demonstrated the expression of T2R4, T2R14, T2R38, and T2R46 in monocyte-derived macrophages (Gopallawa et al. 2020). The T2R14 in unprimed macrophages was activated by *P. aeruginosa* derived QSMs along with other known bitter agonists such as quinine, denatonium benzoate, and flufenamic acid (FFA) (Gopallawa et al. 2020). The T2R dependent calcium signaling in macrophages resulted in increased cGMP and NO dependent phagocytosis of bacteria. This recognition of bacteria and their metabolites by T2Rs is consistent with observations in other species as well. A recent study also demonstrated increased expression of T2Rs in rainbow trout fish (*Oncorhynchus mykiss*) treated with bacteria *Flavobacterium columnare*, infectious hematopoietic necrosis virus (IHNV), and *Ichthyophthirius multifiliis* (Liu et al. 2020). Collectively these studies suggest T2Rs as one of the innate immune targets during bacterial infections.

5 Challenges in T2R Molecular Pharmacology

Significant progress has been achieved over the past decade in characterizing the molecular pharmacology of T2Rs. However, there remain significant challenges, some of them are addressed in this section.

5.1 T2R Ligand Specificity

The experimental evidence from several studies reveals that T2Rs exhibit a substantial degree of agonist non-specificity. The bitter agonists identified thus far are known to activate multiple T2Rs and a single T2R is known to recognize several bitter molecules. For example, the bitter compound denatonium benzoate is known to activate 4 T2Rs (T2R4, T2R8, T2R10, and T2R13), caffeine is known to activate 5 T2Rs (T2R7, T2R10, T2R14, T2R43, and T2R46), chloroquine is known to activate 5 T2Rs (T2R3, T2R7, T2R10, T2R14, and T2R39) and the widely used bitter compound quinine is known to activate 9 T2Rs (T2R4, T2R7, T2R10, T2R14, T2R39, T2R40, T2R43, T2R44, and T2R46) (Meyerhof et al. 2010; Pydi et al. 2012). Similar pattern of activation of multiple T2Rs is observed with QSMs and antibiotics. Recent studies using heterologous cells revealed activation of T2R4, T2R14, and T2R20 by AHLs and by broad-spectrum antibiotics such as tobramycin, levofloxacin, and azithromycin (Jaggupilli et al. 2019). In airway epithelial cells these AHLs and quinolones are shown to activate multiple T2Rs (T2R4, T2R10, T2R14, T2R16, and T2R38 (Lee et al. 2012; Freund et al. 2018). Such broad-spectrum T2R agonist activity possesses significant challenges in delineating T2R specific physiological functions. In contrast, the lack of broad-spectrum T2R blockers is a major challenge.

Over the past decade, T2R structure-function studies have identified bitter blockers, antagonists, and inverse agonists. For example, Nα, Nα-bis (carboxymethyl)-L-Lysine (BCML), γ-aminobutyric acid (GABA), and peptides isolated from beef protein hydrolysates (ETSARHL and AGDDAPRAVF) were effective in blocking quinine and PQS mediated calcium responses with an IC_{50} concentrations ranging from 50 nM to 150 μM, respectively (Pydi et al. 2014b; Freund et al. 2018; Zhang et al. 2018). The broadly expressed T2R14 receptor is blocked by plant flavonoids 4′-fluoro-6-methoxyflavonone, 6,3-′-dimethoxyflavanone, and 6-methoxyflavanone (Roland et al. 2014; Hariri et al. 2017). However, the blocking ability of these compounds has been characterized mostly in heterologous systems. In endogenous cell systems and in-vivo studies the expression of other T2Rs might affect the ability of these bitter blockers to effectively block the response generated by a single receptor.

Constitutive receptor activity and inverse agonism are very important concepts in pharmacology and are of immense value in understanding receptor structure-function and mechanisms, as well as guiding new drug discovery. In the T2R field only few studies have addressed this topic. Structure-function studies on T2R4 identified the first constitutively active mutant (or CAM), S285A in TM7 that

showed ≈3-fold agonist-independent activity over wild type receptor (Pydi et al. 2012). This was followed by the discovery of many CAMs in T2Rs that was reviewed elsewhere (Pydi et al. 2014a). The discovery of T2R4 CAMs led to the characterization of the first inverse agonist, BCML for T2R4 (Pydi et al. 2014b). An interesting study on the primate lemur identified the β-glucoside arbutin acted as an agonist in the ring-tailed lemur but as an inverse agonist in black and black-and-white ruffed lemurs (Itoigawa et al. 2019). This constitutive activity was attributed to a naturally occurring mutation in TM7 of T2R16. This intriguing study opens the possibility of naturally occurring and constitutively active T2Rs existing in humans. Though, this would add another layer of complexity in T2R pharmacology. There is an urgent need for broad-spectrum T2R blockers. Until such blockers are available the field must rely on blocking T2R downstream signaling partners Gβγ, PLC_{β}, IP_3R or using specific T2R knockdown approaches, all of which are tedious and cumbersome.

5.2 T2R Expression and Detection

Different methods have been employed to express T2Rs in heterologous systems. Earlier studies used epitope tagged T2R constructs for expression in HEK293 cells that yielded low plasma membrane expression (Adler et al. 2000; Chandrashekar et al. 2000). The chaperoning of sensory receptors was identified as important for proper folding and targeting to the plasma membrane (Baker et al. 1994; Dwyer et al. 1998). In fact, a recent study has demonstrated T2R14 to be chaperoned to the plasma membrane by β_2-AR (Kim et al. 2016). Interestingly, a pharmacochaperone activity with the bitter agonist quinine was observed for T2R4, T2R7, T2R10, T2R39, and T2R46 (Upadhyaya et al. 2016). Studies have shown T2R chimeric protein sequences to be efficiently targeted to the plasma membrane. For example, rhodopsin-T2R chimera with the first 39 amino acids of bovine rhodopsin added to the N-terminus allowed mT2R5, hT2R4, and mT2R8 to be effectively targeted to the plasma membrane (Chandrashekar et al. 2000). Later it was concluded that the rhodopsin N-terminus 33 amino acids are sufficient to enhance expression of T2R4 by 2.5-fold (Pydi et al. 2013). Another common approach is to tag the N-terminus of T2Rs with the first 45 amino acids of the rat somatostatin receptor 3 (SSTR3) to enable efficient cell membrane localization of T2Rs (Lossow et al. 2016). Hence the use of T2R chimeras can be employed to achieve efficient cell surface expression without substantially compromising the structural integrity and functionality of the receptors.

The characterization of endogenous T2R expression is mostly done at mRNA levels using qPCR and RNA in-situ hybridization approaches (Chen et al. 2019; Taher et al. 2019). To date there are few studies that correlate mRNA and T2R protein expression in cell systems (Tran et al. 2018; Shaik et al. 2019; Gopallawa et al. 2020; Singh et al. 2020). A limiting factor is the availability of T2R antibodies that can target the extracellular epitopes. This is useful in determining the cell surface expression of T2Rs without permeabilizing the cells. Due to the lack of monoclonal

and/or highly specific polyclonal antibodies most of the studies rely on T2R blockers (antagonists or inverse agonists) and siRNA approaches to confirm protein expression of T2Rs. Hence there is an urgent need to develop specific antibodies to detect T2R conformations (active or inactive) and cell surface expression.

6 Conclusion and Future Directions

Over the past few years novel insights about the pathophysiological and pharmacological roles of T2Rs have been obtained. Conventional knowledge of T2Rs being expressed only in gustatory tissues has been overturned. The knowledge gained from recent studies has established T2Rs as potentially important GPCRs in mediating innate immune responses. These findings also led to identification of bacterial QSMs as a new class of ligands for T2Rs. By specifically recognizing certain QSMs, the T2Rs might well be acting as microbe-associated molecular patterns (MAMPs). The lack of high-resolution 3D crystal or cryo-electron microscopic structures of T2Rs bound to ligands or G proteins makes understanding their pharmacology complex. Given the challenges, future studies should be focused on obtaining high-resolution 3D structures of T2Rs, discovery of microbiota derived molecules that activate or block T2Rs, characterizing the T2R mediated innate immune responses to these novel endogenous ligands and elucidating biased T2R signaling in oral and extraoral tissues.

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Bitter Taste Receptors in the Airway Cells Functions

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Contents

Abstract

G protein-coupled receptors (GPCRs) play a central role in regulating the functions of a diverse range of cell types in the airway. Taste 2 receptor (T2R) family of GPCRs is responsible for the transduction of bitter taste; however, recent studies have demonstrated that different subtypes of T2Rs and key components of T2R signaling are expressed in several extra-oral tissues including airways with many physiological roles. In the lung, expression of T2Rs has been confirmed in multiple airway cell types including airway smooth muscle (ASM)

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cells, various epithelial cell subtypes, and on both resident and migratory immune cells. Most importantly, activation of T2Rs with a variety of putative agonists elicits unique signaling in ASM and specialized airway epithelial cells resulting in the inhibition of ASM contraction and proliferation, promotion of ciliary motility, and innate immune response in chemosensory airway epithelial cells. Here we discuss the expression of T2Rs and the mechanistic basis of their function in the structural cells of the airways with some useful insights on immune cells in the context of allergic asthma and other upper airway inflammatory disorders. Emphasis on T2R biology and pharmacology in airway cells has an ulterior goal of exploiting T2Rs for therapeutic benefit in obstructive airway diseases.

Keywords

Airway remodeling · Asthma · Bitter taste receptors · Contraction · COPD · GPCRs · Inflammation · Obstructive lung disease · Relaxation · T2R

Abbreviations

ASM	Airway smooth muscle
CamK	Calmodulin kinase
COPD	Chronic obstructive pulmonary disease
GPCRs	G protein-coupled receptors
IP_3	Inositol 1,4,5-trisphosphate
MLCK	Myosin light chain kinase
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PLCβ	Phospholipase c beta
SR	Sarcoplasmic reticulum
T2R	Type 2 taste receptor
VDCC	Voltage-gated calcium channels
β_2AR	Beta 2 adrenergic receptor

1 Introduction

G protein-coupled receptors (GPCRs) play a central role in regulating the functions of a diverse group of cell types in the airways (Deshpande and Penn 2006; Rosenbaum et al. 2009; Wendell et al. 2020). Airway smooth muscle (ASM) plays an important role in maintaining the diameter of the airways while airway epithelium is critical in maintaining the conduit of air to and from alveoli in addition to its role as the first line of defense against invading airway pathogens. Many well-known GPCRs influence airway tone and thereby the dynamics of respiration. It is well established that contractile-related outcomes in ASM are elicited by the neurotransmitter at the postsynaptic terminal of the parasympathetic nerves, acetylcholine, or inflammatory mediators via activation of GPCRs coupled to the heterotrimeric G protein Gq (Billington and Penn 2003; Deshpande and Penn 2006). Conversely,

Gq-coupled receptor antagonists and Gs-coupled GPCR agonists, which inhibit ASM contraction or promote relaxation have been used to elicit bronchodilation (Deshpande and Penn 2006; Pera and Penn 2016; Wright et al. 2013). However, there is a significant knowledge gap concerning the presence of other GPCR subtypes in the respiratory tract and their effects on airway physiology in both health and disease. GPCRs are also involved in a broad range of physiological functions, including the chemosensory perception of vision, smell, and taste (Rosenbaum et al. 2009). Taste 2 receptors (T2Rs) involved in bitter taste sensation represent a diverse family of GPCRs. T2Rs are described either as a separate putative family or as distantly related to class A (rhodopsin like) GPCRs. Moreover, the newly adapted GRAFS classification system classifies T2Rs in a distinct cluster within the frizzled/taste2 family (Adler et al. 2000; Fredriksson et al. 2003). T2Rs consist of about 30 members in mammals (25 in humans) and are structurally similar to the opsins and the olfactory receptors (Adler et al. 2000; Brockhoff et al. 2010; Chandrashekar et al. 2000; Matsunami et al. 2000; Meyerhof 2005; Mueller et al. 2005). Now it is known that these low-affinity receptors that are broadly tuned to interact with numerous chemically diverse substances are expressed in several extra-oral tissues including on airway cells and regulate many physiological functions (Brockhoff et al. 2010; Chandrashekar et al. 2000; Kinnamon 2012; Shaik et al. 2016; Wu et al. 2002).

Technological advancement in molecular biology along with the production of several lines of transgenic mice expressing key components of T2R signaling has revealed the expression of T2R subtypes in many cell types. In the lung, expression of T2R subtypes has been confirmed in multiple airway cell types including ASM cells, various epithelial cell subtypes, and on both resident and migratory immune cells (Deshpande et al. 2010; Ekoff et al. 2014; Finger et al. 2003; Lin et al. 2008; Maurer et al. 2015; Orsmark-Pietras et al. 2013; Pulkkinen et al. 2012; Sbarbati et al. 2004; Shah et al. 2009; Tan and Sanderson 2014; Tizzano et al. 2011, 2010; Tran et al. 2018; Zhang et al. 2013). Therefore, it has been theorized that ectopic expression of T2R subtypes in the airway may play a role in the innate immune response and the expulsion of harmful exogenous substances from the respiratory tract (Sbarbati et al. 2009). Furthermore, multiple T2R subtypes are expressed in ASM where they have been shown to facilitate airway relaxation and attenuate ASM cell hyperplasia (Deshpande et al. 2010; Sharma et al. 2016, 2017). These findings suggest that T2Rs play an important role in physiological and immune-mediated responses to foreign airway irritants and T2R-mediated effects on multiple airway cell types are beneficial (Conaway Jr. et al. 2020; Nayak et al. 2019a, b).

In this chapter, we discuss the expression of T2R subtypes and the mechanistic basis of their function in the lung. More specifically, in various airway cells, identifying T2Rs expression and understanding their physiological functions in health and disease will lead to advancement in exploiting T2R subtypes for therapeutic benefit in obstructive lung disease.

2 Expression Profile of T2R Subtypes in Multiple Airway Cells

2.1 Expression on Airway Smooth Muscle

Like in many hollow organs, the smooth muscle cells in the airways regulate airway tone and thus act as a key determinant of lung physiology in both health and disease (AJ and P S 2011; Gerthoffer et al. 2012; Hirota et al. 2009; Prakash 2016; Sharma et al. 2010). A study by Deshpande et al. was the first to report the expression and signaling associated with T2R subtypes in cultured ASM cells from human lung donors (Deshpande et al. 2010). The human ASM cells express a repertoire of at least fifteen different T2R subtypes (-10, -14, -31, -5, -4, -19, -3, -20, -45, -50, -30, -9, -13, -42, -46). More importantly, six T2R subtypes (-10, -14, -31, -5, -4, -19) have greater than 1.0-fold abundance of mRNA compared to the beta 2 adrenergic receptor (β_2AR) encoding gene *ADRB2,* while mRNA expression of three subtypes of T2R (-10, -14, -31) was threefold to fourfold higher when compared to the β_2AR gene. Since these seminal findings, multiple investigators have confirmed and validated T2R expression in ASM cells from humans and other species. Grassin-Delyle et al. using human bronchi (freshly isolated from surgical tissue samples) showed expression of eight different T2R subtypes with predominant expression of T2R -5, -10, and -14 (Grassin-Delyle et al. 2013). A study by Pulkkinen et al. in the guinea pig trachealis further confirmed the expression of various subtypes of T2R, namely -3, -10, -4, -14, and -16 (Pulkkinen et al. 2012), followed by independent confirmation of expression of T2R107 (a mouse ortholog of the human T2R10) from two laboratories in murine ASM using different approaches (Tan and Sanderson 2014; Zhang et al. 2013). These investigations suggest that the expression of T2Rs is evolutionarily conserved in ASM across multiple species. Besides, efforts have been made to confirm the expression of T2R subtypes at the protein level although much remains to be done in terms of fully characterizing multiple subtypes at both the transcript and protein level in the future. In the study by Tan and Sanderson, the authors used an immunofluorescence approach in murine tissues and demonstrated expression of T2R107 in ASM by co-staining with the smooth muscle cell marker α-actin. How and what regulates expression of a select subset of T2R subtypes and their level of expression in ASM cells is an exciting riddle for future investigations in addition to identifying subtype-specific functional role(s) for these T2Rs.

2.2 Expression on Various Airway Epithelial Cell Subtypes

Epithelial integrity is critical to maintaining lung homeostasis as the epithelium acts as a first line of defense to any foreign substance entering the airways. Recently, expression of T2R subtypes has been characterized in highly specialized epithelial cells while their exact functional role(s) is still emerging. Primary human airway epithelial cells have been shown to express certain T2R subtypes and some of their downstream signaling effectors (Shah et al. 2009). In this case, the receptors are found on the ciliated cells, rather than on the entire epithelium. Interestingly,

subsequent studies have failed to find evidence of T2R signaling on ciliated airway cells of rodents (Tizzano et al. 2011). This contrast is possibly due to species differences or an effect of cell culture conditions that were used in the study by Shah et al. It will be important to document the presence of these receptors on freshly isolated human airway epithelium or in intact human lung tissue sections in the future.

In the rat, Tizzano et al. showed expression of transcripts of several T2R subtypes, namely T2R-119, -126, -105, -123, -134, -107, -121, and 13 in the epithelium of upper airways, i.e. in the nasal cavity, larynx, and trachea. While T2R13 was absent, the abundance of the above T2R subtypes was confirmed in rat bronchi by these authors. Interestingly, the authors also detected T2R119 and T2R126 transcripts in the whole rat lung, without any indication which cell population contributed to their expression (Tizzano et al. 2011). Expression of T2R38 has been shown by Lee et al. in the human upper airway epithelium (sino-nasal), although this has not been confirmed in the lower airways in the lung (Lee et al. 2012). Another subtype of epithelial cells is *solitary chemosensory cells* (SCCs), found in the mucosal epithelium throughout mammalian organs (Sato 2007; Sbarbati and Osculati 2005; Schneider et al. 2019). Defined as an epithelial lineage as per the core gene signatures (Reid et al. 2005; Sato 2007), they are known by various names in different organs, and sometimes confused with other similar cells (O'Leary et al. 2019; Reid et al. 2005; Sato 2007; Sbarbati and Osculati 2005). In mice, at least eighteen (out of 35 encoded in the mouse genome) T2R subtypes are expressed in tracheal chemosensory cells (Bankova et al. 2018; Krasteva-Christ et al. 2015; Liu et al. 2017; Montoro et al. 2018; Nadjsombati et al. 2018) with T2R126 (Krasteva-Christ et al. 2015) and T2R135 (Bankova et al. 2018; Liu et al. 2017; Nadjsombati et al. 2018) being highly expressed.

Very recently, *rare solitary chemosensory cells* have been described in the airways. Originally identified as tuft cells, these cells have been extensively studied in the gut by the Locksley Lab for their immunomodulatory function (Nadjsombati et al. 2018; O'Leary et al. 2019; Schneider et al. 2019). In human airways, they have been described as the *pulmonary brush cells*, found on the airway linings but with greater abundance around the alveolar region in the lung (Reid et al. 2005). In rodents, these cells have a similarity to the chemosensory (taste) cells and express some of the cardinal markers such as GNAT3, PLCβ2, TRPM5, and T2R108 (Hofer et al. 1996). While these cells have been observed throughout the respiratory tract in rats, they are nearly absent in the intrapulmonary airways below the bronchial branch point in mice. Very recently, heterogeneity between murine airway tuft cells has been demonstrated and can be distinguished into subtypes: immature tuft cells, tuft-1cells and tuft-2 cells; however, it remains to be seen whether these cells express a repertoire of T2Rs (Montoro et al. 2018). Future studies with chemosensory and other markers and cell-sorting techniques should enable us to determine the expression profile of various T2R subtypes in these cells and will also point to their exact role in airway physiology and upper airway and obstructive lung diseases.

2.3 Expression on Airway Immune Cells

Though this chapter is mainly focused on the T2R biology of airway structural cells, we would like to highlight some of the emerging evidence for T2R subtypes in immune cell functions in the context of airway diseases. Very recently, Tran et al. demonstrated expression of T2R38 on freshly isolated human peripheral blood neutrophils, monocytes, and lymphocytes (Tran et al. 2018). Authors reported differential expression of T2R38 among lymphocyte subpopulations and among different immune cell population, suggesting a varied immunomodulatory role of T2R38 (Tran et al. 2018). Similarly, the expression of T2R38 on numerous immune cells has been confirmed by other investigators in humans (Maurer et al. 2015). Ekoff et al. demonstrated mRNA expression for many T2Rs, namely -3, -4, -5, -10, -13, -14, -19, -20, -46 in human mast cells (Ekoff et al. 2014). Orsmark-Pietras et al. demonstrated expression of mRNA of eleven different subtypes of T2R (-4, -5, -10, -13, -14, -19, -20, -31, -45, - 46, and -50) in mixed-blood leukocytes from healthy children and children with severe asthma. Also, authors also found a differential expression of many T2R subtypes in the immune cell population in asthmatics vs healthy subjects (Orsmark-Pietras et al. 2013). Expression of T2R subtypes on various immune cells suggests a wider physiological function for these receptors. For example, Malki et al. recently showed that expression of T2R61 and T2R64 mediate human blood PMN chemotaxis, therefore work as markers for subpopulations of circulating leukocytes (Malki et al. 2015). While the studies have described the physiological role of T2R subtypes in ASM functions in a variety of model systems (in vitro, ex vivo, and in vivo), the significance of T2R subtypes individually in the regulation of ASM functions is not yet established. Further investigation is needed in developing tools to manipulate signaling via individual T2R subtypes (e.g., receptor subtype-specific agonists/antagonists or knockout model). Expression of subtypes of T2Rs on different airway and immune cells is summarized in Table 1.

Table 1 Species and cell-specific expression of various T2R subtypes in the airways

Species/ cell-type	Airway smooth muscle	Epithelial cells	Immune cells
Human	T2R-10, -14, -31, -5, -4, -19, -3, -20, -45, -50, -30, -9, -13, -42, -46, -1 and -8	T2R38	T2R-4, -5, -10, -13, -14, -19, -20, -38, -31, -45, -46, -50, -61, -64
Mouse	T2R107	T2R108	
Rat	T2R-4, -14, -10	T2R-119, -126, -105, -123, -134, -107, -121, 13	
Guinea pig	T2R-3, -10, -4, -14, and -16		

3 Mechanisms and Functional Outcomes of T2R Subtypes in Airways

While expression and function of T2R subtypes are still being investigated in different cell types of the lung, it is intriguing how these receptors are capable of detecting structurally diverse bitter compounds and eliciting unique functions in airway cells that were not thought of previously. Further, the pharmacological properties of T2Rs include broad tuning and low affinity for bitter agonists. The product of these two features is a unique responsiveness and/or activation of T2R subtypes by individual agonists with some degree of overlap in their functional responses in airway cells as described below.

3.1 T2R Signaling Mechanisms in Regulating ASM Contraction and Relaxation

The discovery of expression of T2R subtypes on ASM cells has opened a new era of GPCR knowledge in ASM physiology and pharmacology. Activation of T2Rs on ASM with a variety of chemically diverse classes of bitter tastants (T2R agonists) induces relaxation of ASM and efficacious bronchodilation. T2R-mediated ASM relaxation was demonstrated using ASM cells and airway tissues obtained from human, mice, and guinea pig lungs, and in vivo evidence of bronchodilation was obtained using a murine model (Camoretti-Mercado et al. 2015; Deshpande et al. 2010; Grassin-Delyle et al. 2013; Pulkkinen et al. 2012; Sharma et al. 2017; Tan and Sanderson 2014; Zhang et al. 2012, 2013). Bitter tastant treatment of ASM cells or tissues robustly reverses contraction induced by contractile agonists that are regulators of physiological (e.g., acetylcholine) and pathological (e.g., histamine, 5-hydroxytryptamine) bronchoconstriction. Most importantly, bronchodilation by bitter tastants is preserved in a murine model of asthma and human lung tissues obtained from asthmatic donors (Robinett et al. 2014; Sharma et al. 2017).

Although activation of T2Rs has been shown to elicit relaxation in ASM, the signaling mechanism responsible for this outcome differs from the classical G_s-coupled GPCR signaling, which facilitates bronchodilation through an increase in levels of cyclic adenosine monophosphate (cAMP) and activation of protein kinase A. In ASM, T2R activation elicits an elevation of intracellular calcium concentration ($[Ca^{2+}]_i$) similar to that observed with G_q-coupled signaling; however, T2R activation evokes ASM relaxation, in stark contrast to G_q-mediated ASM contraction (Fig. 1). Under homeostatic conditions, the binding of bronchoconstrictive agonists to G_q-coupled GPCRs in ASM cells leads to G alpha subunit (Gα)-mediated phospholipase C beta (PLCβ) activation (Billington and Penn 2003; Deshpande and Penn 2006). Following PLCβ-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol triphosphate (IP_3), IP_3 diffuses from the plasma membrane through the cytosol and binds to the IP_3 receptor (IP_3R) on the sarcoplasmic reticulum (SR). The subsequent opening of IP_3-sensitive stores results in a rapid rise in $[Ca^{2+}]_i$ which then binds to and forms a complex with

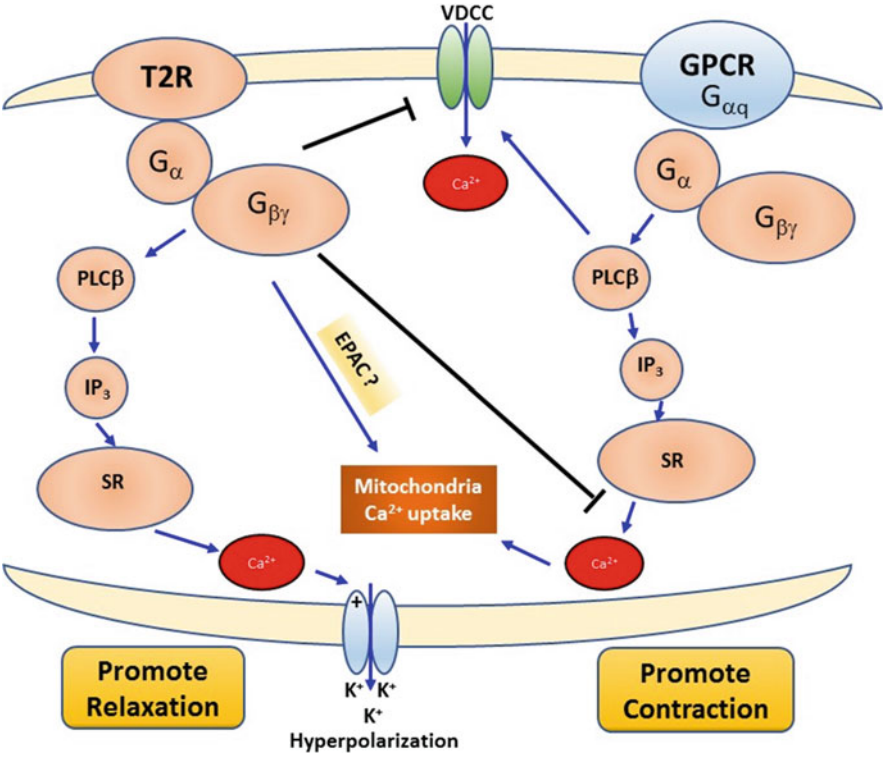

Fig. 1 Mechanisms of T2R-mediated signaling in promoting ASM relaxation. Ligand binding to T2Rs leads to its activation and initiation of downstream signal transduction in ASM cells to promote airway relaxation. As detailed in the text, fundamental mechanisms that are being tested and experimentally validated are: opening of large conductance Ca^{2+} activated K^+ (BK_{Ca}) channels leading to membrane hyperpolarization; inhibition of IP_3R-mediated increase in $[Ca^{2+}]_i$ from the SR; and Gβγ subunit-mediated inhibition of voltage-dependent calcium channel (VDCC) which is activated by contractile agonists resulting in reduced calcium levels and thus inhibiting ASM contraction. Although the inhibition of VDCC or IP3R is Gβγ subunit-mediated, the exact mechanism of inhibition is yet to be fully elucidated. Recently, it has been shown that T2R activation can reduce GPCR-mediated $[Ca^{2+}]_i$ by enhancing mitochondrial calcium uptake thereby decreasing contraction

calmodulin. This active complex activates calmodulin kinase (CamK) and myosin light chain kinase (MLCK). MLCK is responsible for the phosphorylation of myosin light chain (MLC), a protein that is critical for the formation of the myosin-actin cross-bridge necessary for the "*power stroke*" that drives ASM contraction. T2R signaling leads to $[Ca^{2+}]_i$ flux and ASM relaxation in a G beta gamma subunit (Gβγ)-, PLCβ- and IP_3-mediated fashion (Deshpande et al. 2010). Gβγ- and PLCβ-dependent signaling by T2Rs was also demonstrated in murine ASM cells by the ZhuGe group (Zhang et al. 2013). Additionally, T2R subtypes in gustatory cells are known to be coupled to the gustducin (G_{gust}) family of G proteins. However, there is some evidence that suggests that T2Rs couple with heterotrimeric G proteins

belonging to G_i family G proteins in airway smooth muscle cells (Kim et al. 2017). Whether all the subtypes of T2Rs in ASM couple to only one class of G proteins or if there is a difference in G protein-coupling by different T2R subtypes needs to be established. This type of receptor subtype-specific coupling to G proteins has been shown in ASM cells (e.g., muscarinic acetylcholine receptor M2 is coupled to Gi family G proteins whereas M3 receptors couple to Gq family G proteins).

Although there are similarities with some of the downstream components shared between T2R and G_q-coupled signaling, the dynamics of calcium mobilization and localization differ between these opposing pathways. ASM cells treated with bitter taste agonists experience rapid calcium mobilization (2.5 s) and localization of $[Ca^{2+}]_i$ to the sarcolemmal domains. Conversely, the treatment of ASM cells with the contractile agonist histamine results in a delayed increase in $[Ca^{2+}]_i$, which then diffuses throughout the cell indiscriminately (Deshpande et al. 2010). There is evidence that suggests that the rapid T2R-mediated increase in $[Ca^{2+}]_i$ leads to the activation of large-conductance calcium-activated potassium (BK_{Ca}) channels causing hyperpolarization of ASM cells and thus ASM relaxation (Deshpande et al. 2010). Interestingly, T2R-mediated $[Ca^{2+}]_i$ increase was not observed in freshly isolated murine ASM cells and in murine lung slices which is contrary to what was seen in cultured human ASM cells (Tan and Sanderson 2014; Zhang et al. 2013). In a variety of in vitro, ex vivo, and in vivo approaches, T2R agonists induced a relaxation response with a significant reversal or prevention of Gq-mediated contraction of ASM (Camoretti-Mercado et al. 2015; Deshpande et al. 2010; Grassin-Delyle et al. 2013; Pulkkinen et al. 2012; Tan and Sanderson 2014; Zhang et al. 2012, 2013). Differing mechanisms explaining the calcium dynamics involved in T2R-mediated relaxation have also been proposed. One mechanism suggests that T2R-activated Gβγ binds to the IP_3R and attenuates any subsequent G_q-mediated release of $[Ca^{2+}]_i$ from IP_3 stores (Tan and Sanderson 2014). Instead of BK_{Ca} channel activation, another study suggests that T2R-activated Gβγ can directly inhibit the influx of extracellular calcium through voltage-dependent calcium channels (VDCC) necessary to sustain prolonged ASM contraction (Zhang et al. 2012). A study by Camerotti-Mercado et al. demonstrated that bitter tastant-mediated inhibition of contractile agonist-induced calcium elevation and tone in ASM is heterogeneous and dependent on both the T2R agonist and Gq-coupled GPCR agonist. For example, chloroquine was able to inhibit histamine-induced calcium elevation and contraction in human ASM cells but not that induced by endothelin (Camoretti-Mercado et al. 2015). Another recent study demonstrated that T2R activation leads to an influx of calcium into mitochondria, thereby decreasing the contractile agonist-induced increase in cytosolic calcium concentration (Talmon et al. 2019).

In summary, these findings demonstrate airway relaxation by a diverse group of T2R agonists in multiple species: mice (Deshpande et al. 2010; Tan and Sanderson 2014; Zhang et al. 2013), humans (Belvisi et al. 2011; Grassin-Delyle et al. 2013) and guinea pigs (Pulkkinen et al. 2012). Dynamics of calcium regulation by T2Rs alone or in the presence of a contractile agonist are complex and need additional investigation. The above observations warrant further investigation into the interplay

between T2R-induced Ca^{2+} release and the relative contribution of cellular compartments (mitochondria, caveolae, and SR) in terms of their physical proximity to modulate T2R-mediated responses in ASM.

3.2 T2R Activation and Contractile Agonist Specific Regulation of ASM Function

More recently the idea of differential relaxation of ASM by T2R agonists has emerged (Camoretti-Mercado et al. 2015). This has been shown for chloroquine and denatonium in guinea pig ASM when pre-contracted with different contractile agonists (Pulkkinen et al. 2012). Specifically, denatonium (agonist for T2R -4 and -10) preferentially inhibits cholinergic stimulation of guinea pig trachea, while chloroquine (agonist for T2R -3 and -10) can inhibit ASM contraction to a wide range of contractile agonists. Similarly, chloroquine and aristolochic acid (in human ASM) have been shown to differentially inhibit $[Ca^{2+}]_i$ release induced by histamine and endothelin in vitro (Camoretti-Mercado et al. 2015). The idea of agonist- and T2R subtype-specific activation of T2R signaling in ASM cells has been strengthened by antibody- and genetic-based approaches in elucidating the dynamics of $[Ca^{2+}]_i$ mobilization and ASM relaxation facilitated by T2R signaling. For example, the silencing of T2R10 mRNA expression using siRNA significantly reduced the $[Ca^{2+}]_i$ signal induced by strychnine. This finding was recapitulated when blocking T2R10 with anti-T2R10 polyclonal sera. Additionally, the siRNA-based knockdown of the lesser-known subtype T2R46 resulted in a significant reduction of absinthin-mediated attenuation of histamine-induced $[Ca^{2+}]_i$ release (Talmon et al. 2019). These approaches have been useful in establishing the specificity of certain bitter taste agonists to elicit canonical T2R signaling in ASM. Further, lesser-studied subtypes like T2R46 not only reduce histamine-mediated $[Ca^{2+}]_i$ release but also facilitate calcium flux into mitochondria in addition to the canonical events observed withT2R signaling (Talmon et al. 2019). Furthermore, the effects of T2R-mediated changes in mitochondrial dynamics on the ASM function have yet to be studied extensively. Potency of various T2R agonists in cell- and tissue-specific ASM models has been summarized in Table 2. Identifying and discovering T2Rs subtype-specific agonists and antagonists has been a fundamental roadblock to further the understanding of physiological role of individual T2R subtypes in ASM. However, efforts are being made in this direction. There is a limited evidence that antagonists and inverse agonists (based on structures of endogenous ligands) of T2R4 exist (Pydi et al. 2014). Similarly, natural sesquiterpene lactones are described as antagonists for T2R46 (Brockhoff et al. 2011). More recently, synthetic new chemical entities (NCE) that can antagonize T2R subtypes – mainly T2R14/39 (dual) – have been described in literature, as these structures are mainly based on the flavonoid core (Gopallawa et al. 2020; Roland et al. 2014). These continual drug discovery efforts will help discover new T2Rs subtype-specific antagonists and will therefore facilitate physiological studies to investigate their individual role in ASM function in health and pulmonary disease.

Table 2 Potency of T2R agonists in human and murine ASM cell- and tissue-models

T2R Agonist	~EC_{50} (μM)	Model system	Methodology
*Chloroquine**	6	m-PCLS	MCh-mediated contraction (Tan and Sanderson 2014)
Denatonium	15	m-PCLS	MCh-mediated contraction (Tan and Sanderson 2014)
Diphenidol	25	m-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
Phenanthroline	30	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
Flufenamic acid	30	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
*Quinine**	50–60	m-PCLS	MCh and 5-HT-mediated contraction (Tan and Sanderson 2014)
Carisoprodol	60	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
*Chloroquine**	60	h-ASM cells	Ca^{2+} transients (Deshpande et al. 2010)
*Chloroquine**	60	m-trachea h-bronchi m-PCLS	ACh and 5-HT-mediated contraction (Mikami et al. 2017) Histamine-mediated contraction (Grassin-Delyle et al. 2013) 5-HT-mediated contraction (Tan and Sanderson 2014)
Denatonium	60	m-trachea	ACh-mediated contraction (Deshpande et al. 2010)
Dapsone	60	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
Erythromycin	60	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
Saccharin	100	h-ASM cells	Ca^{2+} transients (Deshpande et al. 2010)
Strychnine	100	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
Caffeine	150	h-bronchi	Histamine-mediated contraction (Grassin-Delyle et al. 2013)
*Quinine**	150	m-trachea m-bronchi h-PCLS	Ach and 5-HT-mediated contraction (Deshpande et al. 2010) MCh-mediated contraction (Zhang et al. 2012) IL-13-mediated contraction (Robinett et al. 2014)

5-HT serotonin, *ACh* acetylcholine, *Ca^{2+}* calcium, *IL-13* interleukin 13, *MCh* methacholine, *PCLS* precision-cut lung slices, *h* human, *m* murine

Agonists with multiple tissue- and contractile agonist-specific EC_{50} values are denoted with an asterisk (*)

3.3 T2R Activation in the Regulation of ASM Cell Proliferation

In the airways, ASM cell proliferation and migration are plausible mechanisms by which changes in ASM mass may occur in obstructive lung diseases (Gerthoffer et al. 2012; Hirst et al. 2004; Prakash 2013). Multiple studies have validated the signaling mechanisms that regulate ASM proliferation by T2R subtypes (Kim et al. 2019; Pan et al. 2019, 2017; Sharma et al. 2016) (Fig. 2). Stimulation with various growth factors results in proliferative signaling orchestrated by sustained activation of distinct proliferative pathways. Growth factors can activate their cognate tyrosine-kinase receptors to transduce the mitogen-activated protein kinase (MAPK) pathway that promotes cell proliferation. Additionally, activated receptors can also recruit cytosolic phosphoinositide-3-kinase (PI3K) that phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP_2) to form phosphatidylinositol 3,4,5-triphosphate (PIP_3), which is a second messenger that activates protein kinase B (Akt), which in turn can regulate activation of enzymes and transcription factors that promote cell growth (Sharma et al. 2016, 2008).

We have shown that T2Rs regulate mitogen-induced ASM proliferation, with the bitter tastants chloroquine and quinine specifically inhibiting growth factor-induced ASM cell proliferation (Sharma et al. 2016). In terms of the signal transduction

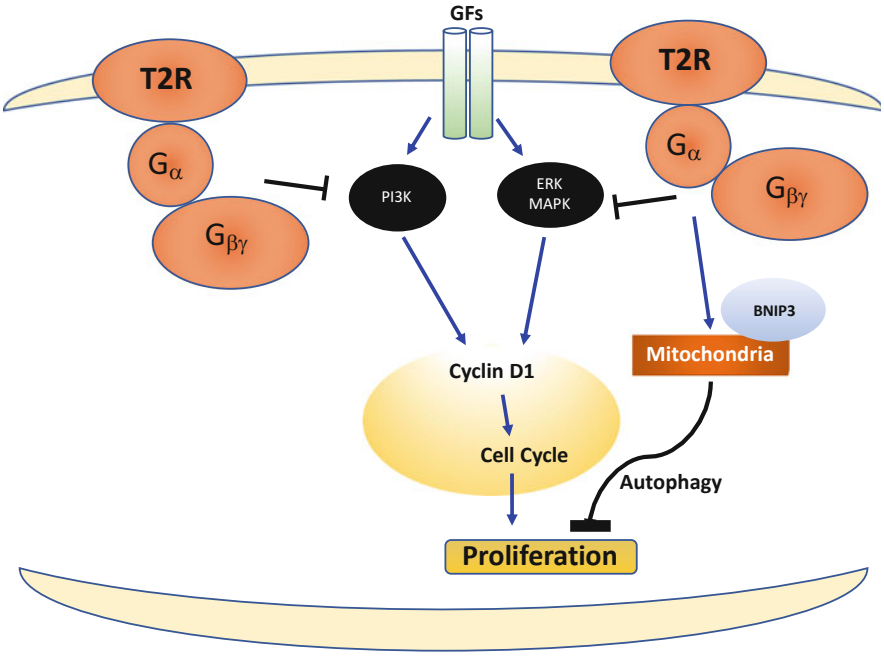

Fig. 2 Mechanisms of T2R-mediated inhibition of ASM proliferation. As detailed in the text, in the ASM cell T2R agonists can inhibit growth factor-induced proliferation of ASM cells by inhibiting PI3K and MAPK signaling pathways. Another mechanism by which T2R activation can decrease ASM proliferation involves mitochondrial fragmentation and autophagy

mechanism involved in the anti-mitogenic effect of T2R agonists, it has been demonstrated that bitter tastants inhibit phosphorylation of Akt and p70S6 kinase independent of PIP_3 regulation, suggesting that it occurs downstream of PI3K. Further investigation revealed that T2R activation inhibited activation of the transcription factors AP-1 (Activator protein-1) and E2F with an associated decrease in expression of cell cycle genes including cyclin D1, which are essential for cell cycle progression (Sharma et al. 2016). Acute treatment (less than 30 min) of ASM cells with bitter tastants did not perturb activation of MAP kinase pathways, including p42/44 and p38 signaling in ASM cells. More recently, a study by Kim et al. demonstrated that T2R agonists retain the capacity to inhibit the proliferation of ASM cells derived from asthmatic donors in an ERK-MAPK dependent manner (Kim et al. 2019). Further, the authors in this study alluded to differential activation of T2Rs by a structurally diverse class of bitter compounds and suggested that multiple mechanisms may contribute to this effect in vitro (Kim et al. 2019). New studies are still emerging on signaling mechanisms by which T2R activation can elicit an anti-proliferative effect in ASM. It was recently demonstrated that activation of T2Rs can alter mitochondrial function and induce autophagy, thereby providing evidence for another signaling axis that may be important in T2R-mediated anti-proliferative effects in vitro (Pan et al. 2017). Further characterization of this complex signaling axis (mitophagy) revealed that Bnip3 is an important regulator of mitochondrial functions upon T2R activation, which also regulates expression of key proteins that influence ASM cell adhesion, migration, and proliferation (Pan et al. 2019).

In summary, mechanistically, T2R agonists inhibit ASM proliferation by multiple mechanisms: inhibiting the growth factor-activated protein kinase B (Akt) phosphorylation, inhibiting of transcription factors AP-1, STAT3, E2F, and NFAT, inhibiting genes associated with cell cycle progression and inducing selective (mito)-autophagy (Pan et al. 2019, 2017; Sharma et al. 2016). These lines of evidence suggest that T2R-mediated signaling can be exploited for reducing increased ASM mass as observed in many obstructive lung diseases (described below). It remains to be seen which particular subtype of T2Rs contributes greatly to this process.

3.4 T2Rs Signaling Mechanisms in Epithelial Cell Subtypes

As described above, although the expression of T2R subtypes (Table 1) may vary in different epithelial cell types, the signaling per se as shown in Fig. 3 is invariant and results in elevation of $[Ca^{2+}]_i$ upon stimulation of chemosensory airway epithelial cells with bitter tastants. Also, although not much is known specifically about signaling in epithelial cells of the lower airways, knowledge gained from upper airway epithelial cell signaling has provided insights into its role in T2R-mediated innate immune responses (Lee and Cohen 2015b; Vetlugina et al. 1989). It is believed that there is a huge diversity in specific signaling pathways that contributes to these responses, more specifically in the sino-nasal epithelium (Hariri and Cohen 2016; Lee and Cohen 2015a; Vetlugina et al. 1989). It has been shown that T2R38

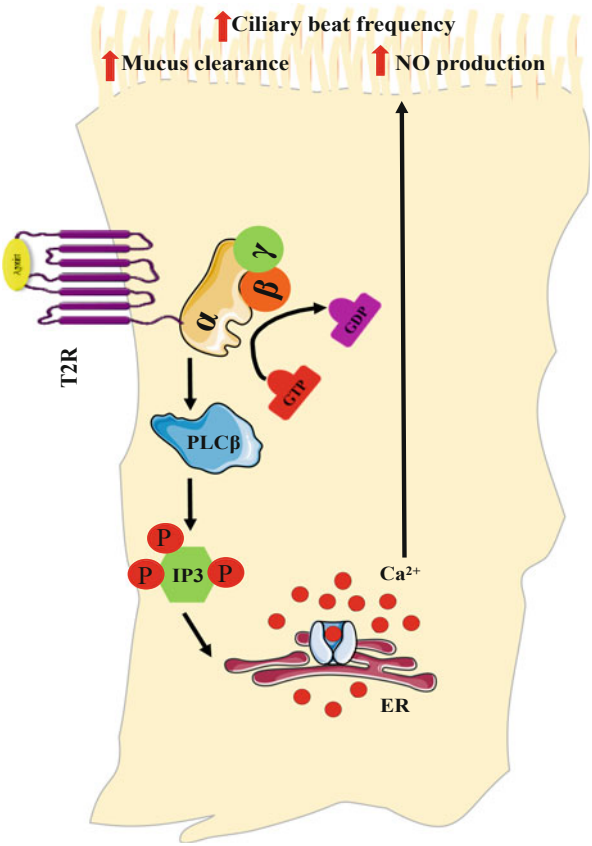

Fig. 3 Signaling and functional outcomes of T2R activation in the airway epithelium. T2R activation in specialized ciliated airway epithelial cells leads to signaling through PLCβ-IP_3 resulting in elevation of intracellular calcium that leads to an increase in the ciliary beat frequency. In addition, it has been shown that T2R activation in the upper airway epithelial cells can lead to increase in NO production

can directly recognize N-acyl homoserine lactones (AHL) (a bitter-tasting compound) secreted from gram-negative bacteria (*P. aeruginosa*) (Lee et al. 2012; Maurer et al. 2015). Increased production of nitric oxide has been demonstrated upon T2R activation, as a mechanism to fight invading pathogens (Lee et al. 2014). Further, Tizzano et al. validated these findings and demonstrated that T2R–AHL interaction results in a calcium-dependent NO production, increased ciliary beat frequency, and increased mucociliary clearance of inhaled pathogens (Tizzano et al. 2006). While activation of T2R38 on ciliated cells in the tracheal epithelium generated bactericidal nitric oxide (Lee et al. 2014, 2012), stimulation of T2Rs on SCCs in the respiratory epithelium stimulated the release of acetylcholine (Finger et al. 2003; Krasteva et al. 2011, 2012; Saunders et al. 2013; Tizzano et al. 2010). In ciliated epithelial cells, T2Rs are located both extracellularly on the motile cilia and the apical membrane of the cell and their activation induces an increase in $[Ca^{2+}]_i$, leading to increased ciliary beat frequency. Possible mechanisms of this functional outcome include induction of nitric oxide and the cGMP-protein kinase G pathway (Salathe 2007). Similarly, activation of T2Rs on SCCs stimulates a calcium wave within the cell (Bezencon et al. 2008). We believe the advancement in T2R biology

and the availability of reagents and tools will allow researchers to capture a unique repertoire of T2R expression in a wide variety of distinct airway epithelial cell subtypes in the lung and establish unique signaling mechanisms in each of these cell types. These insights will aid in our understanding of epithelial-specific T2R expression and their functional role in the airways in both health and disease.

3.5 Functional Outcomes of T2Rs Activation in the Airway Epithelium

There is limited evidence of the exact functional role of T2R activation in the airway epithelium. Our current knowledge is derived from the role of T2Rs in mucus production and regulation of ciliary beat frequency both in vitro and in vivo. More specifically, it has been demonstrated that T2R activation in human ciliated epithelial cells can increase ciliary beat frequency (by increasing calcium) and thereby promote mucus clearance (Shah et al. 2009). This fundamental effect of T2R activation may be highly beneficial in many obstructive lung diseases as current therapeutics have very limited effect if any on mucus production. This effect has been shown in the lower airway epithelial cells (murine models of allergic asthma), where two different T2R agonists, chloroquine and quinine, inhibited mucus staining in the airway lumen (Sharma et al. 2017). Of note, in this model, T2R activation also resulted in an increased airway relaxation (reduced AHR, as described above) and therefore, it remains to be tested in this model whether the effect on mucus production was a result of direct T2R-mediated action in the epithelium or by increased mucus clearance as a result of increased airway relaxation. Furthermore, it has been shown that activation of T2Rs on SCCs (in respiratory epithelium) can induce acetylcholine release, which has been shown to promote neurogenic inflammation (also a preventative measure to reduce the respiratory rate to an inhaled allergen) (Finger et al. 2003; Krasteva et al. 2011, 2012; Saunders et al. 2013; Tizzano et al. 2010). Moreover, multiple lines of evidence emanating from the work carried out in the upper airways suggest that activation of T2Rs in the airway epithelium may be important in pattern recognition. Therefore, T2Rs in the airway epithelium may act as a component that recognizes conserved structures in pathogens and how the body senses pathogen invasion, triggers innate immune responses, and primes antigen-specific adaptive immunity (Lee and Cohen 2015b). Further characterization of T2R function in the lower airway epithelium is needed to advance our efforts in undertaking how specific agonists of T2Rs can be used to study their precise role in lung physiology for a therapeutic gain.

4 T2Rs in Lung Health and Disease: Focus on Features of Allergic Asthma

Physiologic evidence suggests that T2R expression evolved to enhance the immune response to foreign agents like AHL, which are bitter taste molecules secreted by bacteria (Eberl 1999). In support of this, studies show T2R expression in SCCs and immune cells and their ability to respond to AHL as a protective mechanism reinforce the theory that T2Rs on SCCs play a critical role in recognizing airway pathogens (Finger et al. 2003; Lee et al. 2014; Shah et al. 2009). In addition, multiple studies on T2Rs in airway cells have focused on obstructive lung diseases, more specifically asthma. Herein, we describe the role of T2Rs in regulating lower airway cell functions by focusing on ASM, epithelial cells, and infiltrating and/or resident immune cells in the context of allergic asthma. In allergic asthma, the role of various epithelial T2R subtypes in the regulation of allergen-induced airway inflammation is not fully known and the relative contribution of specialized cells (such as tuft cells) and their crosstalk with infiltrating immune cells in the airways remain to be investigated.

The primary function of ASM encircling the airway lumen is to maintain airway tone and at the same time limit bronchoconstriction. Dysfunction in ASM functional capacity to keep the airway open or to shorten more has been observed in many obstructive lung diseases, therefore agents promoting ASM relaxation are highly beneficial in pathological conditions (for example, inhaled bronchodilators for reversing airway obstruction in asthma and COPD). It has been shown that agonists of T2Rs are efficacious bronchodilators providing far more effective relaxation when compared to existing bronchodilators (Deshpande et al. 2010; Grassin-Delyle et al. 2013; Pulkkinen et al. 2012; Tan and Sanderson 2014). Beginning from our serendipitous observation almost a decade ago, several studies have laid the foundation for investigating agonists of T2Rs as effective bronchodilators in the future (Deshpande et al. 2010). It was further demonstrated that targeting T2R subtypes is effective in treating multiple features of asthma. Using murine models of allergic asthma, we have shown that bitter tastants when delivered directly into the lungs not only prevented the development of allergic asthma but were able to reverse the established asthma associated pathology. Further, T2R activation resulted in a robust reduction of allergen-induced immune cell influx and cytokine release (Sharma et al. 2017), confirming the previously reported anti-inflammatory property of T2R agonists in the upper airways (Lee et al. 2014, 2012). Additionally, T2R agonists were able to inhibit the migration of immune cells obtained from human donors to a chemotactic gradient, suggesting a possible mechanism of reducing airway inflammation in asthma. These studies further confirmed earlier findings where T2R agonists were shown to inhibit IgE-induced activation of mast cells and degranulation as measured by inhibition of PGD_2 and histamine (Ekoff et al. 2014). In summary, studies from both murine models and in human cells demonstrated T2R agonists can dampen allergen-induced airway inflammation and thus can be a viable drug target for allergic asthma.

Desensitization of GPCR signaling is frequently encountered in both in vitro and in vivo systems. This is also observed clinically with chronic use of β_2AR agonists in asthmatics which leads to worsening of asthma-related symptoms and loss of asthma control (Crane et al. 1989; Pearce et al. 1995, 1997; Salpeter et al. 2006, 2010; Turki et al. 1995; Walker et al. 2011). We have shown in the murine model of asthma that the T2R agonists inhibited methacholine-induced bronchoconstriction despite modest bronchodilation by a β2AR agonist (Deshpande et al. 2010). Further, studies carried out in the Liggett Lab demonstrated that T2R signaling was not hindered by asthma despite β2-adrenergic receptor tachyphylaxis (An et al. 2012). The authors further reported that T2R agonists were effective in inducing relaxation of asthmatic ASM cells and lung tissue slices, and the T2Rs expression and related signaling in asthmatic ASM cells remain unchanged in inflammatory conditions (An et al. 2012; Robinett et al. 2014). These observations demonstrate that bitter tastants are appealing therapeutic candidates for the management of airway diseases in which beta-agonists fail to provide protection due to receptor desensitization and tachyphylaxis.

It is now well recognized that ASM not only regulates airway tone but also participates in a variety of synthetic functions that maintain the ASM phenotype in vivo. In many obstructive lung diseases, these long-term changes in the ASM phenotype as a result of repeated insult can lead to a change in patency of the airway (broadly known as airway remodeling). Current pharmacologic asthma therapeutics are limited in their role to act on ASM-remodeling (Prakash et al. 2017). To this end, T2R pharmacology provides an attractive option for mitigating ASM-remodeling and facilitating effective airway relaxation. Studies on human ASM cells have shown that T2R agonists curtail growth factor-stimulated cell proliferation obtained from healthy and asthmatic donors alike in a dose-dependent manner (Kim et al. 2019; Sharma et al. 2016). The mechanism by which activation of T2Rs signaling provides beneficial anti-proliferative effects is described above (Fig. 3). What is exciting and highly significant is that the T2Rs employ a distinct signaling pathway in eliciting an anti-mitogenic effect in ASM cells which is independent of PKA activation as seen with β_2AR agonists (Deshpande et al. 2010; Robinett et al. 2011). Furthermore, the anti-mitogenic effect of T2R agonists was also evident in pre-clinical models of asthma. The effects of activation of T2R signaling in preventing or reversing ASM-remodeling (i.e., ASM mass and remodeling features) were also studied using a model of allergic asthma. It has been discovered that T2R activation by chloroquine and quinine reduced the expression of the ASM markers smooth muscle α-actin, calponin, and smooth muscle myosin heavy chain (markers of ASM phenotype). Furthermore, levels of key profibrotic markers were significantly lower in the tissues following treatment with T2R agonists. Collectively, these in vitro and in vivo studies suggest that T2R agonists mitigate ASM-remodeling.

Another aspect that needs equal consideration before targeting various T2R subtypes for clinical gain is the T2R gene polymorphisms (Chamoun et al. 2018; Ueda et al. 2001). Several studies have reported an association between genetic variation in T2R genes, namely T2R-16 and -13, and specific diseases such as alcohol dependence and head and neck cancer (Dotson et al. 2012; Hinrichs et al. 2006; Mangold et al. 2008). In the upper airways, there is a clear evidence for

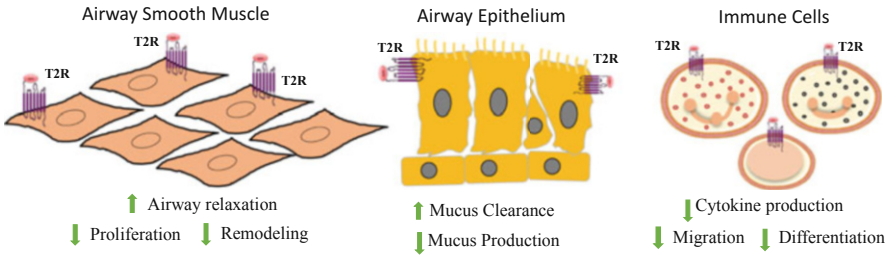

Fig. 4 T2R-mediated effects on multiple airway cell types. T2Rs are expressed on the airway smooth muscle cells, various airway epithelial cell subtypes, and on immune cells. Ligand binding to the cognate T2R and its activation is followed by downstream signal transduction mechanisms resulting in various functional outcomes in multiple airway cell types. In airway smooth muscle, activation of the T2R induces airway relaxation which promotes bronchodilation. Additionally, T2R activation inhibits ASM cell proliferation and profibrotic signaling, resulting in reduction in secretion and/or accumulation of matrix proteins such as collagen-1 and fibronectin (collectively called as "remodeling"). In airway epithelial cell subtypes, activation of T2R signaling causes increased ciliary beat frequency leading to enhanced mucus clearance. Activation of T2R signaling in immune cells mediates reduction in migration and differentiation of immune cells and cytokine production. The multi-modality of T2R-mediated signaling provides a strong rationale for their use in obstructive lung diseases such as asthma

polymorphisms in T2R and its association with disease severity in chronic rhinosinusitis (Adappa et al. 2016; Behrens and Meyerhof 2006; Lee et al. 2012; Triantafillou et al. 2018). More recently, Yoon et al. demonstrated an association between polymorphisms in T2R genes with asthma; more specifically, the genetic variation in T2R114 was positively associated with bronchodilator reversibility in asthmatics (Yoon et al. 2016). Of note, T2R activation in ASM leads to bronchodilation by several mechanisms as shown above (Deshpande et al. 2010; Tan and Sanderson 2014; Zhang et al. 2012, 2013), and these results suggest that there might be a crosstalk between various T2R subtypes and the β_2AR which must be thoroughly investigated in future. Future studies on T2R gene polymorphisms will provide useful information on not only individual differences in ASM sensitivity to these agents but will also elucidate the molecular mechanism by which these diverse and subtype-specific ligands can promote ASM relaxation with concomitant β_2AR therapy. Collectively, while these association studies have identified a possible link between T2R polymorphisms and airway diseases, a causal link is yet to be established and the specific contribution toward (patho)physiological processes remains to be defined.

In summary, multiple lines of evidence emanating from both the animal model and human cells/tissue studies have demonstrated that T2R agonists are highly efficacious in promoting ASM relaxation as well as being effective in mitigating allergen-induced airway inflammation and ASM-remodeling. Thus, T2Rs have emerged as viable drug targets for multi-modal protection in allergic asthma as summarized in Fig. 4.

5 Summary and Future Directions

Many of the research efforts have been devoted to refining the existing bronchodilators by improving their duration of action (e.g., long-acting beta 2 agonists) and in preventing loss of responsiveness with chronic use. In this context, the discovery of T2R subtypes on human airway cells (in particular ASM) has opened a new and exciting area of investigation focusing on an entirely novel way of eliciting bronchodilation and possibly providing the much-needed relief for asthmatics. While T2R biology and pharmacology in other airway cells are still being investigated, the bronchoprotective effect of bitter tastants is intriguing. In addition, T2R subtypes expressed on specialized airway epithelial cells have been shown to provide unique protection against airway infection which can further worsen asthma-related symptoms. The physiological roles of T2Rs go beyond taste and tastant detection; therefore, a potential non-gustatory function with immense therapeutic potential must be taken into account and must be a subject of intensive research. Areas of future studies include identification of subtype-specific signaling and functional effects in airway cells, development of subtype-specific agonists with improved pharmacodynamics properties using advanced computational approaches, and finally, development of a crystal structure for a prototype subtype of T2R that will enable future drug discovery efforts.

T2Rs have short N-terminal domains, with ligand binding in the extracellular loops and transmembrane domains. Recent data suggest that carboxy-terminal regions are particularly important for agonist selectivity (Brockhoff et al. 2010). T2Rs have been considered to function as monomers and while oligomers of T2Rs have recently been discovered, the functional role of T2R oligomers is not fully elucidated (Kuhn et al. 2010). However, it remains a major obstacle that T2Rs only exhibit very minor homology with other GPCR families and that experimental structures of T2Rs are lacking. To date, a considerable number of studies have been performed on multiple human and non-human T2Rs, providing insight into their architectures of binding sites and how a diverse set of compounds can be accommodated. Also, when compared to other GPCRs, T2Rs are rather insensitive in detecting their agonists at concentrations between the mid nanomolar and the low millimolar range, which poses a greater challenge for their use clinically. Different approaches currently being used to investigate T2Rs heavily rely on i) obtaining experimental structures, ii) performing homology modeling, and iii) functionally promoting heterologous expression in various model systems. However, these efforts will get a real boost if a crystal structure for a prototype T2R is solved.

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Mechanisms for the Sour Taste

Jin Zhang, Hojoon Lee, and Lindsey J. Macpherson

Contents

Abstract

Sour, the taste of acids, provides important sensory information to prevent the ingestion of unripe, spoiled, or fermented foods. In mammals, acids elicit disgust and pain by simultaneously activating taste and somatosensory neurons innervating the oral cavity. Early researchers detected electrical activity in taste nerves upon presenting acids to the tongue, establishing this as the bona fide sour taste. Recent studies have made significant contributions to our understanding of the mechanisms underlying acid sensing in the taste receptor cells at the periphery and the neural circuitry that convey this information to the brain. In this chapter,

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we discuss the characterization of sour taste receptor cells, the twists and turns eventually leading to the identification of Otopetrin1 (OTOP1) as the sour taste receptor, the pathway of sour taste signaling from the tongue to the brainstem, and other roles sour taste receptor cells play in the taste bud.

Keywords

Acid · Circuit · Otopetrin · Prodynorphin · Proenkephalin · Somatosensory · Sour · Taste · Taste receptor

1 Introduction

A spritz of lemon juice or vinegar elevates the flavor of a dish. These seasonings supply their own scents and tastiness, but the acidity also prompts chemical reactions in other ingredients, exposing new sensations. Vinegar has been a staple of civilization, dating back thousands of years – ancient Babylonians were making vinegar from wine. However, our love for acidity in food may well be attributed to learning and experience, since the basic sense of taste for acidity, or "sour," usually generates immediate and innate aversive responses of disgust in mammals. This disgust prevents ingestion of potentially harmful substances such as unripe fruits or spoiled food. In the kitchen, we can gauge the freshness of food items by sensing the souring caused by bacteria.

Dramatic changes in pH are detrimental to cellular physiology, and animals have evolved mechanisms to detect these in the external environment (Bessou and Perl 1969), while tightly balancing proton concentration internally. For example, Acid-Sensing Ion Channels (ASICs) are proton-gated channels well conserved in deuterostomes (Lynagh et al. 2018). ASICs convert decreased extracellular pH into excitatory sodium currents. In addition, a number of Transient Receptor Potential (TRP) family proteins, such as TRPA1 and TRPV1 channels, are activated by acids. Interestingly, TRPA1 also functions as a cognate receptor for pungent chemicals in garlic (allicin) or mustard (allyl isothiocyanate), and TRPV1 detects the spiciness of capsaicin (Julius 2013). In the oral cavity, somatosensory afferents originating from the trigeminal ganglia express these pH-sensitive nociceptors, contributing to the painful, burning sensation of acids (Kichko et al. 2018; Tominaga et al. 1998; Wang et al. 2011). However, unlike garlic and hot peppers, which are limited to evoking somatosensation, acids can also directly activate the taste system.

The sense of taste is initiated by specialized taste receptor cells (TRCs), grouped into taste buds scattered around the tongue and the soft palate in the oral cavity. The anatomical structure of taste buds in mammals was first described in the mid-nineteenth century (Loven 1867; Schwalbe 1867), however, their physiological mechanisms remained a mystery for another hundred plus years. Pioneering work by Yngve Zotterman focused on the next station of the taste neuraxis and measured electrical activity of afferent nerve fibers that receive information from the taste buds. Zotterman hooked up electrodes to the chorda tympani, a branch of the cranial nerve VII, and recorded neural responses elicited upon presentation of tastant

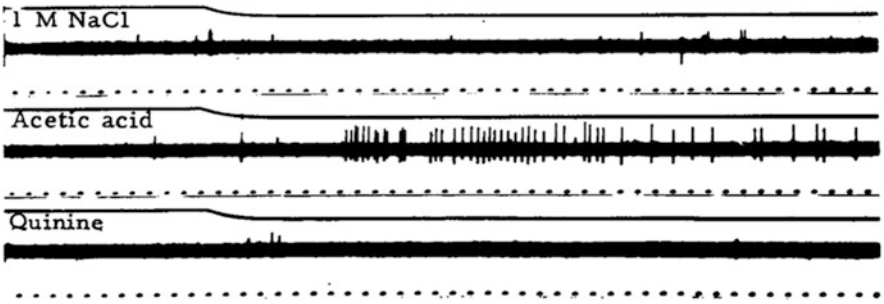

Fig. 1 Single fiber recordings from the chorda tympani nerve of a rhesus monkey. This fiber responds to acetic acid, but not to NaCl or quinine. Reproduced here with permission from Gordon et al. (1959)

solutions to the tongue. Action potentials in taste fibers were observed with various stimuli, including acetic acid – the taste of sour (Fig. 1) (Zotterman 1939).

2 Search for the Sour Receptor

With the advent of molecular biology, identification of genes encoding taste receptors for sweet, bitter, and umami soon ensued (Liman et al. 2014; Yarmolinsky et al. 2009), delineating the coding logic of taste receptor cells. The mutually exclusive expression patterns of these taste receptors within the tongue were particularly illuminating, suggesting that each TRC was specialized for detecting a single taste quality. More recently, functional imaging of taste buds with fluorescent reporters detecting intracellular calcium produced during cellular firing (Chandrashekar et al. 2010; Han and Choi 2018; Roebber et al. 2019) supports the notion that a TRC is specialized to detect one of the basic taste qualities: sweet, bitter, umami, salty, or sour.

So, how are acids detected by the taste system? Over the years, a number of candidate acid-sensitive or proton-gated ion channels have been proposed to mediate sour taste. These included epithelial sodium channels (Gilbertson et al. 1992; Lin et al. 2002), acid-sensitive ion channels (ASICs) (Lin et al. 2002; Ugawa et al. 1998, 2003), hyperpolarization-activated cyclic nucleotide-gated channels (HCNs) (Stevens et al. 2001), chloride channels (Miyamoto et al. 2000), resting two-pore K+ channels (KCNK) (Lin et al. 2004; Richter et al. 2004), and the inward rectifier potassium ion channel $K_{IR}2.1$ (Challis and Ma 2016; Ye et al. 2016). Although strategies for validating candidate taste receptors had been laid out by previous work on other taste receptors, the sour taste receptor posed additional challenges. For example, testing candidates in heterologous cell systems was tricky because many cells are already acid-sensitive and express a variety of proton-conducting channels to maintain intracellular pH. In addition, taste behavior assays are strongly influenced by avoidance behavior from nociceptors reacting to acids. Nevertheless,

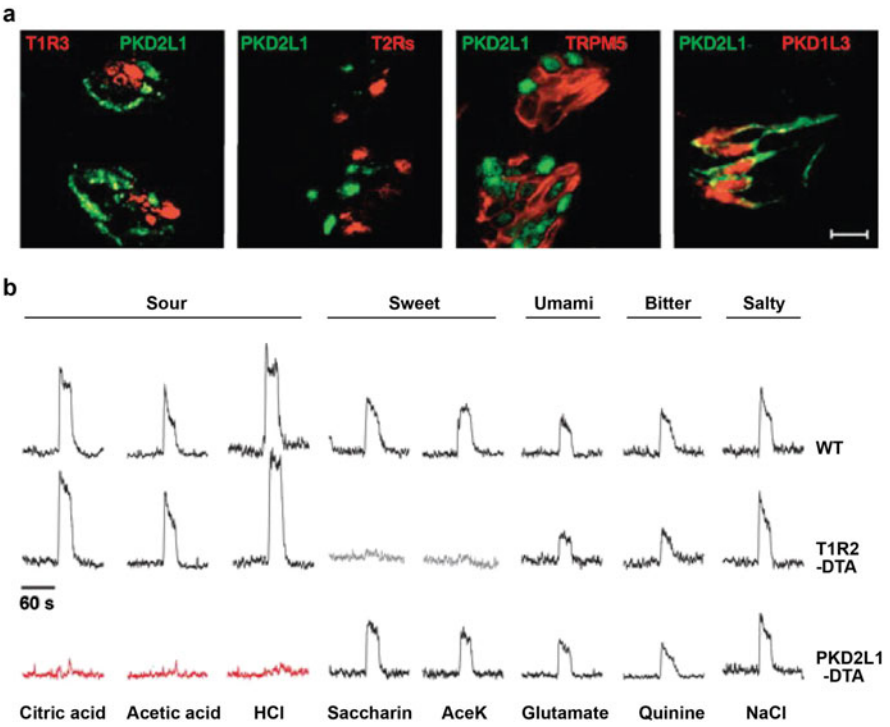

Fig. 2 Pkd2l1-expressing taste receptor cells mediate acid-sensing. (**a**) In situ hybridization shows Pkd2l1 does not co-label with RNA probes against T1R3 (sweet and umami cells), a mixture of 20 T2Rs (bitter cells) or Trpm5 (sweet, umami, and bitter cells). The last panel shows co-labelling with anti-PKD2L1 antibody and an antisense *Pkd1l3* RNA probe. Scale bar, 10 μm. (**b**) Targeted ablation of discrete TRC populations produces animals with selective deficits in taste responses. Chorda tympani nerve recordings were performed in wild-type mice (WT), showing responses to sour, sweet, umami (amino acid), bitter, and salty tastants. However, ablation of sweet cells using diphtheria toxin (T1R2-DTA) generates animals with a marked loss of sweet taste. In contrast, ablation of PKD2L1-expressing cells (PKD2L1-DTA) eliminates responses to all acid stimuli. Reproduced here with permission from Huang et al. (2006)

most of these candidate channels were broadly, rather than selectively, expressed in TRCs and/or other tissues, and none provided conclusive evidence as cognate sour receptors.

An interesting candidate for the mammalian sour receptor was Polycystic Kidney Disease 2-Like 1 (PKD2L1; a TRP channel also known as TRPP2). This and another closely related ion channel, PKD1L3, were found by bioinformatic search for transmembrane motifs among gene transcripts enriched in TRCs (Huang et al. 2006). Although its exact molecular function was unclear, PKD2L1 expression marked a subpopulation of TRCs distinct from bitter-, sweet-, and umami-TRCs (Fig. 2a). Huang et al. then genetically ablated PKD2L1-expressing (PKD2L1^{+}) cells with diphtheria toxin. In these mice, the chorda tympani nerve no longer showed response to acids (Fig. 2b). The sour taste receptor *cells* had been identified!

Subsequent studies showed that knockout of both PKD2L1 and PKD1L3 channels only slightly attenuated nerve responses to acid stimuli (Horio et al. 2011; Nelson et al. 2010), casting doubt as to whether these were the actual receptors for sour taste. Nevertheless, *Pkd2l1* is a highly expressed marker gene for sour TRCs and provides a reliable platform for isolating, characterizing, and manipulating these cells. Gene expression profiling determined that, unlike other previously characterized TRC populations, the PKD2L1$^+$ cells expressed many components of synaptic machinery, such as SNAP25 (Yang et al. 2000) and voltage-gated calcium channels (VGCCs) (Roberts et al. 2009). Notably, early electron micrograph studies had characterized taste buds as consisting of three main types of cells (Murray and Murray 1971): Glia-like "support" cells (type I), elongated "receptor" cells (type II) and cells with synaptic machinery (type III). Researchers had reasoned that type II cells would detect tastants and subsequently pass that information onto type III cells, which had synaptic connections established with the afferent fibers. Although synaptic markers are present in PKD2L1$^+$ cells, this hypothesis was largely disproven when ablation of PKD2L1$^+$ TRCs had no effect on other taste qualities (Fig. 2b). Instead, this result signified that each TRC can independently convey taste information to their respective partner neurons. In spite of the "type" designation of taste bud cells having gained broad acceptance, most studies have not reconciled ultrastructure with gene expression and future work will need to focus on characterizing nuanced subpopulations of TRCs.

Although PKD2L1 and PKD1L3 do not appear to be the primary pH sensors in sour TRCs, recent work on their function is worth noting. These atypical calcium channels are enriched in the primary cilia of cells and maintain a high local calcium concentration near the ciliary membrane (DeCaen et al. 2013). Indeed, mice lacking *Pkd2l1* have a high prevalence of intestinal malrotation, suggesting abnormal ciliary sonic hedgehog activity during early development (Delling et al. 2013). PKD2L1 is enriched in the taste tissues and its role in transducing sour taste – or other – information remains to be elucidated.

3 Identification of the Sour Taste Receptor

After many years of futile searches, a novel family of proton-selective ion channels, Otopetrins, recently emerged as strong candidates for the elusive sour receptor. A key insight was the identification of a Zn^{2+}-sensitive proton conductance in PKD2L1$^+$ TRCs that responds to extracellular acidification (Bushman et al. 2015; Chang et al. 2010), setting the stage for finding proton channels in sour TRCs with such properties. Liman and colleagues screened 41 candidate proton channels expressed in PKD2L1$^+$ sour TRCs using electrophysiological assays in gene-transfected human embryonic kidney 293 (HEK293) cells or *Xenopus* oocytes (Tu et al. 2018). In this breakthrough study, they demonstrated that Otopetrin 1 (*Otop1*) expression generated Zn^{2+}-sensitive currents when the extracellular pH was lowered. Notably, OTOP1 is proton-specific, but not appreciably permeable to

Na^+, Cs^+, Li^+, or K^+. Other family members including murine OTOP2 and OTOP3, and a Drosophila ortholog dmOTOPLc were also found to encode proton channels.

The otopetrin family is conserved from nematodes to humans, and it was originally identified in the vestibular system (Hughes et al. 2004, 2007, 2008; Hurle et al. 2003). OTOP1 is required for the formation of otoconia and otoliths, calcium carbonate biominerals within the inner ear that are used for the detection of linear acceleration and gravity. Mouse strains bearing single-point mutations in *Otop1*, *tilted* (*tlt*) and *mergulhador* (*mlh*), exhibit impaired otoconia morphogenesis and vestibular defects (Hurle et al. 2003; Lane 1986; Ornitz et al. 1998).

Structural analysis of otopetrins provided important insights into their proton-conducting function (Chen et al. 2019; Saotome et al. 2019). Cryo-EM structures of zebrafish OTOP1 and chicken OTOP3 (Saotome et al. 2019) and Xenopus OTOP3 (Chen et al. 2019) revealed a dimeric architecture, in which each subunit forms 12 transmembrane helices divided into two structurally similar domains – the amino and the carboxy domains. In molecular dynamics simulations, hydrophilic vestibules formed by the two domains and in the interface between them create conduits for water entry into the membrane core, suggesting three potential proton conduction pathways (Saotome et al. 2019). In future studies, it will be instrumental to understand the gating and proton selectivity mechanisms of otopetrins.

Members of the otopetrin proton-selective family exhibit distinct expression patterns (Tu et al. 2018). In addition to taste receptor cells, OTOP1 is expressed in vestibular cells, brown adipose tissue, heart, uterus, dorsal root ganglion, adrenal gland, mammary gland, and stimulated mast cells, while OTOP2 is highly expressed in stomach, testis, and olfactory bulb. Therefore, it will be interesting to examine the role of otopetrins in these physiological settings.

OTOP1 is highly expressed in taste cells, but is it responsible for sensing the sour taste? *Otop1* was found in an independent search for gene candidates encoding sour receptors using bulk and single-cell RNAseq of $Pdk2l1^+$ TRCs (Zhang et al. 2019). Two recent studies engineered *Otop1* homozygous knockout ($Otop1^{-/-}$) mice and demonstrated that *Otop1* is essential for sour sensing (Teng et al. 2019; Zhang et al. 2019). Like *tlt* mice, these $Otop1^{-/-}$ mice exhibited tilted head positioning and balancing deficits. $Otop1^{-/-}$ mice had a dramatic loss of gustatory nerve response to a wide range of sour stimuli, including strong and weak acids, while responses to other taste qualities were unaffected (Teng et al. 2019; Zhang et al. 2019). In addition, whole-cell patch clamp recording and intracellular pH imaging in isolated sour TRCs demonstrated that OTOP1 is required for cellular and neural responses to sour stimuli (Teng et al. 2019); and functional calcium imaging of the geniculate ganglion (one of the first neuronal stations in transmitting taste information from the tongue to the brain, see Sect. 4 for details) confirmed that $Otop1^{-/-}$ mice lost acid-evoked responses in sour neurons that receive information from sour TRCs (Zhang et al. 2019).

It is worth noting that residual responses to sour stimuli were observed in $Otop1^{-/-}$ mice in gustatory nerve recordings (Teng et al. 2019; Zhang et al. 2019). Are there additional sour-sensing mechanisms in the gustatory system? It was previously hypothesized that, in addition to proton conductance, weak acids

could decrease intracellular pH and induce sour responses by diffusing across the plasma membranes as undissociated molecules (Challis and Ma 2016; Lyall et al. 2001; Ogiso et al. 2000; Richter et al. 2003; Roper 2007; Ye et al. 2016). The acid-sensitive K+ channel $K_{IR}2.1$ was shown to be blocked by intracellular acidification, thus amplifying the responses to proton influx in sour cells (Ye et al. 2016). Another hypothesis is that weak acids may be more effective sour stimuli because they serve as a source of H^+, protecting protons from being absorbed by salivary proteins and buffers (Teng et al. 2019; Ye et al. 2016). As sour cells in *Otop1*$^{-/-}$ mice exhibited similarly abolished responses to a wide range of acids including weak organic acids (e.g., citric acid and tartaric acid) and strong acids (e.g., HCl), it is less likely that intracellular acidification plays a critical role in sour sensation in mice as previously hypothesized (Teng et al. 2019; Zhang et al. 2019). To identify the source of the acid sensitivity retained in *Otop1*$^{-/-}$ mice, Zuker and colleagues reasoned that bitter TRCs could be responsible, as a subset of T2R-expressing bitter TRCs were shown to respond to acid stimuli (Barretto et al. 2015; Oka et al. 2013). Indeed, when AITC was applied on the tongue of *Otop1*$^{-/-}$ animals to acutely block bitter TRC activity (Oka et al. 2013), residual responses to acid stimuli in these animals were abolished (Zhang et al. 2019). This finding also suggests that OTOP1 is not responsible for sour sensing in bitter cells, but instead some T2R GPCRs or other channels are sensitive to low pH in bitter TRCs.

These studies of *Otop1*$^{-/-}$ mice demonstrated that OTOP1 is essential for acid sensing in sour TRCs, but is it the sour receptor, or merely part of the machinery transducing acid signals? If OTOP1 alone functions as the sour receptor, *Otop1* expressed ectopically in another TRC cell type should endow the recipient cell with sensitivity to acid stimuli. To investigate this, the *T1R3-Otop1* knock-in animals were engineered where the *Otop1* gene was inserted into the sweet receptor locus to target Otop1 expression in sweet TRCs. Indeed, sweet neurons of *T1R3-Otop1* knock-in mice responded not only to sweet tastants, but over 95% of them were also activated by sour stimuli (compared to less than 5% in wild-type siblings; Zhang et al. 2019). These loss-of-function and gain-of-function experiments together provide compelling evidence that OTOP1 is the sour taste receptor.

4 The Sour-Sensing Pathway

How is sour taste information transmitted from the tongue to the brain? In general, gustatory information recognized by TRCs is relayed by peripheral sensory neurons of the geniculate/petrosal ganglia that innervate the taste buds and project to the rostral nucleus of the solitary tract (rNST) (Fig. 3). In rodents, rNST taste neurons project to the parabrachial nucleus (PBN) before the taste signals reach the ventroposterior medial nucleus of the thalamus (VPMpc). In primates, rNST neurons directly project to VPMpc. From the VPMpc, the gustatory fibers then project to the primary gustatory cortex (GC) in the insula (Carleton et al. 2010; Smith and St John 1999; Yarmolinsky et al. 2016).

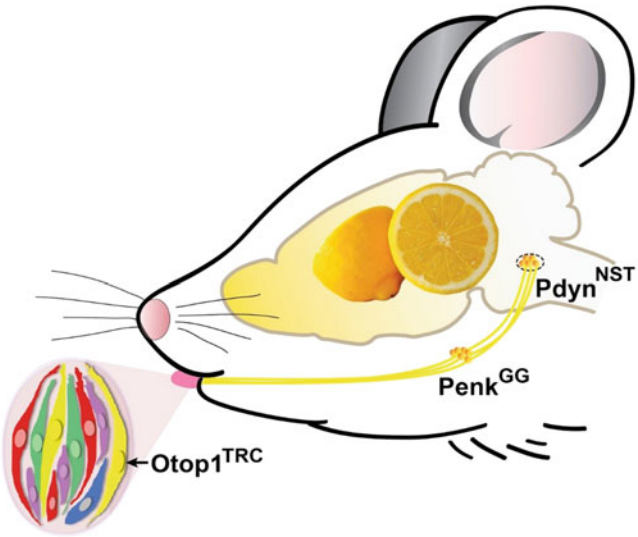

Fig. 3 The sour-sensing taste pathway in mice. A representative taste bud is depicted, with each color representing a population of taste receptor cells (TRCs) mediating a taste quality. Cells responding to the sour taste are colored yellow

At the first neural relay of the taste circuit, gustatory information is transmitted through the taste ganglia to the brainstem. Studies were carried out to characterize the tuning properties of taste ganglion neurons. Using functional calcium imaging of the geniculate ganglion, Barretto et al. found that ganglion neurons primarily respond to a single taste quality (Barretto et al. 2015). Similar results were shown in an independent study, although the authors also found ganglion neuron responses to multiple taste stimuli when tastant concentration increased (Wu et al. 2015). These studies suggest that TRCs tuned to individual taste qualities are innervated by a matching set of dedicated ganglion neurons. In support of this, Lee, Macpherson, and colleagues discovered that bitter and sweet TRCs provide specific instructive signals to bitter and sweet ganglion neurons via different guidance molecules (Lee et al. 2017). Moreover, studies from the Finger lab suggest that sour taste may be transmitted through dedicated nerve fibers (Larson et al. 2015; Stratford et al. 2017a). They found that gustatory nerve fibers expressing the serotonin receptor HTR3A terminate in close proximity to the Type III TRCs. They hypothesized that these contacts are potentially serotonergic synapses where serotonin released from Type III TRCs during sour taste stimulation would directly activate HTR3A receptors on the gustatory fibers innervating those cells.

If taste information travels through dedicated neuronal populations, it should be possible to identify and label groups responding to each taste quality. To achieve this, Zhang et al. first segregated geniculate ganglion (GG) neurons into distinct clusters based on their transcriptional profiles obtained by single-cell RNA sequencing. The tuning properties of each of the 5 *Phox2b*-positive (i.e., taste) clusters were

then determined using a combination of genetic knockouts, neuronal ablation, or functional imaging experiments. When genetically encoded calcium indicators (GCaMP) were targeted into a GG cluster marked by Proenkephalin ($Penk^{GG}$) and live-animal Ca^{2+} imaging were performed, $Penk^{GG}$ specifically responded to sour stimuli (Zhang et al. 2019). Another single-cell RNA sequencing study corroborated Penk as a marker for a transcriptionally distinct cluster of GG neurons, however, the role of this cluster was not pursued (Dvoryanchikov et al. 2017). In the next station, the rNST, the sour-sensing population is labeled by Prodynorphin ($Pdyn^{rNST}$), which receive direct inputs from the Penk-expressing sour ganglion neurons (Zhang et al. 2019). Optogenetic activation of $Pdyn^{rNST}$ elicits immediate taste aversion just as a sour chemical on the tongue would evoke, and activation of these neurons was recognized and reported as a sour stimulus in a learned taste behavioral assay.

Together, these results delineate how sour taste information travels from the tongue to the brain through dedicated sour cells: $Otop1^{TRC} \rightarrow Penk^{GG} \rightarrow Pdyn^{rNST}$ (as illustrated in Fig. 3) (Krashes and Chesler 2019; Zhang et al. 2019). How does sour taste information continue to be transmitted and processed in the brain? Additional sour-responding neurons have yet to be discovered (e.g., along the taste pathway of PBN → VPMpc → GC). Recently, a Satb2-expression population of PBN neurons were found to selectively transmit sweet taste signals to the gustatory thalamus (Fu et al. 2019). In the gustatory cortex, Chen et al. identified four separate areas ("hotspots") each representing sweet, bitter, salty, and umami using in vivo two-photon calcium imaging (Chen et al. 2011). Conspicuous in its absence is sour: is there a yet-to-be-detected sour hotspot in the insula? A recent calcium imaging study of awake-behaving animals detected neurons selectively activated by licking acids to be distributed broadly in the gustatory cortex (Chen et al. 2021). Optogenetic activation of the sweet or bitter areas of the gustatory cortex elicits appetitive or aversive responses (Peng et al. 2015); and their projections terminate in separate sub-regions of the amygdala to encode taste valence (Wang et al. 2018). If sour is represented by a distinct group of neurons in the gustatory cortex, do they project to sub-regions of amygdala, and do those projections code for aversion?

In addition to the taste pathway, acid is detected by free nerve endings of trigeminal neurons and travels through the somatosensory system. Animals lacking sour TRCs (by genetic ablation of $Pkd2l1^{+}$ taste receptor cells with diphtheria toxin) still exhibit strong aversion to acids (Huang et al. 2006). As anticipated, sour taste receptor knockout ($Otop1^{-/-}$) animals showed similar aversion to acid solutions compared with wild-type siblings. However, the sour-evoked aversion is largely diminished when trigeminal $TRPV1^{+}$ neurons were abolished in $Otop1^{-/-}$ animals (Zhang et al. 2019), supporting that the taste and somatosensory are the main pathways responsible for aversive responses to acid stimuli. Similar to the gustatory nerves, oral afferents of the trigeminal nerve project centrally to the brainstem. To locate the brainstem sour-responding neurons activated through non-taste pathways, Stratford et al. utilized the "taste blind" *P2rx2/P2rx3* double knockout mice and found acid-induced cFos-activity in the dorsomedial trigeminal brainstem nucleus situated laterally adjacent to the rostral NST (Stratford et al. 2017b). This dorsomedial nucleus is innervated by trigeminal nerve fibers labeled with calcitonin

gene-related peptide (CGRP), which is particularly interesting as many of these neurons also express the acid-responsive TRPV1 channel. Nonetheless, many questions remain about the transmission of sour information in the somatosensory system, and how these two sour-sensing pathways may interact.

5 Other Roles for "Sour" TRCs

In addition to detecting acids, "sour" TRCs are involved in sensing the carbon dioxide in fizzy drinks, play a role in aversive high salt taste, and are potentially responsible for water taste signaling. For all three of these roles, the enzyme carbonic anhydrase is required. Carbonic anhydrases are a family of enzymes that interact with carbon dioxide in many different systems. CAs catalyze the interconversion of CO_2 and water into bicarbonate ions and free protons ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$) (Baird et al. 1997). Carbonic anhydrase 4 (CAR4) is found in sour TRCs, with its expression closely overlapping with PKD2L1 and other type III cell markers (Chandrashekar et al. 2009; Lossow et al. 2017). CAR4 is localized to the exterior of the cell, attached to the plasma membrane by a glycophosphatidylinositol (GPI) anchor. This location is important for its function – not as a traditional receptor, but as a mediator producing free protons that could then be gated by nearby otopetrin channels. Although this has yet to be formally tested, *Otop1*$^{-/-}$ mice should show significant loss of CO_2 responses, comparable to *Car4*$^{-/-}$.

Sour taste receptor cells, together with bitter TRCs, have been shown to be involved in mediating amiloride-insensitive high salt taste (Lewandowski et al. 2016; Oka et al. 2013). In mice, there are two components of salt taste. First is an attractive component, mediated primarily through the epithelial sodium channels (ENaC), which can be blocked by the drug amiloride. Another component, "high salt taste" is characterized by amiloride insensitivity (i.e., not mediated by ENaC) and is activated by high concentrations of NaCl (>300 mM), KCl, $CaCl_2$, N-methyl-d-glucamine (NMDG) Cl, and other salts. Using chorda tympani (CT) nerve recording and transgenic mouse models, Oka et al. demonstrated that high salt responses are mediated by the activation of bitter and sour TRCs (Oka et al. 2013). Mice blind to both bitter- (via *Trpm5*$^{-/-}$) and sour-tastes (via *Pkd2l1-TeNT*) no longer displayed aversion to high salt. Lewandowski et al. followed up on these studies, using calcium imaging of isolated circumvallate taste receptor cells to better understand the cellular populations and probe the mechanisms leading to TRC activation by high salt stimuli (Lewandowski et al. 2016). They found a subpopulation of sour-responding TRCs did indeed respond to high salt stimuli, but that the mechanism was still unclear.

Recently, Roebber et al. used calcium imaging of semi-intact fungiform taste buds, and found activation of *Plcβ2*$^+$ (type II) cells, but surprisingly, not Car4$^+$ (type III) cells by NaCl (Roebber et al. 2019). The authors suggest that the engagement of sour taste cells in high salt detection may be relegated only to sour TRCs within circumvallate and not fungiform papillae taste buds, but there are some problems with this interpretation. Electrophysiological recordings from the chorda tympani

nerve (which innervates the fungiform papillae) from *Trpm5*$^{-/-}$ mice still show considerable amiloride-insensitive salt responses (Oka et al. 2013). Only when both type II and type III cells were silenced (*Pkd2l1-TeNT*; *Trpm5*$^{-/-}$), did CT nerve responses or behavioral aversion cease to high salt stimuli. Additional evidence for the involvement of type III cells in amiloride-insensitive salt taste is provided by an investigation of the *Skn1a*$^{-/-}$ mouse which lacks type II cells (Larson et al. 2020). An electrophysiological examination of these mice found that there is a significant amiloride-insensitive NaCl CT nerve response remaining in *Skn1a*$^{-/-}$, consistent with the findings of Oka et al. (2013) and Lewandowski et al. (2016).

The mechanisms leading to activation of the sour taste pathway by high concentrations of sodium and potassium salts are still unclear, but anion effects, osmolarity, and ionic strength affecting carbonic anhydrase activity are all potential contributors. Since the high-salt response is mediated by both type II and type III cells, it is more difficult to tease apart these contributions. However, several lines of evidence indicate a role for carbonic anhydrase in the activation of sour taste cells by high salt stimuli. The hypothesis is that the carbonic anhydrase enzyme is inhibited by the ionic strength of the concentrated salt solution, causing a local shift in pH towards more acidic, therefore activating the proton channel OTOP1. Three experiments support this hypothesis: (1) *Car4*$^{-/-}$ mice have attenuated CT nerve responses to KCl after blockade of bitter TRCs with AITC, (2) CAR4 inhibitors considerably reduce sour-cell mediated responses to KCl, CaCl2 and NMDG Cl, and (3) manipulating pH affects the CT nerve responses to high salt stimuli, all pointing to a role for CAR4 in this process (Oka et al. 2013). If this mechanism is correct, *Otop1*$^{-/-}$ should also display reduced responses to high salt stimuli like KCl. These experiments would need to be performed using CT nerve recording or intact taste bud imaging to prevent the activation of voltage-gated calcium channels directly.

The proposed involvement of sour taste receptor cells in detecting water taste is intriguing, but still controversial. Zocchi et al. used CT nerve recording to demonstrate that nerve responses to the rinse-out of artificial saliva (4 mM NaCl, 10 mM KCl, 6 mM $KHCO_3$, 6 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.24 mM K_2HPO_4, 0.24 mM KH_2PO_4. pH 7.4–7.6) with deionized water is dependent on PKD2L1-expressing taste receptor cell function and on CAR4 (Zocchi et al. 2017). The authors hypothesize that the washout of bicarbonate from the saliva causes a CAR4 mediated increase in proton concentration. This decreased pH would presumably be detected by OTOP1 channels (yet to be tested). While this mechanism makes sense in principle, the consequences of water activating sour cells are counterintuitive given that sour taste is thought to elicit aversive, not attractive behaviors. Zocchi et al. go on to show that stimulation of PKD2L1^{+} TRCs via the expression of blue-light activated Channelrhodopsin (ChR2) elicits strong attractive behaviors in mice. A recent follow-up study using a different *Pkd2l1-Cre* knock-in mouse model with more restricted Cre-dependent ChR2 expression showed that blue light on the tongue elicited no attraction, and instead mild aversion (Wilson et al. 2019). Given several reports highlighting the importance of other peripheral circuits for the regulation of thirst and satiation (Augustine et al. 2019; Zimmerman et al. 2019),

it is unclear if and how this proposed sour-mediated water taste pathway contributes to these processes.

So far, we have presented "sour" or "type III" cells as an identical, single cell type. This may not necessarily be the case. Heterogeneity has been observed in these cells: there are differences in mRNA and protein expression, as well as varied responses to stimuli in calcium imaging assays (Dutta Banik et al. 2020; Lewandowski et al. 2016; Lossow et al. 2017; Roebber et al. 2019; Sukumaran et al. 2017). One subtype of type III cells, recently described by Dutta Banik et al. are particularly interesting. In this study, calcium imaging of isolated taste receptor cells is used to define a population of "Broadly-Responsive" type III taste cells which respond to multiple taste stimuli besides sour, including bitter, sweet, and umami, but not sodium salt. These cells were initially identified as type III because of their VGCC-mediated responses to KCl and defined by the expression of *Plcβ3*. The authors estimate that this population makes up approximately 25% of type III cells, but further characterization of this population is warranted. It will be interesting in the future to test the activation of this particular subset (via opto- or chemo-genetic mechanisms) to understand their role in taste signaling in intact mice.

Research on the cellular and molecular basis of sour and salt taste specifically in humans is more limited and somewhat contradictory. There is still a debate about whether ENaC channels play a role in human salt taste or not (reviewed by Bigiani (2020)). Without clarification of the cell type(s) involved in low-salt detection in humans, it is difficult to speculate how type III cells alone could produce both appetitive and aversive reactions. If there is a yet-to-be-identified (or potentially still ENaC-expressing) low-sodium cell analogous to mouse TRCs, then this problem resolves itself. If not, and Type III TRCs are responsible for all salt taste and sour taste transduction in humans, it becomes more paradoxical. Very high salt in humans is aversive, although potentially not as aversive as in mice (Cowart and Beauchamp 1986; Leshem 2009; Moder and Hurley 1990). As with mice, there may be two components to this high salt aversion – through bitter cells and through sour. Interestingly, in humans high salt-induced hypertension reduces TRPM5 expression, inhibiting the aversive component of high salt taste signaling, perhaps leading to continued consumption of high salt (Cui et al. 2019). Are type III sour cells included in high-salt signaling in humans? We're not aware of any data yet to confirm or reject this to date.

6 Perspectives and Open Questions

What is sour taste? This remains a complicated question. While we all are familiar with the shock of biting into a lemon wedge or the sharpness of a vinaigrette, this experience is confounded by the integration of many sensory inputs – sour taste, acid somatosensation, and olfactory and other stimuli. Given that there are multiple routes for detecting acids, why do we have a dedicated population of TRCs for detecting sour? It has been hypothesized that sour TRCs may be much more sensitive to organic acids like citric acid that are present in foods as compared to

inorganic acids like HCl (Ramos Da Conceicao Neta et al. 2007). Possibly the position of sour TRCs, with their apical membranes exposed to the oral cavity through the taste pore, gives them a sensitivity advantage over acid-sensitive somatosensory neurons.

While acid detection is the most obvious function of sour TRCs, this undervalues their other roles in the taste bud. In addition to sensing carbonation, high salt, and potentially water taste, these cells also actively signal to other TRCs in the taste bud. Sour cells are the only TRC type to contain synaptic vesicles and synaptic release machinery (Yang et al. 2000). Activation of the sour TRCs by acid or optogenetic stimulation inhibits bitter and sweet taste responses (Formaker et al. 2009; Vandenbeuch et al. 2020), potentially via GABA and serotonin release from synaptic vesicles (Huang et al. 2008, 2009, 2011; Tomchik et al. 2007). This mechanism may help to enhance sour signals among taste mixtures and provide for better sour taste detection and discrimination. Another puzzling aspect about sour TRC signaling is that although the cells are equipped with synaptic vesicles, only GABA and serotonin have been observed to be released, not ATP (Huang et al. 2008, 2011; Larson et al. 2020). Yet ATP signaling is required for activation of gustatory nerve fibers by sour stimuli (Finger et al. 2005; Vandenbeuch et al. 2020). Sour cells do not express the CALHM ATP channels found in sweet, umami, bitter, and salt cells (Nomura et al. 2020; Taruno et al. 2013, 2020). So how do sour TRCs relay signals to gustatory ganglion neurons? This is still an active line of investigation, with several laboratories engaged in pursuing an answer.

There remain many outstanding questions as we have laid out throughout this chapter. However, identification of OTOP1 as a sour receptor, and mapping the sour taste circuit from the periphery to the brainstem opens exciting new avenues to explore taste signaling and multisensory integration. For example, at what point, if ever, do sour taste and acid somatosensory signals converge? How is sour aversion suppressed by other appetitive stimuli, or through learning and experience? Perhaps this is common knowledge for others, but as taste researchers, we were surprised to discover that it is a standard husbandry practice (e.g., the Jackson Laboratory) for rodents to be reared in water that is acidified with HCl to a pH of 2.5–3.0 to control the growth of Pseudomonas. These laboratory animals spend their entire lives drinking noticeably sour water. Do their responses to sour stimuli differ from mice who are reared with water at a neutral pH? While we are not aware of studies that have addressed this for taste sensitivity or signaling, acidified water does affect the microbiome (Barnett and Gibson 2019), and should not be overlooked as a potential confound. For us, even the idea of ordering a bottle of Perrier with lemon at a cafe is so refreshing and invigorating, but if a mouse placed their drink order, the waiter would likely return with a glass of tap water, flat and neutral.

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Cellular and Molecular Mechanisms of Fat Taste Perception

Aziz Hichami, Amira Sayed Khan, and Naim Akhtar Khan

Contents

Abstract

During the last couples of years, a number of studies have increasingly accumulated on the gustatory perception of dietary fatty acids in rodent models and human beings in health and disease. There is still a debate to coin a specific term for the gustatory perception of dietary fatty acids either as the sixth basic taste quality or as an alimentary taste. Indeed, the psycho-physical cues of orosensory detection of dietary lipids are not as distinctly perceived as other

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taste qualities like sweet or bitter. The cellular and molecular pharmacological mechanisms, triggered by the binding of dietary long-chain fatty acids (LCFAs) to tongue taste bud lipid receptors like CD36 and GPR120, involve Ca^{2+} signaling as other five basic taste qualities. We have not only elucidated the role of Ca^{2+} signaling but also identified different components of the second messenger cascade like STIM1 and MAP kinases, implicated in fat taste perception. We have also demonstrated the implication of Calhm1 voltage-gated channels and store-operated Ca^{2+} (SOC) channels like Orai1, Orai1/3, and TRPC3 in gustatory perception of dietary fatty acids. We have not only employed siRNA technology in vitro and ex vivo on tissues but also used animal models of genetic invalidation of *STIM1, ERK1, Orai1, Calhm1* genes to explore their implications in fat taste signal transduction. Moreover, our laboratory has also demonstrated the importance of LCFAs detection dysfunction in obesity in animal models and human beings.

Keywords

CD36 · Fatty acids · Fat taste · Lipids · GPR120 · Microbiota · Obesity

Abbreviations

CaM	Calmodulin
CIF	Ca^{2+} influx factor
DAG	Diacylglycerol
IP_3	Inositol-tris-phosphate
LCFAs	Long-chain fatty acids
PIP_2	Phosphatidyl-inositol-bisphosphate
PLC	Phospholipase C
PTK	Protein tyrosine kinase
STIM1	Stromal interaction protein-1
TBC	Taste bud cell
VEG	von Ebner's glands

1 Introduction

It is now generally accepted that, in our daily food, Western diet brings more than 40% of fat whose intake, in the long run, may contribute to the incidence of obesity which is generally associated with a number of pathologies, for instance, type 2 diabetes mellitus, hypertension, and cancer. Interestingly, obese subjects have been shown to exhibit a higher spontaneous preference for fat than lean subjects (Drewnowski et al. 1985; Mela and Sacchetti 1991), suggesting the existence of a selective detection of fat-rich foods that might operate in the buccal cavity.

Taste is one of the chemosensory components that enable orosensory perception of food. There are well-known five basic taste qualities, i.e., sweet, sour, bitter, salt,

and umami (Herness and Gilbertson 1999). The taste buds, localized in gustatory papillae, are distributed throughout the tongue epithelium. There are principally 3 forms of gustatory papillae, i.e., circumvallate, fungiform, and foliate, and each of which comprises of almost an equal number of taste buds (Suzuki 2007). The circumvallate papillae are present on the posterior region of tongue, while fungiform and foliate papillae are located on dorsal surface of the anterior 2/3 and sides of the posterior 1/3 of the tongue, respectively (Fig. 1). Each *taste bud* contains 4 types of taste bud cells, TBC (Takeda and Hoshino 1975): type I cells (also called glial-like cells) respond to salt taste, type II cells express taste receptors for sweet, bitter, and umami, type III cells (also called pre-synaptic cells) communicate via a synapse with the gustatory nerves and are thought to be involved in the perception of sour or acid molecules, and finally the type IV cells that act as progenitors for three types of TBC (Miura et al. 2006). In this chapter, we will not go into details on the classification of TBC and different taste modalities as these aspects will be dealt in other chapters.

2 Orosensory Detection of Dietary Fat

"Fat" is the term used to refer to triglycerides and esterified fatty acids which represent a component of the traditional lipid-rich food. Generally, high-fat foods are highly appreciated because they confer textural properties to food, making it more palatable. Moreover, the hedonic property of fat is a driving force, promoting its preferential consumption. Humans and rodents exhibit a spontaneous attraction and preference for fat-rich foods (Mattes 2009) and long-chain fatty acids (LCFAs), but not for triglycerides or esterified fatty acids (Hiraoka et al. 2003). It has been proposed that during food mastication, the free LFCAs will be released in the buccal cavity by the action of lingual lipases, secreted by von Ebner's glands (VEG) which are located in the vicinity of lingual papillae (Kawai and Fushiki 2003).

As described for basic taste modalities, fat taste perception also involves the following steps: (1) the expression of specific taste receptors in TBC, (2) the coupling of signaling pathways downstream to these receptors, leading to increases in free intracellular calcium concentrations, $[Ca^{2+}]i$, and the release of neurotransmitters, (3) transfer of the gustatory message to afferent nerves, leading to the brain, and (4) physiological events, like pancreatobiliary secretions. All these conditions have been confirmed (Besnard et al. 2016; Gilbertson and Khan 2014; Khan et al. 2020) and will be explained in the following sections.

2.1 Experimental Evidences

Two-bottle preference test represents a classical measure of behavioral responsiveness to taste stimuli. In this test, animals are allowed to make a choice between a control solution (containing a vehicle) and a test solution (containing a tastant), and the intake is recorded over a specified period of time. In the study of Tsuruta et al. (1999), rats were allowed to make a choice between a bottle containing

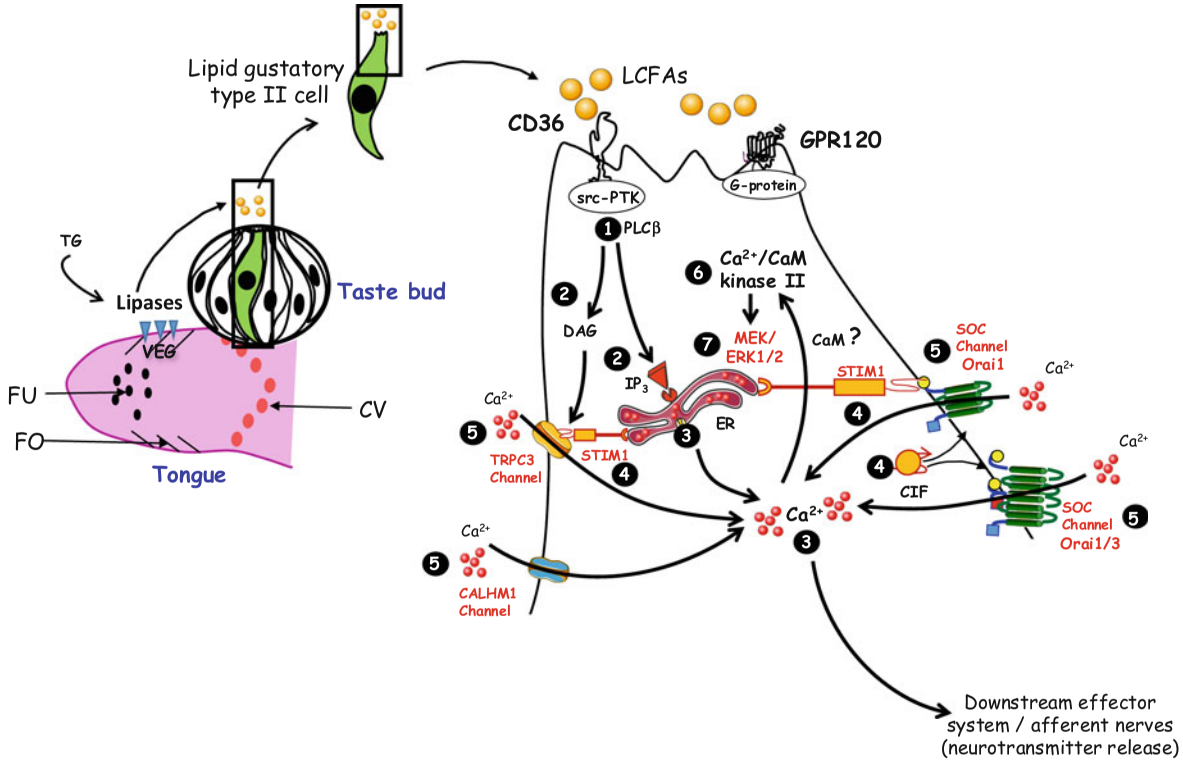

Fig. 1 Lipid receptors-coupled cell signaling cascade in a type II gustatory cell. The long-chain fatty acids (LCFAs), released by the VEG lipases, bind to CD36 and GPR120 receptors. LCFAs-binding then induce the phosphorylation of *src*-PTK which in turn activates/phosphorylates PLCβ. This phospholipase hydrolyzes PIP_2 to give rise to second messengers, IP_3 and DAG. IP_3 binds to its receptor located on the endoplasmic reticulum (ER), inducing the release of Ca^{2+} from the internal stores. As per capacitative model, the release of Ca^{2+} from the ER triggers Ca^{2+} influx via the opening of SOC channels like Orai1 and

Orai1/3, orchestrated by STIM1. During the translocation of STIM1 to the plasma membrane, CIF is also released from the ER and translocated towards the SOC channels. Calhm1 channels also leak Ca^{2+} that is probably involved in CaM activation to phosphorylate the cascade of MAP kinases via Ca^{2+}/CaM kinase II. Simultaneously, DAG opens TRPC3 channels that favor Ca^{2+} influx, also under the control of STIM1. For the sake of simplification, the figure only shows the CD36-activated mechanisms; however, GPCRs are also known to induce an increase in Ca^{2+}, according to the capacitative model. The numbers in the circles show the sequences of the events, triggered by CD36. We do not show the DRK channels which are supposed to act downstream to Ca^{2+}signaling (Gilbertson and Khan 2014). Some of the components of cell signaling are marked with red ink to demonstrate that their silencing or genetic ablation in animal models resulted in decreased gustatory perception of dietary fatty acids. The question mark (?) shows unresolved pathway. The figure also shows the presence of 3 types of papillae on the tongue epithelium. The human tongue contains 6 to 9 CV papillae as mentioned here; however, mice contain only one CV papilla. The VEGs are shown adjacent to foliate papillae for the simplification, but they are also found in close proximity to CV papillae. *CaM* calmodulin, *CIF* Ca^{2+}-influx factor, *CV* circumvallate, *FO* foliate, *FU* fungiforme, *PTK* protein-tyrosine kinase, *VEG* von Ebner's gland, *DAG* diacylglycerol, IP_3 inositol-tris-phosphate, *PLC* phospholipase C, *ER* endoplasmic reticulum

either a solution of 0.1% LCFA dissolved with 0.3% xanthan gum in tap water or a control solution, containing only 0.3% xanthan gum. These investigators observed that rats preferred LCFAs in the following order: linolenic acid > linoleic acid > oleic acid. However, fatty acid derivatives (methyl oleate, oleyl alcohol, methyl linoleate, and linolyl alcohol) in which carboxyl group was modified were not preferred by rats. Moreover, the rats preferred LCFAs than triglycerides (TG) or medium-chain fatty acids (MCFA) with a number of carbons from 8 to 14 (Tsuruta et al. 1999). Hence, the chain length as well as the carboxyl group was crucial in the detection and preference for LCFAs (Tsuruta et al. 1999). It has been suggested that mice and rats could always detect and prefer LCFAs even when textural, olfactory, and post-ingestive cues are simultaneously minimized (Takeda et al. 2001; Fukuwatari et al. 2003). Laugerette et al. (2005) demonstrated that, when the post-ingestive cue was neutralized by an esophageal ligation in mice, oral detection of fat persisted. Similar findings were also observed during licking test, which employs a computer aided contact-lickometer and determines the number of licks during brief access (10 s–60 s) to a tastant. It was observed that the number of licks for a mineral oil solution containing 0.2–4% linoleic acid was significantly greater than the number of licks for a similarly textured solution (Yoneda et al. 2007).

2.2 Clinical Evidences

The three-alternative forced choice (3-AFC) ascending test is one of the classical and commonly employed procedures to investigate the taste sensitivity in human subjects. Hence, volunteers are offered with solutions of tastants at the lowest concentrations, with a progressive increase after each tasting, until the test sample is correctly identified for three consecutive times. This concentration is designated as the detection threshold of the test substance (Mattes 2008). Free fatty acids seem to be orally perceived as tastants or irritants, depending on their concentration. At higher concentrations, they might cause a "scratchy" sensation, whereas "fatty" attribute is more obvious at lower concentrations (Galindo et al. 2012). Chalé-Rush et al. (2007) observed that healthy subjects were able to correctly identify saturated and unsaturated fatty acids in test solutions when the olfactory (using nose clips), visual (experiment conducted in red light), and textural (adding textural agents as mineral oil and acacia gum) cues were masked. In humans, the mean detection threshold for oleic acid was 2.2 mM, ranging from 0.26 to 12 mM (Stewart et al. 2010). These investigators classified individuals as hypersensitive (detection threshold of oleic acid <3.8 mM) or hyposensitive (detection threshold of oleic acid >3.8 mM).

We have pointed out here-before that VEG, adjacent to the papillae, are the source of lipases (LIP) which release free fatty acids during mastication. Although a preduodenal lipase (LIPPF) has been shown to be synthesized and secreted by VEG in rodents (Hamosh and Scow 1973), the presence of lingual lipase in human has been debated. Spielman et al. (1993) reported that LIPPF expression is absent in human VEG; nevertheless, a lipase activity leading to a partial TG

breakdown of FFA is likely to occur in human saliva (Neyraud et al. 2012; Pepino et al. 2012; Stewart et al. 2010) as the addition of orlistat (a lipase inhibitor) increased fatty acid thresholds (Pepino et al. 2012). Indeed, a recent study has revealed the presence of alternative lipase (LIP) isoforms (LIPK, LIPM, LIPN) in human buccal epithelium (Voigt et al. 2014).

3 Fat Taste Receptors

3.1 CD36

Cluster of differentiation 36 (CD36), which belongs to the class B scavenger receptor family, was first identified as a platelet integral membrane glycoprotein (glycoprotein IV) (Tandon et al. 1989). CD36 is a glycoprotein, composed of 471 amino acids with a molecular weight of approximately 53–80 kDa depending on the degree of glycosylation (Oquendo et al. 1989). CD36 consists of a large extracellular hairpin-like structure and two intracellular transmembrane -NH_2 and -COOH terminals. CD36 is functionally associated with proteins, involved in endocytosis and cell signaling, including Caveolin-1 and *src*-PTK, respectively (Huang et al. 1991; Ring et al. 2006). CD36 expresses the binding sites for various ligands including thrombospondin-1, oxidized low-density lipoproteins (LDL), advanced glycation end products (AGE), growth hormone releasing peptides, a membrane protein expressed by *Plasmodium falciparum*-infected erythrocytes and free fatty acids (Silverstein and Febbraio 2009). Several reports have documented the expression of CD36 in lingual gustatory cells in humans (Ozdener et al. 2014) and other mammals (Laugerette et al. 2005; Fukuwatari et al. 1997; Montmayeur et al. 2001; Gaillard et al. 2008; Abdoul-Azize et al. 2013). We have isolated CD36-positive cells from mouse taste buds and reported that CD36 is expressed on type II cells identified by co-expressed α-gustducin (El-Yassimi et al. 2008).

3.2 GPCR

The fatty acids also exhibit binding affinity to a number of transmembrane receptor proteins that belong to G-protein-coupled receptor (GPCR) superfamily. GPR43 has been shown to bind to short chain (C2:0–C6:0) saturated fatty acids, whereas GPR84 exhibits high affinity for medium chain (C8:0–C14:0) fatty acids (Wang et al. 2006). It has been reported that GPR113 is expressed by circumvallate (CV) papillae in humans, primates, and rodents (LopezJimenez et al. 2005). GPR113 seems necessary for normal responsiveness to dietary oils such as soybean oil and corn oil as well as fatty acids such as linoleic acid (LA) and oleic acid. Indeed, GPR113-knock-out mice exhibited impaired responsiveness to fat stimuli in a behavioral paradigm (US Patent 2019 265231A1). GPR40 and GPR120 bind to, respectively, short medium-chain and LCFAs (Itoh et al. 2003; Cartoni et al. 2010). GPR40 and GPR120 share only 19% sequential homology, indicating that they might be

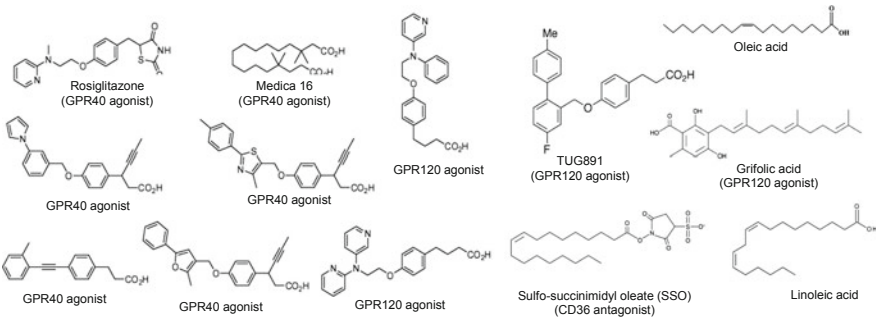

Fig. 2 Modulators of GPR40, GPR120, and CD36 described in the chapter

involved in different roles (Costanzi et al. 2008). Cartoni et al. (2010) suggested that the attenuated expression of GPR40 and GPR120 was associated with a reduced preference for a lipid taste in mice. Although GPR120 has been shown to be expressed by type II cells in circumvallate papillae in mice (Matsumura et al. 2009) and in humans (Galindo et al. 2012; Ozdener et al. 2014), the role of GPR40 remains debatable in humans.

GPR120 is activated only at high concentrations of fatty acids (Ozdener et al. 2014). Its activation on the tongue has been reported to stimulate a specific type of fiber, called fatty acid (F)-type fiber, to transfer gustatory signal afferently to the brain (Yasumatsu et al. 2019). However, contrary to the findings of Cartoni et al. (2010), a more recent report has indicated that GPR120$^{-/-}$ and GPR40$^{-/-}$ mice were not different from wild-type mice in their preference for intralipid (Sclafani et al. 2013). Godinot et al. (2013) investigated whether GPR40 and GPR120 agonists may trigger a fatty sensation in rodents and humans. Hence, they tested several non-fatty acid agonists of GPR40 and GPR120 as possible activators of fat taste. Interestingly, five GPR40 agonists and two GPR120 agonists (Fig. 2) activated the glossopharyngeal nerve in mice. Although none of these agents could induce a preference for fat over control in rodents, two GRP120 agonists (rosiglitazone and Medica 16) were perceived as "fatty" at 50 μM and 100 μM by human volunteers during 2-alternative forced choice tests. Similarly, we have employed TUG-891, a novel GPR120 agonist, that binds to lingual taste bud GPR120 and exerts its action on the "tongue-brain-gut" loop by increasing CCK and GLP-1 secretion, leading to less food intake in obese mice (Murtaza et al. 2020b).

As observed with other taste modalities such as sweet, bitter, or umami (see chapters 2 through 5 in this book), taste recognition threshold for fatty molecules should be correlated with their potency (EC_{50}) at activating their respective receptors or their respective affinity. Oh et al. (2010) used a cell-based reporter system by transfecting HEK293 cells with constructs for GPR120, they reported that DHA, EPA, and palmitic acid (C16:1n7), all activated the SRE-luc reporter with an EC_{50} of 1–10 μM, while saturated fatty acids were without any effect. Consistent with this, a rise of intracellular calcium levels in response to LA was higher than that induced by oleic acid in HEK293 cells, expressing GPR120 (Galindo et al. 2012, Adachi et al.

2014). Ozdener et al. (2014) reported that LA at 20 μM triggered GPR120-mediated responses in STC-1 cells. However, orosensory detection of FFAs in human occurs at much higher concentrations, between 0.26 and 12 mM (Stewart et al. 2010). These inconsistencies could result from assay-dependent variables (Charlton and Vauquelin 2010) as well as differences among native and recombinant cells and intact tissues with respect to receptor reserve, G-protein stoichiometry, coupling efficiency, and biased agonism (Kenakin 1997; Kenakin 2019).

3.3 Delayed Rectifying Potassium Channels

At least, nine different isoforms of delayed rectifying K^+ (DRK) channels are known under three subfamilies. Among them, three isoforms (KCNA, KCNB, and KCNC) are expressed by rat fungiform papillae, but only KCNA channels are exclusively sensitive to LCFAs (Liu et al. 2005). Gilbertson et al. (1997) suggested a role of DRK channels in the orosensory perception of dietary lipids. It was observed that extracellular application of *cis*-polyunsaturated fatty acids (PUFA), but not *trans*-PUFA, on rat TBC inhibited DRK channels and, thereby, caused the inhibition of K^+ efflux which, subsequently, resulted in fast cellular depolarization due to an accumulation of positive charge. The concentration of PUFA to exert an inhibitory effect on DRK channels was dependent on the degree of unsaturation, but was independent of the chain length or the position of the double bonds. These authors found an inhibition constant of 1.02 μM with an inhibition of 83% of DRK at 10 μM concentration of arachidonic acid (Gilbertson et al. 1997). In contrast to PUFA, saturated fatty acids, varying in chain length from 6 to 20 carbons (10–25 μM), did not influence the activity of DRK channels (Gilbertson et al. 1997). The fat taste receptors (CD36 and GPR120) are expressed on the apical region of the papillae, whereas the DRK channels are localized in the basolateral region. We have concluded that DRK channels might act downstream of fat taste receptor activation (Gilbertson and Khan 2014).

4 Fat Taste Signaling

Sclafani et al. (2013) reported that α-gustducin-knock-out mice exhibited less taste preference for an oil-containing solution in a two-bottle choice test. Though CD36 is co-expressed in α-gustducin-expressing type II cells, this finding is surprising since CD36 is not directly coupled to a G-protein. However, it is possible that, in analogy to T lymphocytes, after the binding of the agonist to its receptor some intracellular signal transducers like protein-tyrosine kinases (PTKs) may be recruited to the intracellular domains of the receptor to assure the activation of some small G-proteins for signal transduction. We have conducted a number of experiments and demonstrated that *src*-protein-tyrosine kinases (*src*-PTKs) are involved in the signal transduction during the orosensory detection of dietary fat. Hence, the LCFAs were found to induce the activation of Fyn[59] and Yes[62] types of *src*-PTKs in mouse

TBCs (El-Yassimi et al. 2008). Phosphorylation of *src*-PTKs may then trigger the activation of PLCβ2 which will hydrolyze the phosphatidyl-inositol-bisphosphate (PIP_2), giving rise to inositol tris-phosphate (IP_3) and diacylglycerol (DAG) and ultimately leading to an increase of intracellular Ca^{2+} concentration (Fig. 1). Transient receptor potential cation channel subfamily M member 5 (TRPM5) channels is a calcium-activated, non-selective, cation channel that is known to induce depolarization in response to increase in free intracellular calcium. This channel has been implicated in the orosensory detection of bitter, sweet, and umami tastes. TRPM5 channel also plays a critical role in fat taste detection as LA-induced increases in free intracellular calcium concentration were significantly curtailed in taste bud cells from *TRPM5*$^{-/-}$ mice (Liu et al. 2011). Indeed, the spontaneous preference for fatty acids observed during double bottle choice tests was abolished in *TRPM5*$^{-/-}$ mice, which confirms their role in gustatory perception of dietary fatty acids (Sclafani et al. 2007; Liu et al. 2011). TRPM5 and DRK channels might be involved in TBC depolarization, inducing the release of ATP through CALHM1 channels (Taruno et al. 2013). The activation of CALHM1 is also critical for fat taste perception as increases in $[Ca^{2+}]i$ and spontaneous preference for lipid solutions in mice were curtailed in *CALHM1*$^{-/-}$ mice (Subramaniam et al. 2016) (Fig. 1). Whether released ATP exerts its action on P2X2 or P2X3 receptors, leading to the release of neurotransmitters like serotonin from TBCs, is not known, though we have reported that serotonin is released by some TBC during their activation by dietary LCFAs (El-Yassimi et al. 2008). Interestingly, mice knocked out for P2X2/P2X3 receptors also displayed a reduced fat preference (Sclafani and Ackroff 2014). In analogy to sweet taste perception, we can propose that serotonin, released by some TBC, will send the lipid gustatory message to afferent neurons, connecting the nucleus of solitary tract (NTS) via gustatory nerves VII/IX or F-waves.

As far as Ca^{2+} signaling is concerned, the TBC activation follows the capacitative model of Ca^{2+} homeostasis, proposed by Putney Jr (1999). The binding of IP_3 to its receptors (IP_3R), located on the endoplasmic reticulum (ER), will result in ER Ca^{2+} store depletion, followed by Ca^{2+} influx (El-Yassimi et al. 2008). We would like to mention here briefly the implication of STIM1 in Ca^{2+} signaling during gustatory perception of fatty acids. STIM1 acts as a Ca^{2+} depletion sensor, localized in close proximity (10–25 nm) to the ER membrane (Liou et al. 2005). In unstimulated conditions, EF-hand motif of STIM1 is bound to Ca^{2+}, and cell activation, leading to the release of Ca^{2+} from ER pool, causes the oligomerization of STIM1 and its translocation towards ER-plasma membrane, in association with cell plasma membrane (Fig. 1). STIM1 then interacts with store-operated Ca^{2+} (SOC) channels, leading to rapid influx of Ca^{2+} to refill the intracellular pool. The SOC channels are composed of four channel forming units, encoded by *Orai* genes which are molecular constituents of the Ca^{2+}-release activated Ca^{2+}-currents, I*CRAC* (Mignen et al. 2009). We observed that TBC expressed both mRNA and protein of Orai1 and Orai3 channels, and that they played the role of SOC channels to assure Ca^{2+} influx after LCFAs-induced depletion of ER pool. We have demonstrated that the STIM1 is indispensable for the perception of lipid taste (Dramane et al. 2012). Indeed, STIM1

knock-out mice completely lost preference for an LCFA in a two-bottle preference test (Dramane et al. 2012).

The Ca^{2+}-influx factor (CIF) is another important component of Ca^{2+} signaling. The CIF was discovered in human T cells as an agent, which is released from ER during store depletion and, consequently, favors Ca^{2+} influx in order to refill the intracellular pools, and this agent can be detected as early as 20–25 s after store depletion (Bolotina and Csutora 2005). We purified CIF from mouse LCFAs-activated TBC and demonstrated that taste bud CIF can be a universal factor as the TBC CIF triggered Ca^{2+} influx in mouse TBC, human T lymphocytes and HEK293 cells (Dramane et al. 2012). CIF failed to trigger any Ca^{2+} influx in Orai-null and Orai3-silenced mouse TBC.

To this series of intracellular events, we have recently added the implication of transient receptor potential canonical-3 (TRPC3) channels that allows Ca^{2+} influx, under the control of STIM1 (Murtaza et al. 2020a). The TRP proteins are non-selective cation channels that are activated by endogenous DAG, produced as a result of PIP_2 hydrolysis (Fig. 1). We have demonstrated that mouse as well as human TBC co-expresses CD36 and GPR120 with TRPC3, but not TRPC6 and 7, channels. TRPC3-null mice lost the preference for dietary LCFAs in lickometer, gustometer, and two-bottle preference tests.

We have also demonstrated that the MAP kinase cascade is activated upstream to CALHM1, in human TBC (Subramaniam et al. 2016), and that MAPK activation was functionally coupled to the above-reported *src*-Fyn[59] phosphorylation in the raft fractions of human TBC (Fig. 1). The key importance of MAPK-CALHM1 was affirmed by the fact that genetic ablation of *Erk1* or *Calhm1* genes impaired preference for dietary fat in mice (Subramaniam et al. 2016). Similarly, a MEK inhibitor applied on the tongue of human healthy participants decreased the orosensory detection capacity of dietary LCFAs (Subramaniam et al. 2016).

5 CD36 and GPR120 Play Alternative Roles

Another important point is the role of CD36 and GPR120 in fat taste signaling. The question, naturally, arises why do we need two proteins for one function? The difference is that the former exhibits high affinity whereas the latter possesses low affinity for dietary fatty acids. We have further resolved this enigma by conducting experiments on isolated raft and non-raft fraction from mice TBC under fasting conditions (Ozdener et al. 2014). We have demonstrated that CD36 is highly localized in raft fractions under these circumstances; however, an acute contact with LCFAs induces the translocation of CD36 to the cytosolic fraction and at the same time, GPR120 is upregulated in the raft fractions (Fig. 3). We can state, alternatively, that LCFAs, after their contact with lipid receptors, induce their downregulation or upregulation to assure, respectively, immediate detection and sustained lipid signaling on the arrival of fat-rich food. These experimental observations have been recently confirmed in obese participants, maintained for 8 weeks on a low-calorie diet (Costanzo et al. 2019). These investigators observed

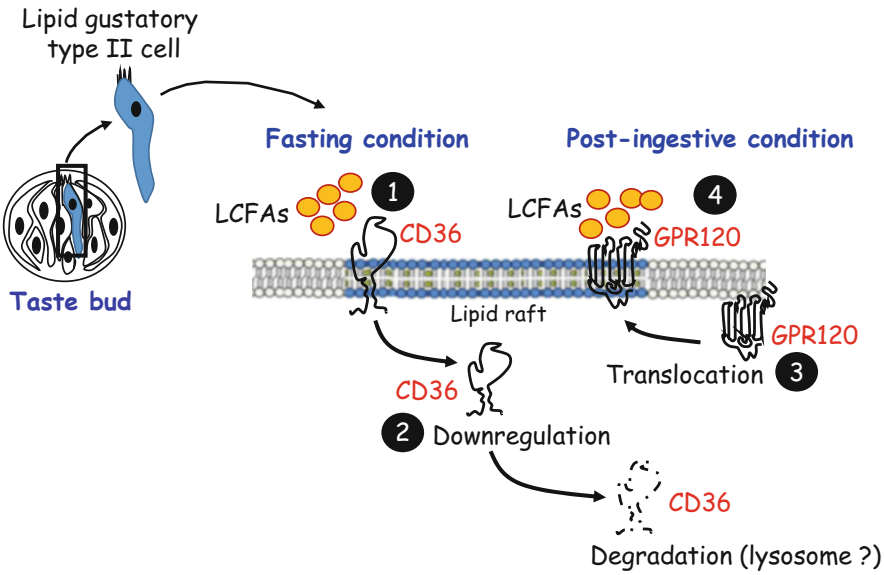

Fig. 3 The complementary roles of CD36 and GPR120 in gustatory perception of dietary fatty acids. Under a fasting condition, the CD36 expression in lipid rafts of type II TBC is very high, whereas GPR120 expression is low (Ozdener et al. 2014). The free LCFAs released in the mouth cavity (for example, at the beginning of breakfast) bind to high affinity CD36 whose levels are very high under fasting or pre-meal conditions. The activated CD36 assures the orosensory detection of free LCFAs and informs the brain on the arrival of dietary fat. The interaction CD36-LCFAs triggers the downregulation of CD36 from the rafts and its degradation (probably via a lysosomal mechanism). This event is followed by the upregulation/translocation of GPR120 to the rafts in order to regulate the post-ingestive mechanisms of fat eating behavior. Both the lipid receptors are coupled to Ca^{2+} signaling, one of the earliest gustatory key signals, that assures the transfer of taste message from the tongue to the brain. The numbers in the circles show the sequences of the events, triggered by CD36 and then followed by GPR120

that CD36 and GPR120 mRNA in the taste papillae exhibit alternative expression before and after dieting. Another recent report of Choo et al. (2020), which studied the effect of maternal obesity on taste bud expression of receptors in the offspring, observed that a reduction in CD36 expression in the taste bud of female offspring of HFD fed mice was accompanied by an increase in GPR120 in the TBC.

6 Dietary Fatty Acids Activate Tongue-Brain-Gut Loop

All of the gustatory messages from tongue end in different regions of the brain, leading to identification and amplification of hedonic perception of the sapid agents. We have observed that dietary LCFAs, applied onto the mouse tongue activated both gustatory and reward brain centers in these animals (Peterschmitt et al. 2018). The gustatory information is first conveyed to the NST (Fig. 4) which is the first relay of gustatory pathway. Parabrachial nucleus (PBN) of the pons makes the second relay

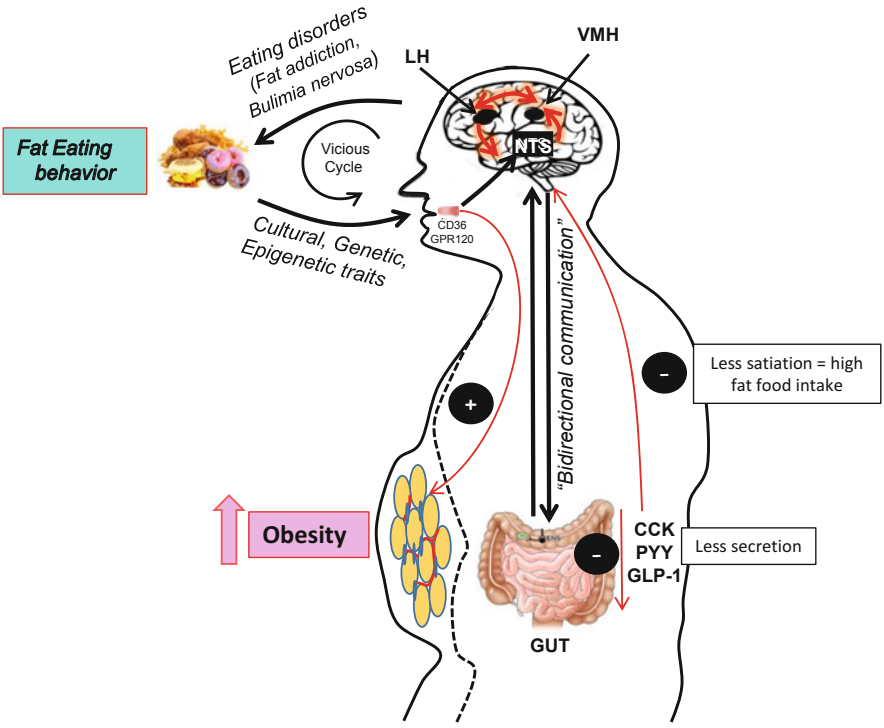

Fig. 4 The implication of high preference/intake of dietary lipids in obesity. Fat eating behavior is essentially regulated by physiological needs and governed by different factors. To simplify, we would like to state that fat-rich food, being a part of our social life and under the influence of socio-economic and cultural traits, may be dysregulated by genetic (for example, genetic polymorphism of lipid receptors like CD36 & GPR120) or epigenetic (hypermethylation of lipid receptors) alterations that could result in high fat intake which, in the long run, may induce fat addictive behavior (Sarkar et al. 2019). Fat-rich palatable food also increases the hedonic preference and, thus, may be preferred during some eating disorders like bulimia nervosa. All of these factors, associated with high fat intake, may contribute to obesity. Fat addiction and cultural/genetic factors might create a "vicious cycle" that may aggravate or increase body weight gain. Fat-rich diet is also known to induce lesser satiation than protein-rich diets, associated with lesser release of satiety factors like CCK, PYY, and GLP-1 from the gut. This phenomenon further reinforces high fat intake leading to obesity. The figure also shows the "bidirectional communication" between brain and gut where different brain areas like NTS (relay center) are involved in coordination with LH (satiety center) and VMH (hunger center) during fat eating behavior. The (+) and (−) signs show, respectively, stimulatory and inhibitory actions. *CCK* cholecystokinin, *GLP-1* glucagon-like peptide 1, *PYY* peptide-YY, *LH* lateral hypothalamus, *VMH* ventro-median hypothalamus

nucleus for the ascending fat taste signal. Ventroposterior medialis parvocellularis (VPMPC) of the thalamus makes the third relay which then transmits taste message to the insular cortex (Khan et al. 2020). Brain reward area comprises ventral tegmental area (VTA), nucleus accumbens (NAcb), and ventral pallidum. The VTA constitutes the mesolimbic dopaminergic system. We have demonstrated that LCFAs, by binding to the tongue receptors, activate VTA, but to the lesser extent the

NAcb (Khan et al. 2020). It is noteworthy that LCFAs deposition onto the rodent tongue, with an esophageal ligation, also led to a rapid and sustained increase in efflux and protein contents of pancreatobiliary secretions (Laugerette et al. 2005; Murtaza et al. 2020b). This effect was fully abolished in CD36 knock-out mice. These findings demonstrate that lingual CD36 participates to the digestive anticipation, through the activation of a reflex loop involving tongue/NTS/peripheral loop, which results in pancreatobiliary secretions preparing the body for digestion the fat incoming.

7 Fat Taste and Obesity

7.1 Altered Functions of Fat Receptors

A number of studies suggest an association between obesity and decreased orosensory detection of LCFAs (Fig. 4). In fact, diet-induced obesity was associated with high detection thresholds for fatty acids in rats and mice during brief licking tests (Shin and Berthoud 2011; Chevrot et al. 2013). There exists an inverse correlation between body mass and the ability to detect lower concentrations of fatty acids during behavioral tests (Christine Feinle-Bisset et al. 2010; Shin and Berthoud 2011; Chevrot et al. 2013). Interestingly, the decrease in taste sensitivity in diet-induced obesity is also associated with decreased Ca^{2+} signaling in gustatory papillae of these animals, leading to a decrease in neurotransmitter release (Ozdener et al. 2014).

With regard to clinical studies conducted hitherto, there was a positive correlation between obesity and fat detection thresholds; the higher was the body mass index (BMI), the higher was the orosensory detection threshold in obese participants. Conversely, caloric restriction and bariatric surgery were able to reverse these alterations in taste sensitivity in rodents and humans (Miras and Le Roux 2010; Berthoud and Zheng 2012). Obese patients who had undergone gastric bypass surgery reported fat-rich foods less pleasant (Miras and Le Roux 2010).

Zhang et al. (2011) have reported that the expression of CD36 was decreased in the circumvallate papillae of diet-induced obese rats. These authors suggested that the decreased expression of CD36 was associated with reduced oral sensitivity to dietary lipids. This resulted in an increased fat intake and, consequently, sustained obesity in these animals (Zhang et al. 2011). In another study, Chen et al. (2013) observed that experimental attenuation of CD36 expression resulted in decreased preference for lipids in both obesity-prone Osborne-Mendel (OM) and obesity-resistant S5B/Pl (S5B) rats. Similarly, it has been reported that lipids can trigger immediate downregulation of CD36 after an acute exposure to fatty acids in mouse TBC (Chevrot et al. 2013). Hence, it can be assumed that over consumption of fat, before the appearance of obesity, could reduce CD36 level in taste buds. Furthermore, it has been shown that genetic polymorphism of fat receptors is associated with decreased orosensory fat detection in obese participants (see the next subsection). Possibly, chronic overconsumption of saturated fat could affect the distribution

of CD36 by decreasing plasma membrane fluidity. Changes in hormonal status during obesity (e.g., insulin resistance, hyperleptinemia, and decrease in the plasma GLP-1 levels) may also contribute to fat taste alteration (Bartoshuk et al. 2006). Consistent with this, insulin, leptin, and GLP-1 receptors are found in TBC (Loper et al. 2015). Furthermore, the localization of CD36 plasma membrane is controlled by insulin in myocytes (Van Oort et al. 2008).

7.2 Genetic and Epigenetic Modifications of Fat Receptors

It has been widely known that CD36 polymorphism is closely related to the disorders linked with excess of body lipids. Ma et al. (2004) reported that a common haplotype at the CD36 locus was associated with high free fatty acid levels and increased cardiovascular risk in Caucasians (Ma et al. 2004). Love-Gregory et al. (2011) showed that variants in CD36 gene were associated with the metabolic syndrome and high-density lipoprotein cholesterol. Bokor et al. (2010) have reported the relationship between rs1527483 polymorphism and obesity, high BMI and percentage of body fat. The CD36 SNP rs3212018 was associated with BMI and waist circumference (WC) in African-American participants (Keller et al. 2012). Pepino et al. (2012) also reported the association of CD36 polymorphism, taste perception for high fat foods and obesity in human beings. Obese subjects were divided into three groups on the basis of rs1761667 allele distribution. It was observed that subjects with homozygous group for AA had eight times higher detection threshold for oleic acid and triolein than homozygous allele GG while the heterozygous subjects had intermediate values. In another study on African-American participants, Keller et al. (2012) observed that AA genotype of rs1761667 was associated with greater perceived creaminess of the salad dressings, regardless of the fat concentration. Our team has conducted a number of studies in different regions of north Africa. The CD36 A-allele of rs1761667 was correlated with decreased orosensory detection of fat, while G allele was associated with low detection thresholds in obese, but not in lean, Algerian children (Sayed et al. 2015). These findings were reproduced in Algerian teenagers (Daoudi et al. 2015) and Tunisian (Mrizak et al. 2015; Karmous et al. 2018) and Moroccan (Bajit et al. 2020) obese participants wherein we reported that AA and AG genotypes were more frequent in obese than in lean subjects. It is noteworthy that the A allele of rs1761667 is associated with decreased expression of CD36 (Love-Gregory et al. 2011; Ghosh et al. 2011), suggesting reduced fatty acid taste detection may be mediated by low CD36 present on the TRC.

There are also epigenetic mechanisms potentially regulating fatty taste detection. A recent report by Berrichi et al. (2020) has investigated the relationship between CD36 gene methylation and obesity in young Algerian children. DNA methylation, observed at CpG and non-CpG dinucleotides, represents the most common epigenetic modification (Ehrlich 2002). These CpG dinucleotides are grouped in specific regions termed "CpG islands," which reside in or near to the promoters in 50% of human genes (Jin et al. 2016). CpG methylation may lead to inhibition of binding of

trans-activating factors and, consequently, to gene silencing as reported for CD36 (Keller et al. 2016) and GPR120 (Díaz et al. 2016). Obese children presented higher methylation level of the CpG sites than lean participants, and CD36 and GPR120 gene methylation was associated with high lipid detection thresholds in obese participants (Berrichi et al. 2020). These findings strengthen the notion that variations in the expression of fatty acid receptor CD36 may directly affect the gustatory perception of fat and, hence, obesity.

8 Fat Taste Regulation

Orosensory detection of fat is modulated by several hormones linked to obesity and eating behavior. Oxytocin, originally known for promoting delivery and milk ejection in mammalian females, also exerts an anorectic effect. In addition to acting within central circuits, oxytocin also seems to influence the peripheral taste system. Indeed, mouse TBC has been shown to express the oxytocin receptor (OTR) (Sinclair et al. 2010) and oxytocin elicited Ca^{2+} responses when applied to taste bud cells in vitro (Sinclair et al. 2010). OTR appeared to be expressed only by Type I TBC, suggesting that, if confirmed, oxytocin could modulate taste signals via a cell-to-cell communication between type I and type II TBC.

The multifunctional hormone GLP-1 (Baggio and Drucker 2007) is expressed by Type II and Type III TBC, whereas its receptor is expressed on afferent gustatory nerves (Shin et al. 2008). LCFAs lead to the release of GLP-1 in freshly isolated mouse TBC (Martin et al. 2012). As demonstrated in the entero-endocrine cells (Hirasawa et al. 2008), this event seems to be dependent on the activation of lingual GPR120 by LCFA (Martin et al. 2012). Conversely, GLP-1R-null mice displayed low sensitivity to an oily solution (Martin et al. 2012).

Beside autocrine/paracrine regulation, we would like to comment on indirect regulation of fat preference in mice. Generally, there are two classes of taste modifiers, negative allosteric modulators (NAM) and positive allosteric modulators (PAM). These agents do not bind directly to the taste receptor agonist binding site, rather they induce an allosteric conformational change of the target receptor and modulate its functioning. Recently, Shanmugamprema et al. (2020) have emphasized the synthesis of negative modulators, but these agents cannot be used to treat obesity as obese subjects already suffer from low fat taste perception. On the contrary, a PAM or high affinity agonists of the tongue lipid receptors are more likely to prove beneficial. Though we do not have, at the moment, the PAM to mimic fat taste signaling, we have recently identified a unique mechanism to potentially address some pathophysiological issues associated with fat consumption. We have shown that human and mouse TBC express Takeda-G-protein-receptor-5 (TGR5) on human and mouse TBCs (Bensalem et al. 2019), and the receptor activation by a plant terpenoid, Zizyphin ($C_{51}H_{80}O_{18}$), improved glucose tolerance, dyslipidemia, and fatty liver disease in obese mice (Murtaza et al. 2017; Berrichi et al. 2019). Oleanolic acid, another terpenoid present in olive oil from wild trees, was found to exert anti-obesity effects and to improve obesity-associated glucose tolerance,

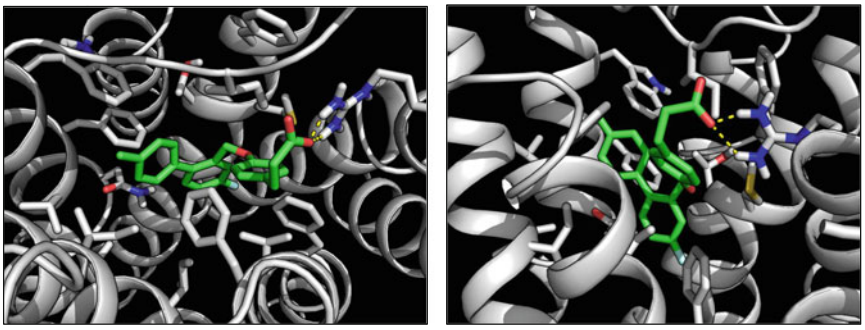

Fig. 5 The binding of TUG891 to GPR120. TUG891 (green) docked in GPR120 (gray) with the most important side chains forming the binding pocket shown, and ionic and hydrogen bonds between the carboxylate of TUG891 and arginine[99] of GPR120, indicated by yellow stippled lines. (Photo, by courtesy of Dr. Trond Ulven)

insulin level, plasma lipopolysaccharide (LPS), and hepatic cholesterol and triglyceride concentrations in mice (Djeziri et al. 2018). This triterpenoid exerted these favorable effects by influencing, in part, CD36 mRNA expression in TBCs and gustatory lipid perception. We have also recently carried out a number of experiments showing that a novel GPR120 non-caloric agonist, TUG891, which exerts an anti-obesity action, binds to lingual GPR120 (Fig. 5) and induces a rapid increase in Ca^{2+} by acting on GPR120 in cultured mouse and human TBC (Murtaza et al. 2020b). TUG891 also induced the activation of ERK1/2 phosphorylation and enhanced in vitro release of GLP-1 from cultured human and mouse TBCs. In situ application of TUG891 onto the tongue of anesthetized mice triggered the secretion of pancreatobiliary juice and CCK, via the *tongue-brain-gut loop.* In behavioral tests, mice exhibited a spontaneous preference for solutions containing either TUG891 or LCFAs instead of a control. This is the first report to demonstrate that fatty acid non-caloric agonists might be anti-obesity agents that will exert their action on the tongue lipid receptors.

9 Conclusions and Challenges

We have seen that fat taste perception activates almost the same signaling pathways as other basic or primary taste qualities. The orosensory detection of dietary fatty acids also leads to the activation of different brain areas which, in turn, trigger the release of gut peptides (Fig. 4). However, experiments conducted with human participants in tasting sessions have not generated quite the same type of physiochemical perceptions as for sweet or bitter tastes. Moreover, some participants felt a "scratchy" or "burning" or other feelings in fatty acid tasting sessions. Dietary lipids may exhibit different oral sensations, depending on the physiological state of the taster and on the nature of fatty acid-containing solution. Hence, fat taste may be an

alimentary taste (Hartley et al. 2019). Alimentary taste, as opposed to the basic tastes, does not have the salient perceptual functions, observed with sweet, sour, salt, and bitter when a tastant is placed on the tongue. The team of Richard Mattes has proposed a Latin word "Oleogustus" where "oleo" means oily or fatty and "gustus" for taste (Running et al. 2015). Moreover, it is also possible that there might be fat detection component independent of oro-gustatory perception as seen in the primates (Rolls 2012).

There is still much to explore with respect to the role of autocrine and paracrine regulation of fat taste perception. The role of buccal microbiota in fat taste perception also should be explored in the future. Duca et al. (2012) have shown that germ free mice had increased taste sensitivity and increased expression of CD36 relative to normal control mice. However, a study conducted on human participants showed no microbiota-dependent effect on the orosensory sensitivity to lipids (Besnard et al. 2018).

Important questions remain with regard to the signaling aspects of fat taste. Since CD36 is expressed in type II cells, known to convey signals from bitter, sweet or umami tastants, how is fat taste coded at the periphery? Is there a special subpopulation of type II cells specific for recognition of lipids and not expressing any of the other taste receptors? We do not know how F-type neurons encode fatty acid information via GPR120 activation (Yasumatsu et al. 2019). Do they communicate with CD36 during gustatory perception of dietary lipids? The activation of MAP kinase cascade (MEK/ERK1/2) is generally achieved by the c-raf protein which is not present in mouse TBC. Do the mouse TBC express b-raf (or RSK or MSK effectors) or other enzyme complex like Ca^{2+}/CaM kinase II as is seen in the neuronal activation in the brain? We have observed that LCFAs phosphorylate not only MEK1/ERK1/2/ETS cascade but also p38 and JNK1/2 kinases (Subramaniam et al. 2016). How do they interact with each other in fat taste signaling? ATP is known to exert its action on purinergic receptors, present on type II and III cells, to convey sweet gustatory message afferently. Similarly, do fat taste receptor cells release ATP during LCFA stimulation? It should also be noted that GPR40 is absent on the human tongue, but is present in mouse taste papillae (Galindo et al. 2012). Are other GPCR like GPR43 and GPR84, identified in human fungiform papillae (Liu et al. 2018), also involved in fat taste perception? STIM1 is central in Ca^{2+} signaling in TBC. Does this agent also orchestrate the opening of Calhm1 channels in TBC? Besides, the implication of several intracellular signaling transducers like STIM1, Orai1, Orai1/3, and TRPC3 has to be investigated in other taste qualities like sweet, sour, acid, bitter, and umami. In the future, the availability of high affinity agonists for GRP120 or CD36 could help understand the role of these receptors in fat detection in humans and could offer an avenue for the development of effective anti-obesity treatments.

Conflict of Interest All the authors declare that they do not have any conflict of interest.

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Immune Regulatory Roles of Cells Expressing Taste Signaling Elements in Nongustatory Tissues

Hong Wang, Ichiro Matsumoto, and Peihua Jiang

Contents

Abstract

G protein-coupled taste receptors and their downstream signaling elements, including Gnat3 (also known as α-gustducin) and TrpM5, were first identified in taste bud cells. Subsequent studies, however, revealed that some cells in nongustatory tissues also express taste receptors and/or their signaling elements. These nongustatory-tissue-expressed taste receptors and signaling elements play important roles in a number of physiological processes, including metabolism and immune responses. Special populations of cells expressing taste signaling

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elements in nongustatory tissues have been described as solitary chemosensory cells (SCCs) and tuft cells, mainly based on their morphological features and their expression of taste signaling elements as a critical molecular signature. These cells are typically scattered in barrier epithelial tissues, and their functions were largely unknown until recently. Emerging evidence shows that SCCs and tuft cells play important roles in immune responses to microbes and parasites. Additionally, certain immune cells also express taste receptors or taste signaling elements, suggesting a direct link between chemosensation and immune function. In this chapter, we highlight our current understanding of the functional roles of these "taste-like" cells and taste signaling pathways in different tissues, focusing on their activities in immune regulation.

Keywords

Acetylcholine · Gustducin · IL25 · Inflammation · Pou2f3 · Solitary chemosensory cells · TrpM5 · Tuft cells · Type 2 immunity

1 Introduction

During the past few decades, our understanding of the molecular and cellular mechanisms of taste reception at the periphery has greatly advanced, and receptors for five basic taste qualities and many intracellular signaling molecules in taste cells have been well characterized (Margolskee 2002; Roper and Chaudhari 2017; Yarmolinsky et al. 2009). For instance, type 1 taste receptors (T1Rs) mediate sweet and umami tastes, whereas type 2 receptors (T2Rs) mediate bitter taste (Adler et al. 2000; Bachmanov et al. 2001; Hoon et al. 1999; Matsunami et al. 2000; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001). T1Rs and T2Rs are G protein-coupled receptors (GPCRs), coupled to taste-cell-expressed trimeric G proteins composed of Gnat3 (also known as α-gustducin), Gnb3 (Rossler et al. 2000), and Gng13 (Huang et al. 1999; McLaughlin et al. 1992). Upon receptor activation, Gnat3 is dissociated from Gβ3γ13 dimer which then activates phospholipase C β2 (PLCβ2), which in turn cleaves phospholipid phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacyl glycerol (Rossler et al. 1998; Zhang et al. 2003). IP3 opens up the type III IP3 receptor to allow calcium mobilization in taste cells (Hisatsune et al. 2007). Rising calcium triggers opening of the transient receptor potential cation channel subfamily M member 4 (TrpM4) and TrpM5 to depolarize taste cells (Dutta Banik et al. 2018; Hofmann et al. 2003; Liu and Liman 2003; Perez et al. 2002; Zhang et al. 2003), which leads to release of the neurotransmitter ATP through the voltage-activated ATP channel CALHM1/3 (Ma et al. 2018; Taruno et al. 2013) to activate afferent gustatory nerves (Finger et al. 2005).

Intriguingly, the expression of taste signaling molecules, including taste GPCRs, is not limited to taste cells in the oral epithelium. They are also expressed in other types of cells outside the oral cavity, such as in respiratory and alimentary organs, and function as chemoreceptors and their signaling molecules (Deckmann et al.

2014; Finger et al. 2003; Ohmoto et al. 2008). One population of the nongustatory cells that express taste signaling elements are the so-called solitary chemosensory cells (SCCs). In mouse airway SCCs show molecular signatures very similar to sweet, umami, and bitter taste cells (i.e., type II taste cells based on morphological classification). They express not only taste signaling molecules such as Gnat3, PLCβ2, and TrpM5 but also T1R and T2R taste receptors (Finger et al. 2003). The generation of these SCCs depend on the transcription factor Pou2f3 (also known as Skn-1a), as do sweet, umami, and bitter taste bud cells (Matsumoto et al. 2011; Ohmoto et al. 2013). However, in striking contrast to taste bud cells, which express T1Rs and T2Rs in separate subsets of cells, SCCs co-express T1R3 and some T2Rs (Adler et al. 2000; Nelson et al. 2001; Ohmoto et al. 2008). SCCs are also found in urethra, trachea, and gingival epithelium (Deckmann et al. 2014; Krasteva et al. 2011; Zheng et al. 2019). Although fish have SCCs on their body surface, fish SCCs do not express any taste-related genes, such as T1R and T2R taste receptors, Gnaia, PLCβ2, or TrpM5 (Ishimaru et al. 2005; Matsumoto et al. 2011; Yoshida et al. 2007). Thus, SCCs in mammals and fish may have species-specific functions.

Intestinal tuft cells also express Gnat3, PLCβ2, and TrpM5 (Gerbe et al. 2016; Howitt et al. 2016; Nadjsombati et al. 2018) and rely on Pou2f3 for their development (Gerbe et al. 2016). SCCs and intestinal tuft cells share similar morphology and molecular features, and several recent publications have referred to SCCs as tuft cells (O'Leary et al. 2019; Sell et al. 2020). However, historically, *SCC* is used more frequently to describe cells expressing taste signaling elements (i.e., Gnat3, TrpM5) in the airway, urinary tract, and so forth, whereas *tuft cell* refers to such cells in the GI tract and thymus. Moreover, recent single-cell RNAseq (scRNAseq) studies indicate that SCCs and tuft cells can be further divided into subtypes based on gene expression signature (Montoro et al. 2018). These subtypes of SCCs and tuft cells may have distinct roles, such as immunological or neuromodulatory roles (O'Leary et al. 2019).

In this chapter, we focus on immune regulatory functions of nongustatory-tissue-expressed taste GPCRs and their downstream signaling elements. We describe our current understanding of the functions of tuft cells in the GI tract, SCCs in airway and gingival tissues, and immune cells that express taste signaling elements (Table 1). Regardless of the localization of these cells, they seem to play a role as chemosensory cells to induce biophylactic responses, such as the release of cytokines and induction of antimicrobial responses.

2 Intestinal Tuft Cells in Type 2 Immunity

The classical model of type 2 immunity involves T helper 2 (T_h2) cells and cytokines produced by these cells, including interleukin-4 (IL-4), IL-5, and IL-13 (Lloyd and Snelgrove 2018). The group 2 innate lymphoid cells (ILC2s) were discovered recently as another major producer of type 2 cytokines (e.g., IL5 and IL-13) and play important roles in type 2 immunity (Fort et al. 2001; Moro et al. 2010; Neill et al. 2010; Price et al. 2010). In mucosal tissues, parasites (e.g., helminths),

Table 1 Nongustatory cell types expressing taste signaling elements and GPCRs that play roles in immune regulation

Cell type	Taste signaling elements and GPCRs	Function	References
Intestinal tuft cells	TrpM5, Gnat3, Pou2f3, T1R3, T2Rs, Sucnr1/GPR91	Type 2 immune response; modulation of gut inflammation	Bezencon et al. (2008), Gerbe et al. (2016), Howitt et al. (2016), von Moltke et al. (2016), Haber et al. (2017), Feng et al. (2018), Lei et al. (2018), Nadjsombati et al. (2018), Schneider et al. (2018), Luo et al. (2019), Howitt et al. (2020), Imai et al. (2020)
Airway SCCs	TrpM5, Gnat3, PLCβ2, Pou2f3, T1Rs, T2Rs	Response to microbial and xenobiotic compounds; antimicrobial defense	Finger et al. (2003), Tizzano et al. (2010), Ohmoto et al. (2013), Lee et al. (2014b), Kohanski et al. (2018), Patel et al. (2018), Perniss et al. (2020), Sell et al. (2020)
Gingival SCCs, epithelial cells	TrpM5, Gnat3, PLCβ2, T2Rs, TAS2R38	Antimicrobial response	Gil et al. (2015), Zheng et al. (2019)
Human blood leukocytes (T cells, B cells, neutrophils)	TAS2R13, TAS2R14, TAS2R19, TAS2R38, TAS2R43, TAS1R2, TAS1R3	Regulation of cytokine production; chemotaxis	Orsmark-Pietras et al. (2013), Malki et al. (2015), Maurer et al. (2015), Tran et al. (2018)
Mouse neutrophils	T1R1, T1R3	Regulation of cytokine production; chemotaxis	Lee et al. (2014a)
Human lung macrophages	TAS2Rs	Regulation of cytokine production	Grassin-Delyle et al. (2019)
Thymic medulla epithelial/tuft cells	TrpM5, Gnat3, PLCβ2, Pou2f3, Tas2r105, Tas2R108, Tas2R113, Tas2R118, Tas2r131, Tas2R137, Tas2r138	Supporting immune cells development and polarization	Panneck et al. (2014), Soultanova et al. (2015), Yamashita et al. (2017), Bornstein et al. (2018), Miller et al. (2018)

allergens, and tissue damage stimulate epithelial and other cells to produce alarmins, such as IL25, IL-33, and thymic stromal lymphopoietin (TSLP), which in turn induce tissue-resident ILC2 proliferation and cytokine production. Type 2 cytokines orchestrate several characteristic responses associated with type 2 immunity, including production of immunoglobulin E, recruitment and activation of eosinophils, and tissue remodeling (Gurram and Zhu 2019). Type 2 immunity is critical for defense against parasites, while its dysregulation contributes to allergic diseases. Although

not fully understood, immune responses to helminths also modulate inflammation mediated by type 1 cytokines (i.e., cytokines produced by T_h1 cells), such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF). Helminthic therapies have shown efficacy against inflammatory bowel disease (IBD) and some autoimmune diseases (Cleenewerk et al. 2020; Rook 2012). Multiple recent studies revealed that intestinal tuft cells and taste signaling elements play critical roles in gut type 2 immune responses. Below we provide a brief review of these studies.

2.1 Intestinal Tuft Cells Express Taste Signaling Elements

Tuft cells (also called as brush cells) are a rare type of cell in the intestinal epithelium. Morphologically, these cells have a tuft of long, rigid microvilli on their apical surface, giving this cell type its name (Hofer and Drenckhahn 1992; Trier et al. 1987). Molecularly, these cells show immunoreactivity to taste signaling elements such as Gnat3, TrpM5, and Pou2f3, suggesting they are "taste-like" cells in the gut. Microarray analyses of sorted TrpM5-GFP$^+$ tuft cells from transgenic mice expressing enhanced green fluorescent protein gene (*Egfp*) under the promoter of the *Trpm5* gene confirm the expression of taste signaling elements such as Gnat3, TrpM5, and Pou2f3 but a lack of or low expression of T1Rs and T2Rs (Bezencon et al. 2008). More recently, scRNAseq analysis of gut epithelial cells and bulk RNAseq analysis of tuft cells have validated the expression of TrpM5, Gnat3, and Pou2f3, but RNAseq showed no detectable expression of taste receptors in these cells, with an exception of low expression of T1R3 (Haber et al. 2017; Nadjsombati et al. 2018). Based on these studies, tuft cells appear to utilize the same taste signaling pathway for their function but have receptor repertoires different from those of taste receptor cells. More recently, expression of some T2Rs has been detected in gut tuft cells in a transgenic mouse model (Imai et al. 2020), and bitter receptors may play a role in sensing worm infection in the gut (Luo et al. 2019). Thus, whether a subset of gut tuft cells express taste GPCRs remains to be determined.

2.2 Intestinal Tuft Cells Detect Gut-Dwelling Worms

Until recently, the function of intestinal tuft cells was unclear. The earliest indication of their function came from the microarray study of sorted TrpM5-GFP$^+$ tuft cells (Bezencon et al. 2008). The enrichment of innate-immunity-related genes was noted in these cells, including the expression of genes involved in the leukotriene pathway (e.g., Alox5), the prostaglandin pathway (e.g., Cox-1, Cox-2), and type 2 immunity (e.g., IL25). Despite this intriguing observation, it took a few more years to uncover the function of these cells. In 2016, three studies (Gerbe et al. 2016; Howitt et al. 2016; von Moltke et al. 2016) showed that tuft cells are sentinel cells for worm detection and initiation of type 2 innate immunity. Using an IL25-RFP mouse line (knock-in mouse that expresses tandem-dimer red fluorescent protein [RFP] from the

Il25 locus), von Moltke et al. (2016) showed that IL25 is exclusively expressed in tuft cells throughout the gut. Using knockout mouse models that ablate IL25, IL13, and IL4ra (the receptor for IL13), respectively, they demonstrated that tuft cells initiate type 2 immunity through a tuft cell–ILC2–epithelium response circuit. In addition to the long-known hallmark features of type 2 immunity, such as expansion of goblet and ILC2 cells, von Moltke et al. (2016) also demonstrated that tuft cell hyperplasia is a key feature of type 2 immunity. All these phenotypic changes are greatly diminished in mice deficient for IL25, IL13, or IL4ra, along with delayed expulsion of *Nippostrongylus brasiliensis*, a well-defined type 2-response-inducing parasite (von Moltke et al. 2016).

In another study, Gerbe et al. (2016) arrived at the same conclusion using a different mouse model in which Pou2f3 was ablated. Because Pou2f3 is a master regulator for tuft cell and taste receptor cell specification, mice deficient for Pou2f3 have no tuft cells or type II taste receptor cells. In the absence of Pou2f3, typical type 2 responses (expansion of tuft, goblet, and ILC2 cells) were not observed. As a consequence of dampened type 2 immunity, worm clearance was delayed in Pou2f3-knockout mice (Gerbe et al. 2016). These studies clearly establish the role of tuft cells in initiating type 2 immunity.

Based on a striking phenotype – expansion of tuft cells in the distal ileum – observed in their mouse colony, Howitt et al. (2016) demonstrated that the protozoan *Tritrichomonas muris* is responsible for inducing tuft cell expansion and type 2 immunity. They further showed the requirement of Gnat3 and TrpM5 for tuft cells to detect and respond to worm infection (Howitt et al. 2016). Together, these elegant studies provide insight into how tuft cells work and bridge a gap in understanding how worms induce type 2 immunity.

2.3 Succinate Activates Intestinal Tuft Cells

Once the role of tuft cells as sentinel cells for detecting worm infection and triggering type 2 immunity was established, research turned to their mechanisms of action. Building on the transcriptome characterization of tuft cells, several groups searched for tuft-cell-expressed receptors, with a particular focus on GPCRs, because of the essential roles of Gnat3 and TrpM5 in mediating tuft cell detection of worms. Analyzing the microarray and scRNAseq data led to identification of a number of GPCRs selectively expressed in tuft cells, including Ffar3, Sucnr1, Gprc5c, P2ry1, and T1R3 (ranking order from high to low) (Haber et al. 2017).

Are these receptors capable of directly recognizing worm-derived metabolites and thus responsible for detecting worms and triggering type 2 immunity? Three labs, including ours (Lei et al. 2018; Nadjsombati et al. 2018; Schneider et al. 2018), directly tested if the ligands for these receptors are able to activate type 2 immunity. Indeed, when mice were provided a drinking bottle containing succinate (~100 mM), the ligand for Sucnr1 (also called Gpr91), for a week or so, tuft cell, and goblet cell expansion was noted in the small intestine. Mice lacking Sucnr1 show none of these changes. Physiologically, *T. muris*-induced type 2 immunity appears to be

dependent on Sucnr1. Furthermore, disturbance of the gut microbiome can lead to elevated succinate in the distal gut. We found this can also result in innate type 2 immunity in the distal ileum in wild-type but not in Sucnr1-null mice. *N. brasiliensis* is known to secrete succinate, yet in mice deficient for Sucnr1 *N. brasiliensis* can still induce robust type 2 immunity (tuft and goblet cell expansion), suggesting that an additional receptor (s) is required for detecting *N. brasiliensis*-secreted metabolites or proteins.

Despite high expression of Ffar3 (free fatty acid receptor 3) in tuft cells based on scRNAseq data, feeding mice short-chain fatty acids did not induce type 2 immunity (Nadjsombati et al. 2018; Schneider et al. 2018). Interestingly, our in situ hybridization study showed no detectable expression of Ffar3 in tuft cells (unpublished data). These data argue against but cannot completely exclude a major role of Ffar3 in mediating detection of worm-secreted metabolites. Gprc5c is an orphan receptor; our unpublished in situ hybridization data show its expression in tuft cells, yet a conventional knockout mouse model that lacks Gprc5c (Sano et al. 2011) still displays robust type 2 responses after *N. brasiliensis* infection (unpublished data). However, whether Gprc5c mediates detection of other worms is as yet unclear. Multiple parasitic worms do induce type 2 immunity and may be sensed by different receptors.

With regard to taste receptors, feeding mice sweeteners or bitter compounds does not appear to induce type 2 immunity (Howitt et al. 2020). Interestingly, we (unpublished observations) and others (Howitt et al. 2020) have noted a substantial reduction of tuft cell number in the intestine in one *Tas1r3*-null strain. However, in another *Tas1r3*-null strain we noted no change in the number of tuft cells (unpublished data). These data suggest that a genetic component of the *Tas1r3* locus, rather than *Tas1r3* gene itself, affects tuft cell number, although the exact mechanism is still unclear.

Which receptor detects parasites remains a puzzling question. GPCRs have been tested quite exclusively by us and others. Other than Sucnr1, no other GPCRs have been clearly identified for such roles. Luo et al. proposed a role for TAS2Rs for worm detection (Luo et al. 2019); this hypothesis remains to be tested with Tas2r knockout models. The IL25 receptor Il17rb, which is not a GPCR, is also selectively expressed in tuft cells but not in other epithelial cells. The Locksley lab tested if tuft-cell-expressed Il17rb could mediate the detection of worms and found that ablation of Il17rb from tuft cells does not affect type 2 immunity (O'Leary et al. 2019). Thus, this receptor is also not directly involved in detecting worm-secreted metabolites. Therefore, the search for the worm receptor(s) continues. It is possible that receptors other than GPCRs may be involved in detecting parasitic worms. For instance, worms may activate IgG-domain-containing proteins (e.g., IgSF) in tuft cells. In agreement with this idea, multiple tyrosine kinases and phosphatases are selectively expressed in tuft cells. Presumably, those IgSF family members can bind to worm-secreted metabolites or proteins to modify tyrosine phosphorylation and subsequently activate tuft cells. This line of research is worth pursuing.

Alternatively, identifying the receptor responsible for worm detection may start with further characterization of worm-secreted metabolites. In *N. brasiliensis*

infection models, worms dwell in the proximal gut, yet type 2 immunity occurs throughout the gut, which suggests worms talk to tuft cells most likely via worm-secreted metabolites rather than physical contact. Not all worms produce the same reactions. For instance, *Heligmosomoides polygyrus* can also induce type 2 immunity (Gerbe et al. 2016; Howitt et al. 2016; von Moltke et al. 2016), but its effect appears to be more regional, indicating that *H. polygyrus*-secreted active metabolites may diffuse only locally or have a shorter half-life and are readily digested by enzymes richly present in the gut lumen. Several labs have performed biochemical analyses of *N. brasiliensis* excretory-secretory products (NES) using proteomics or chemical analyses of small molecules in NES (Balic et al. 2004; Harcus et al. 2009; Sotillo et al. 2014; Wangchuk et al. 2019). Unbiased testing of these metabolites using mice or an ex vivo system such as intestinal organoids (Ootani et al. 2009; Sato et al. 2009) may help identify metabolites able to activate tuft cells. Subsequent work can be conducted to determine the target(s) for such metabolites.

2.4 Tuft-Cell-Secreted IL25, Leukotrienes, Prostaglandins, and Other Effectors Coordinately Trigger Type 2 Immunity

IL25 is a major activator for type 2 immunity. Besides IL25, other immune or neuronal effectors are also produced in tuft cells. For instance, leukotriene signaling elements are selectively expressed in tuft cells but not in other epithelial cells (Bezencon et al. 2008; Haber et al. 2017; Nadjsombati et al. 2018). Recently, the von Moltke laboratory has elegantly demonstrated the involvement of tuft-cell-derived leukotrienes in type 2 immunity. Specific ablation of Alox5 in gut epithelial cells or tuft cells using Vil-Cre or Pou2f3-cre results in reduced type 2 immunity in the gut (McGinty et al. 2020).

The prostaglandin pathway has also been found in tuft cells, including Cox-1 and Cox-2 (Bezencon et al. 2008; Haber et al. 2017; Nadjsombati et al. 2018), suggesting that it may also contribute to tuft-cell-initiated type 2 immunity. No studies have reported whether ablating tuft-cell-expressed Cox-1 or Cox-2 can affect type 2 immunity, but we predict that ablating two Cox genes can have a significant impact on tuft-cell-mediated type 2 immunity.

Aside from immune factors, acetylcholine may be produced in tuft cells as well. For instance, choline acetyltransferase (ChAT), the enzyme responsible for synthesizing acetylcholine, is expressed in tuft cells (Porter et al. 1996). ChAT-GFP^{+} cells from transgenic mice expressing GFP under the promoter of ChAT are immunoreactive for DCLK-1, a marker for tuft cells (Schutz et al. 2015). Our own unpublished in situ hybridization data also show the expression of ChAT in tuft cells. Disrupting ChAT function can affect the sentinel function of airway SCCs (Perniss et al. 2020).

The target cells for tuft-cell-derived acetylcholine are unknown. Although deletion of muscarinic acetylcholine receptor type 3 (M3) can affect type 2 immunity (Darby et al. 2015; McLean et al. 2016), whether tuft-cell-produced acetylcholine acts on the M3 receptor remains to be tested. Interestingly, many enteric neurons use

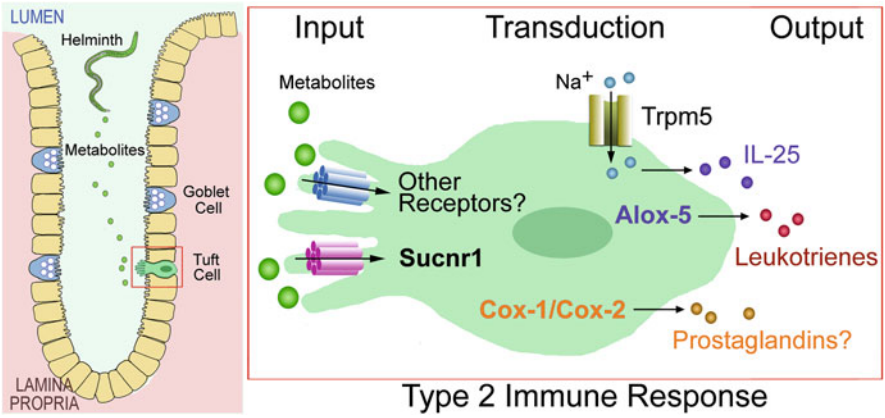

Fig. 1 Schematic drawing of the intestinal epithelium and the interaction of tuft cells and parasitic helminths (left). The illustration at right shows receptors (input), main signaling molecules (transduction), and effectors (output) in tuft cells

acetylcholine as neurotransmitter to regulate smooth muscles in the gut (Schemann et al. 1993). Therefore, there is also a possibility that tuft-cell-produced acetylcholine may coordinate with neuron-produced acetylcholine to activate smooth muscles to enhance gut movement and facilitate clearance of worm. Figure 1 illustrates our current understanding of intestinal tuft cells in type 2 immunity.

2.5 Mechanisms Underlying Tuft Cell Hyperplasia in Response to Worm Infection

A hallmark feature of type 2 immunity is tuft cell hyperplasia (Gerbe et al. 2016; Howitt et al. 2016; von Moltke et al. 2016). Like other epithelial cells, tuft cells derive from $Lgr5^+$ intestinal stem cells in mice with or without worm infection (von Moltke et al. 2016). How type 2 immunity feeds back on intestinal stem cells to alter cell specification is largely unclear. Nevertheless, intestinal tuft cell expansion apparently relies on type 2 cytokines. Treatment of cultured organoids with IL4 or IL13 results in tuft cell hyperplasia (Gerbe et al. 2016; Howitt et al. 2016; von Moltke et al. 2016). Therefore, type 2 cytokines can interact with their receptors such as Il4ra or Il13ra1 to directly impact cell fate determination of intestinal stem cells. Numerous previous studies have demonstrated that Stat6 is a key transcription factor for mediating the effects of IL4 and IL13 on target cells (Urban Jr. et al. 1998). We speculate that Stat6 is also regulated by the IL4/IL13 pathway in $Lgr5^+$ intestinal stem cells. Activated Stat6 may further up-regulate the expression of tuft-cell-specific transcription factors such as Pou2f3 for tuft cell specification. It would be interesting to determine if the genetic locus of Pou2f3 has binding sites for Stat6. This may provide a molecular explanation for tuft cell hyperplasia in the gut.

Taste stem cells and intestinal stem cells are regulated in a similar fashion (Barker et al. 2007; Yee et al. 2013). Taste receptor cells and "taste-like" tuft cells share a great deal of similarity, although as mentioned above, one key difference between these two types of cells is the receptor repertoire: one detects tastants and the other detects worm-secreted metabolites. Given the extensive similarity between these two systems, it would be interesting to investigate if taste receptor cells are also modulated by type 2 immunity (see also O'Leary et al. 2019), for instance, whether elevated type 2 immunity can alter taste cell composition by impacting taste stem cell activity.

3 Taste GPCRs and Their Downstream Signaling Elements in the Regulation of Gut Inflammation

IBD is a debilitating chronic illness that affects millions worldwide. The incidence of IBD appears to be increasing, especially in children (El-Matary et al. 2014). The etiology of IBD remains incompletely understood. Diet, gut microbiome, and genetic factors all play roles in IBD (Kaser et al. 2010). Chronic inflammation associated with IBD is a risk factor for developing gastrointestinal and other cancers (El-Matary and Bernstein 2020; Pedersen et al. 2010; Rubin et al. 2012).

Taste receptor signaling may shape the inflammatory status of the gut through a number of mechanisms, such as by influencing dietary choice, changing gut microbiome, and/or direct regulation of immune responses. Diet has a strong impact on gut health and is heavily influenced by taste perception. T2R bitter receptors recognize many food-derived compounds, and polymorphisms in *TAS2R* genes change perception of these bitter-tasting compounds (Bufe et al. 2002; Roudnitzky et al. 2015). Numerous studies have shown that taste receptor polymorphisms are associated with the consumption of vegetable, fruit, and sugary snacks (Chamoun et al. 2018; Eriksson et al. 2019; Mennella and Bobowski 2015; Reed and McDaniel 2006). Genetic variations in bitter receptor genes, such as *TAS2R38*, *-16*, *-3*, and *-5*, are associated with differential intake of vegetables, such as brassica vegetables, coffee, and alcohol (Chamoun et al. 2018; Hayes et al. 2011). Polymorphisms in the sweet taste receptor gene *TAS1R2* are associated with sugar and carbohydrate consumption (Chamoun et al. 2018; Eriksson et al. 2019; Reed and McDaniel 2006). Variations in CD36, a candidate fat taste receptor, are associated with fat intake (Daoudi et al. 2015). Vegetables contain various biologically active compounds that have anti-inflammatory activities, whereas high-fat and high-sugar diets contribute to gut inflammation (Hou et al. 2011).

Given the well-established roles of taste receptors and signaling elements (e.g., Gnat3 and TrpM5) in sensing and responding to nutrients and bacterial-derived compounds in nongustatory tissues (Jang et al. 2007; Kokrashvili et al. 2009; Lee et al. 2014b; Tizzano et al. 2010), it is conceivable that taste receptor signaling pathways may also regulate gut inflammation through mechanisms other than influencing dietary choices. This notion is supported by our recent research in animal models. We have found that genetic knockout of Gnat3 leads to aggravated gut

inflammation in the dextran sulfate sodium (DSS)-induced IBD model in mice (Du et al. 2018; Feng et al. 2018). In this study, Gnat3-knockout and wild-type control mice were fed the same diet (regular rodent chow). Mice lacking Gnat3 showed more tissue damage and more immune cell infiltration in the colon than did wild-type control mice. Augmented gut inflammation was also observed in TrpM5-knockout mice in the DSS-induced IBD model (unpublished data), supporting that the Gnat3- and TrpM5-mediated taste receptor signaling pathway is involved in regulating gut inflammation.

Gnat3-knockout mice also had higher levels of the inflammatory cytokines TNF and IFN-γ but lower levels of IL5, IL13, and IL10 in the colon after DSS-induction (Feng et al. 2018). This type I versus type II cytokine imbalance suggests that lacking functional taste receptor signaling skews immune responses more toward type I cytokine-mediated inflammation, consistent with the role of gut taste signaling pathways in type 2 immunity (Howitt et al. 2016) and the anti-inflammatory effect of helminthic therapies (Cleenewerk et al. 2020; Rook 2012).

Due to the lack of tissue-specific knockout of Gnat3 and TrpM5, the tissues that require the function of Gnat3 or TrpM5 to regulate gut inflammation remain unclear. Interestingly, Kitamoto et al. (2020) showed that periodontal inflammation exacerbates gut inflammation in mouse models. Periodontitis-associated oral pathobionts, including *Klebsiella* and *Enterobacter*, can translocate to the gut and trigger gut inflammation. In addition, pathobiont-reactive Th17 cells migrate from the oral cavity to the gut, where they can be activated and contribute to the pathogenesis of IBD (Kitamoto et al. 2020). These findings, together with the protective role of taste signaling elements in periodontitis (Zheng et al. 2019) (see below), suggest that the anticolitis function of Gnat3 and TrpM5 may not be limited to the colon. Their roles in protecting gingival tissue inflammation may also be important for dampening gut inflammation.

Another related question as yet unresolved is which types of cells are critical for Gnat3/TrpM5-mediated regulation of gut inflammation. Tuft cells in the gut and SCCs in the gingiva are obvious candidates. Furthermore, as discussed below, some types of immune cells also express taste GPCRs and their signaling proteins, including Gnat3 and TrpM5. Whether these immune-cell-expressed taste signaling proteins are involved in the regulation of gut inflammation is unknown. Nevertheless, the taste signaling protein-mediated pathway provides a new angle for controlling gut inflammation. An in-depth understanding of the underlying mechanisms may lead to novel treatments for IBD.

Chronic gut inflammation is a risk factor for gastrointestinal cancers (El-Matary et al. 2014; Gonda et al. 2009; Rubin et al. 2012), suggesting that reduced function of taste receptors and signaling elements may be associated with cancer risk. Genetic polymorphisms in some *TAS2R* genes, including *TAS2R16* and *-38*, have been implicated in colorectal cancer risk in some studies (Barontini et al. 2017; Yamaki et al. 2017). However, it appears that the association between the polymorphisms of individual *TAS2R* genes and cancer is weak, and further studies are needed to draw firm conclusions. This is not entirely surprising, because humans have around 25 intact T2Rs (Devillier et al. 2015), and many of them recognize overlapping

sets of ligands. The effect of individual T2Rs on health or cancer is likely small. On the other hand, Gnat3 and TrpM5 are common signaling components of taste GPCRs, and manipulating their function may have much greater outcome, although experimental evidence is still lacking.

4 SCCs in the Airway Act as Sentinel Cells to Coordinate Host Defense

SCCs were described in fish some years ago. Around 2003, these cells were detected in rodents by immunostaining using antibodies against taste cell markers (Finger et al. 2003). Like taste bud cells, SCCs express taste signaling elements as well as taste receptors and depend on Pou2f3 for differentiation (Ohmoto et al. 2013). However, rather than being organized in a bud-like structure, they are instead scattered through the airway. Morphologically, these cells show packed apical microvilli, clear cytoplasmic vesicles, and cytoneural junctions. Functionally, SCCs appear to respond to bitter substances via SCC-expressed bitter taste receptors (Tizzano et al. 2010). SCCs are innervated by trigeminal neurons, which they can activate and thus evoke changes in respiratory rate.

Recently, with the demonstration of IL25 as a principal cytokine expressed in intestinal tuft cells, Cohen and his collaborators showed that SCCs in the human airway also express IL25 and secrete antimicrobial products to facilitate host defense (Kohanski et al. 2018; Patel et al. 2018). Particularly, SCCs in the airway may be involved in the pathogenesis of chronic rhinosinusitis with nasal polyps and asthma. A recent review article summarizes the molecular and functional features of SCCs in the airway (Sell et al. 2020).

A clear demonstration of the involvement of SCCs in airway innate immune responses came from a recent study by Perniss et al. (2020). They showed that SCCs sense virulence-associated formylated bacterial peptides and trigger mucociliary clearance through coordinated ciliary beating (Perniss et al. 2020). The innate response depends on taste signaling elements (e.g., TrpM5, Gnat3) but is independent of taste receptors. SCC-expressed acetylcholine appears to be a major effector for paracrine cholinergic signaling. Once again, the work showed that tracheal SCCs integrate chemosensation with innate defense. Yet, which receptor(s) in SCCs detects virulence-associated formylated bacterial peptides remains unclear.

5 Taste GPCRs and Their Downstream Signaling Elements in Gingival Tissues and Their Connections to Periodontitis and Dental Caries

A number of studies have shown that allelic variations in taste receptor genes are associated with dental caries (Arid et al. 2020; Eriksson et al. 2019; Haznedaroglu et al. 2015; Izakovicova Holla et al. 2015; Kulkarni et al. 2013; Robino et al. 2015; Wendell et al. 2010). Wendell et al. (2010) identified significant associations

between genetic polymorphisms in human *TAS2R38* and *TAS1R2* genes and dental caries scores. The *TAS2R38* alleles associated with dental caries protection have P (P49A), A (A262V), and V (V296I) amino acid substitutions, which correspond to taster alleles of the *TAS2R38* gene. People with PAV alleles of *TAS2R38* have high bitter taste sensitivity to phenylthiocarbamide (Drayna 2005). In contrast, nontaster alleles (AVI) of *TAS2R38* are associated with dental caries risk. Two single-nucleotide polymorphisms in the *TAS1R2* gene are also associated with either caries protection or risk (Wendell et al. 2010). Recent studies in several different countries have reported similar findings: allelic variations in *TAS1R2* are significantly associated with dental carries (Eriksson et al. 2019; Haznedaroglu et al. 2015; Izakovicova Holla et al. 2015; Kulkarni et al. 2013; Robino et al. 2015). Genetic polymorphisms in *TAS1R1* (Arid et al. 2020; Eriksson et al. 2019), *TAS1R3* (Haznedaroglu et al. 2015), and *GNAT3* (Eriksson et al. 2019) were also associated with increased incidence of dental caries.

Polymorphisms in taste receptors and signaling molecules can influence dietary choices, which may contribute to either caries protection or risk (Chamoun et al. 2018; Eriksson et al. 2019; Reed and McDaniel 2006; Wendell et al. 2010). Moreover, taste receptor signaling pathways, particularly T2R-mediated pathways, regulate antimicrobial responses in various types of cells by recognizing bacterial quorum-sensing molecules and evoking protective reactions (Lee et al. 2012, 2014b; Tizzano et al. 2010). Gil et al. (2015) showed that the *TAS2R38* gene is expressed in primary gingival epithelial cells, and the cariogenic bacteria *Streptococcus mutans* stimulate expression of *TAS2R38* in these cells. The taster alleles (PAV) of *TAS2R38* are induced to a higher level than the nontaster alleles (AVI). RNA interference experiments showed that T2R38 might be involved in innate immune responses, such as production of antimicrobial peptides and inflammatory cytokines when stimulated by *S. mutans* or by the periodontal pathogen *Porphyromonas gingivalis* (Gil et al. 2015). Allelic variations in *TAS1R1* and *GNAT3* genes were associated with oral microbiota profiles (Esberg et al. 2020). These studies suggest that, in addition to their effects on diet, polymorphisms in genes related to taste receptor signaling can affect oral microbial populations to influence dental caries and periodontal diseases.

Indeed, recently Zheng et al. (2019) reported that SCCs are present in mouse gingival epithelium and express multiple T2Rs and taste signaling molecules, including Gnat3, TrpM5, and PLCβ2. Similar to SCCs in other tissues, gingival SCCs depend on the transcription factor Pou2f3 for differentiation, because Pou2f3-knockout mice lack detectable SCCs in gingiva. One of the gingiva-expressed T2Rs, T2R105, can be activated by the bacterial quorum-sensing molecule acyl-homoserine lactone, as well as by the bitter compounds denatonium and cycloheximide. Gnat3-knockout mice showed accelerated alveolar bone loss, a hallmark of periodontitis (Zheng et al. 2019). The oral microbiota from Gnat3 knockouts were distinct from those in wild-type mice. More important, mice lacking SCC function, due to knockout of either Gnat3 or Pou2f3, developed more severe periodontitis induced by molar ligation. Periodontitis is associated with increased expression of inflammatory cytokines, and Gnat3-knockout mice showed much higher levels of

inflammatory cytokines induced by molar ligation than those in wild-type mice. Conversely, Gnat3 knockout mice showed decreased levels of the antimicrobial peptides β-defensin 1–3 and increased colonization of bacteria around the ligatures. Stimulation of gingival SCCs with denatonium protected wild-type mice against ligature-induced periodontitis (Zheng et al. 2019), demonstrating that T2R-mediated signaling protects gingiva from infection and inflammation.

6 Taste GPCRs and Their Downstream Signaling Elements in Immune Cells and Organs

The expression of taste GPCRs and their downstream signaling elements has been detected in various types of immune cells. Orsmark-Pietras et al. (2013), using microarray gene expression profiling, detected increased levels of multiple *TAS2R* mRNAs in blood leukocytes of children with severe, therapy-resistant asthma compared to healthy controls. Further analyses by quantitative PCR confirmed the findings, and the expression of *TAS2R13*, *-14*, and *-19* was significantly increased in leukocytes from severe asthmatic children (Orsmark-Pietras et al. 2013). In this study, bitter taste receptors showed higher levels of expression in lymphocytes than in neutrophils or monocytes. The bitter compounds chloroquine and denatonium inhibited the production of multiple cytokines induced by lipopolysaccharide (LPS) in blood cells. Malki et al. (2015) also detected the expression of T2Rs, as well as T1Rs, in human blood leukocytes. Their results suggest that different blood cell types, including monocytes, natural killer cells, B cells, T cells, and neutrophils, express varying levels of T2Rs and T1Rs (Malki et al. 2015). Immunohistochemical experiments suggest that T1R2 and T1R3 are expressed in large subsets of neutrophils and T and B cells. Expression of T2R43 and -38 was also detected in large subsets of neutrophils. Saccharin, a ligand for the T1R2/T1R3 sweet receptor and the T2R43 bitter receptor, induced chemotaxis of neutrophils, which can be partially inhibited by small interfering RNA to *TAS1R2*/*TAS1R3* and *TAS2R43*. Lee et al. (2014a) reported that mouse neutrophils express the heterodimeric umami taste receptor T1R1/T1R3. L-alanine and L-serine, ligands of the mouse T1R1/T1R3 receptor, stimulated chemotactic migration of neutrophils and inhibited LPS-induced release of TNF, CCL2, and IL10 (Lee et al. 2014a). Maurer et al. (2015) reported the expression of T2R38 in human neutrophils and proposed this as the receptor for the bacterial quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone (Maurer et al. 2015). Tran et al. (2018) reported the expression of T2R38 in T cells, and activation of T cells by anti-CD3/CD28 antibodies increased levels of T2R38. Goitrin, a ligand for T2R38, inhibited TNF secretion in peripheral blood mononuclear cells from donors with taster alleles of *TAS2R38* but not from those with nontaster alleles (Tran et al. 2018). Similarly, the expression of 16 *TAS2R* genes was detected in human lung macrophages; LPS treatments significantly increased the expression of several *TAS2R* genes, including *TAS2R7* and *-38*, and several bitter compounds inhibited cytokine production by lung macrophages stimulated by LPS (Grassin-Delyle et al. 2019).

Collectively, these studies, largely dependent on expression profiling, suggest that taste GPCRs may be functionally important in various types of immune cells. However, definitive studies are still lacking to understand the physiological significance of this expression pattern. Some of these studies lacked rigorous controls, such as specific knockout of taste receptors or their downstream signaling molecules, so it is difficult to rule out off-target effects of the taste compounds used in the studies. However, this is an important area of study, because taste compounds are ingredients of the everyday diet. Even small effects on immune function could result in significant benefit or harm in the long run.

Interestingly, taste GPCRs and downstream signaling elements are also detected in a subset of thymic medulla epithelial cells. By using transgenic reporter mouse strains, Panneck et al. (2014) first reported that some medullary thymic epithelial cells (mTECs) express ChAT as well as several proteins in the taste transduction cascade, including Gnat3, TrpM5, and PLCβ2 (Panneck et al. 2014). These mTECs express epithelial cell markers, such as cytokeratin-8 (CK8) and CK18, but not the T cell marker CD3. Villin, a tuft/brush cell marker, is also expressed by these thymic cells. This gene expression pattern is analogous to that of tuft cells found in other tissues, such as the intestine. Using Tas2r131-tauGFP transgenic mice, Soultanova et al. (2015) showed that these thymic tuft cells also express *Tas2R131*. RT-PCR also detected the expression of *Tas2r105* and *-108*. Double immunostaining showed that the thymus chemosensory tuft cells are distinct from the autoimmune regulator (AIRE)-expressing thymic epithelial cells involved in negative selection of T cells (Soultanova et al. 2015). Similar to intestinal tuft cells and airway SCCs, the TrpM5-expressing thymic epithelial cells depend on Pou2f3 for differentiation, and these cells are missing in Pou2f3 knockout mice (Yamashita et al. 2017). Two recent publications further confirmed the identity of these thymic tuft cells (Bornstein et al. 2018; Miller et al. 2018). More tuft cell markers were found in this subset of cells, including DCLK-1 and IL25. Compared to tuft cells in the small intestine, thymic tuft cells express several *Tas2rs* at much higher levels, including *Tas2r108*, *-138*, *-137*, *-118*, *-113*, and *-105*. Miller et al. (2018) showed that the thymic tuft cells do pass through an AIRE-expressing stage during development and have the ability to present antigens. Furthermore, TrpM5 is required for their function in supporting invariant natural killer T cell development and polarization. Bornstein et al. (2018) identified four subsets of mTECs by using scRNAseq and epigenetic characterization. They determined that mTEC IV cells are the thymic tuft cells, and their differentiation requires Pou2f3, consistent with tuft cells found in other tissues (Bornstein et al. 2018). Lacking thymic tuft cells resulted in increased levels of thymus-resident ILC2 cells. Again, these studies illustrate the important roles of taste GPCRs and their downstream signaling elements in immune regulation.

7 Conclusion

Taste signaling elements such as TrpM5 and Gnat3 mediate taste transduction in type II taste receptor cells. In some nongustatory tissues, SCCs or tuft cells that express such taste signaling elements or taste receptors appear to directly link chemosensation to immune responses and thus act as sentinel cells for multiple tissues. Some SCCs are also innervated, suggesting they may also trigger neuronal responses. In multiple types of immune cells, taste receptors and/or their signaling elements are expressed and appear to play regulatory roles in cytokine production and chemotaxis. Targeting the taste signaling elements in nongustatory tissues may provide therapeutic opportunities for many immune-related disorders.

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Assessment of Taste Function

Y. Zhu and T. Hummel

Contents

Abstract

Taste disorders, impacting well-being and physical health, can be caused by many etiologies including the use of medication. Recently, taste disturbance is also considered as one of the predominant symptoms of COVID-19 although its pathogenesis requires further research. Localized taste disorders may be overlooked considering that whole-mouth taste perception is insured through several mechanisms. Individuals often fail to discern taste from flavor, and

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interviews/surveys are insufficient to properly assess taste function. Hence, various taste assessment methods have been developed. Among them, psychophysical methods are most widely applied in a clinical context. Less-biased electrophysiological, imaging, or morphological methods are used to a much lesser degree. Overall, more research is needed in the field of taste.

Keywords

Taste · Gustatory · Mouth · Smell · Dysgeusia

1 Introduction

Taste disorders change our lives in many ways. On the one hand, patients with taste disorders have an increased risk to ingest rotten or spoiled foods and, on the other hand, they may lose enjoyment of foods which could further result in significant changes in eating habits (Clark 1998) and mental health, e.g., depression and anxiety (Bergdahl and Bergdahl 2002; Han et al. 2018; Hur et al. 2018). Changes in eating habits may promote malnutrition (Schiffman 1983), metabolic and cardiovascular disease (Sergi et al. 2017; Xue et al. 2020), or obesity (Nasser 2001). Overall, this may result in an impaired quality of life (Risso et al. 2020). But of course, taste impairment is not a life-threatening condition and receives less research interests compared with other sensory disorders. One fact is that isolated taste complaints which seem serious enough to bring patients to special clinics are much less common than, for example, smell disorders (Deems et al. 1991; Pribitkin et al. 2003). However, this does not mean that taste is negligible. On the contrary, taste sensations are so important that robust mechanisms have been developed to preserve them across the life span (Bartoshuk 1989). At first, taste receptor signals are transmitted by numerous cranial nerves; the facial, the glossopharyngeal, and the vagus nerves (Heft and Robinson 2014). Second, studies both in rats and humans support the idea of inhibitory connections among taste nerves (Bartoshuk 1989). For example, anesthetization of the chorda tympani nerve of the tongue could produce an increase in perceived taste intensity of the whole mouth (Ostrom et al. 1985). This concept suggests that, when a single taste nerve was injured, the whole-mouth taste perception may increase due to the loss of the inhibitory effect of one taste nerve on other taste nerves. Apart from that, taste perceived in one area of the oral cavity tends to be projected into the whole mouth (Todrank and Bartoshuk 1991). These mechanisms explain why localized taste dysfunction typically has negligible influence on whole-mouth taste perception. These mechanisms also explain why some people may overlook existing taste problems.

Estimates of the prevalence of taste dysfunction in the general population vary. They range from 0.84% to 74% (Deems et al. 1991; Pribitkin et al. 2003; Vennemann et al. 2008; Welge-Lussen et al. 2011; Correia et al. 2016) due to the use of regional or whole-mouth taste tests. Hence, taste disorders may be underestimated. They may surface when the gustatory system is confronted with other assaults. For example, drugs are among the main causes of taste disorders. A

study based on 408 patients with diminished taste capacity showed that 32% of the patients exhibited drug-induced taste loss. For people over 65 years old, the proportion rose to 47% (Ikeda et al. 2008). These numbers may also depend on the investigated sample. In patients presenting themselves to a tertiary Smell and Taste with diminished taste capacity (quantitative taste disorders) and also taste distortions (qualitative taste disorders) only 4% of 491 patients had a medication-induced taste disorder (Fark et al. 2013). Few drugs have been well investigated in that respect, for example the case of terbinafine, an antimycotic drug (Doty and Haxel 2005) or the case of vismodegib, a drug used in basal cell carcinoma that is not tolerated by some of the patients because of its side effects on taste (Xie and Lefrançois 2018). Considering this it appears necessary to devote more research to the area of taste.

In addition to drug-induced dysgeusia (Ackerman and Kasbekar 1997; Wickham et al. 1999), taste disorders can also be caused by various other etiologies such as systemic diseases (Schelling et al. 1965; Solomons et al. 1977; Atkin-Thor et al. 1978; Burch et al. 1978), zinc deficiency (Yoshida et al. 1991; Sakai et al. 2002), glossitis and stomatitis (Itoh et al. 2002), inflammation of the upper respiratory tract (Henkin et al. 1975), smoking (Schneller et al. 2018), alcohol consumption (Silva et al. 2016), radiation therapies (Deshpande et al. 2018), idiopathic factors (Yoshida et al. 1991; Sakai et al. 2002), and ageing (Doty 2018a).

Taste problems can also be an important sign of disorders such as stroke (Nakajima et al. 2010) and other central nervous system problems (Lang et al. 2006; Landis and Guinand 2009; Shah et al. 2009; Theys et al. 2009), or as a paraneoplastic symptom (Marinella 2008). Still, in most cases, due to the concomitant, more pronounced symptoms of other disorders, taste complaints are often overlooked (Landis and Heckmann 2013).

Recently, taste complaints have received worldwide attention as a frequently mentioned symptom of the coronavirus disease 2019 (COVID-19), with up to 80% of subjects reporting taste disturbances or olfactory-related changes (Cooper et al. 2020). In many cases, however, patients misinterpreted loss of retronasal olfaction/flavor as loss of gustatory function (Hintschich et al. 2020). Many viruses causing upper respiratory tract infections can cause impairments in chemosensation (Mäkelä et al. 1998). However, compared to acute cold patients, taste function seems significantly worse in people affected by COVID-19, particularly for bitter gustatory scores (Huart et al. 2020). This suggests that the pathogenesis of chemosensory impairments induced by COVID-19 could be distinct. It has been proposed that post-COVID19 taste dysfunction is due to interactions between viral S-protein and receptors (such as the angiotensin-converting enzyme 2 (ACE2) receptor) in taste receptor cells (Cooper et al. 2020; Singh et al. 2020) with more research evidence being required (Risso et al. 2020).

It becomes clear that it is important to use less biased ways to assess gustatory function. These various tools to assess gustatory function will be the focus of the present chapter. Nevertheless, there is a large body of literature on the measurement of taste sensations in response to suprathreshold stimulation using various scales, for

example visual analogue scales or labeled magnitude scales. A detailed discussion of these various tools can be found in Snyder et al. 2015.

In the present chapter we describe various methods to assess taste. We grouped them in four categories:

1. *Psychophysical methods*, the most mature and widely applied methods in clinic, are partly based upon interviewing patients and rely upon their cooperation. In cases of insufficient ability to cooperate – e.g., in children or patients with cognitive impairment or lack of motivation to perform the test findings may be difficult to evaluate.
2. *"Objective" tests* include electrophysiological methods and functional MRI. These procedures, granting a higher degree of objectivity, are technically more demanding and less practical than psychophysical tests and require the investigator's expertise, which are primarily used in experimental and legal contexts.
3. *Morphological tests* include the counting of taste papillae, contact endoscopy, and biopsies. The practical value of such tools on an intra-individual level has yet to be clarified. Based on biopsies, molecular localization tools can be utilized.
4. *Behavioral methods* indicate taste tests only used in animals, for example to offer insights into chemotherapy-induced taste disorders in humans.

The present chapter will focus on the various ways to assess taste function in humans and clinical populations, for example based on taste threshold measurements or the recording of gustatory event-related potentials. A specific focus lies on the various tools that can be used to deliver tastes to the tongue.

What also has to be mentioned is that taste does not work in isolation but that taste is closely related to somatosensation (e.g., Boucher et al. 2003; Simon et al. 2006; Just et al. 2007; Just and Hummel 2010; Miles et al. 2018; Berry and Simons 2020) and olfaction (e.g., Welge-Lussen et al. 2005; Welge-Lussen et al. 2009; Landis et al. 2010; Migneault-Bouchard et al. 2020). These aspects will not be discussed in this chapter.

2 Before Taste Testing

Every taste test in humans should be accompanied by a brief smell test and vice versa, as subjects are prone to mistake flavor and aromas for taste sensations, and therefore may mention taste problems instead of smell problems. For example, patients presenting with chemosensory dysfunction exhibit in most cases a smell disorder and very few of them show an isolated taste disturbance (Deems et al. 1991; Fujii et al. 2004). Background of this phenomenon is that most people use "taste" and "flavor" as exchangeable terms, as synonyms. However, smell loss may also be accompanied by loss of gustatory function possibly due to a loss of a central nervous interaction/amplification between the two systems (Gudziol et al. 2007; Landis et al. 2010; Migneault-Bouchard et al. 2020).

A brief inspection of the oral cavity also appears to be important. And of course, prior to applying a test or starting an examination, the patient's history has to be taken even if only briefly. A detailed introduction to this basic interview can be found with Welge-Luessen et al. (2013) or Bromley and Doty (2019). The "gustatory history" should include the following: previous surgery in the head and neck region, trauma, medication (Reiter et al. 2006; Doty et al. 2008), infections, Burning Mouth Syndrome, systemic diseases (such as renal insufficiency, diabetes, metabolic disorders, Sjogren's syndrome), or neurological or psychiatric diseases.

3 Psychophysical Tests

Generally, for psychophysical tests, at least three approaches should be differentiated: (1) Chemogustometry & Electrogustometry – based on the use of either chemical stimuli or electric stimuli. (2) Whole-mouth testing & Regional testing – based on taste tests applied to the whole mouth or distinct oral sites. (3) Taste threshold tests, Taste identification tests, and Taste intensity tests – based on their design principles and goals.

Representative and validated tests will be introduced below. One specific test can belong to more than one classification, e.g. electrogustometry would be applied in both regional testing and taste threshold tests.

4 Chemogustometry and Electrogustometry

In *chemical testing*, usually small amounts of taste substances are applied, e.g. presented by medicine droppers (Henkin and Larson 1972), sprays (Hummel et al. 2013), cotton swabs (Bartoshuk et al. 1983), or filter paper freshly soaked (Ikeda et al. 2005; Nagai et al. 2012) or impregnated and dried (Mueller et al. 2003; Landis et al. 2008) with tastants. After distributing liquids in the oral cavity, the sample is swallowed, and the mouth is rinsed. The ideal solution for rinse should remove the taste from the tongue without producing new taste sensations, it should be tasteless. Deionized water (Hoehl et al. 2010) might be preferred over tap water because electrolytes in tap water can affect taste (Burlingame et al. 2007). Others have suggested "artificial saliva," a tasteless solution (containing the main ionic components of saliva, 25 mM KCl + 2.5 mM $NaCO_3$). FMRI-based studies show that water activates taste-related brain areas in humans when contrasted to "artificial saliva" (de Araujo et al. 2003).

It appears to be an advantage of the filter papers, either soaked or impregnated with tastants that the paper allows localized stimulation of a certain site of the tongue or palate and that removal of the paper also removes the largest portion of the tastant. Hence, lingering of the taste may be less of an issue. One novel material (Smutzer et al. 2008) to present chemical tastants is pullulan (a polysaccharide polymer) combined with the polymer hydroxypropyl methylcellulose. Disks made of this material dissolve directly on the tongue and do not require removal after stimulation.

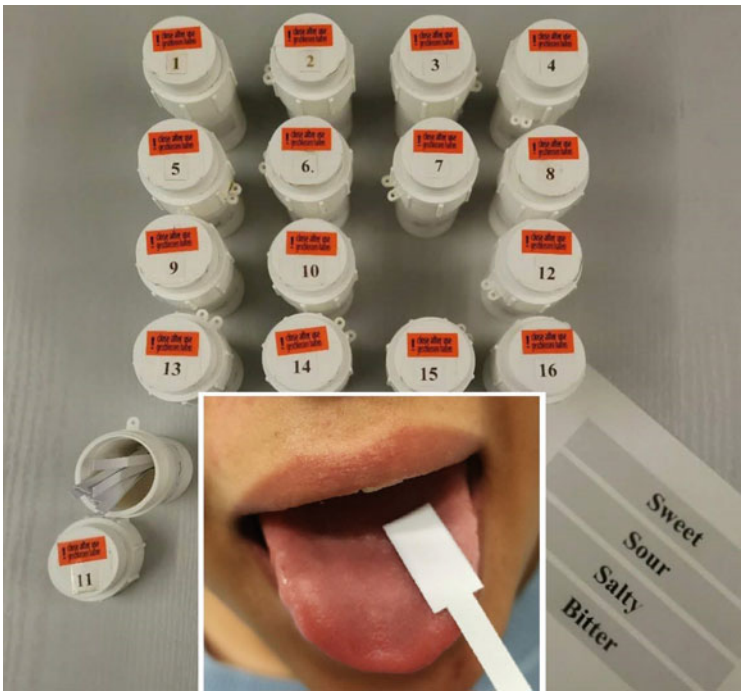

Fig. 1 Taste strips: filter paper strips impregnated with sucrose, citric acid, sodium chloride, and quinine hydrochloride in four concentrations each, to be placed upon the tongue's left or right side, with the patient being required to indicate the perceived taste in a list

Suprathreshold testing can be a simple screening tool which allows to quickly test a patient's ability to identify taste qualities, typically in the form of a forced choice from a list of four identifiers – sweet, sour, salty, and bitter. One example are taste sprays (Welge-Lussen et al. 2011; Hummel et al. 2013) using the natural substances sucrose (1 g in 10 g aqua; sweet), citric acid (0.5 g in 10 g aqua; sour), sodium chloride (0.75 g in 10 g aqua; salty), and quinine hydrochloride (0.005 g in 10 g aqua; bitter). Such suprathreshold testing is also easily possible with other test systems like the "taste strips" or the three drop test (Mueller et al. 2003; Landis et al. 2008; Pingel et al. 2010) (Figs. 1 and 2).

Threshold testing can be performed with the Three Drop Test (Henkin et al. 1963; Pingel et al. 2010) (compare Doty et al. (2001) and Doty (2018b)). Drops (volume < 0.1 ml) of either water or a taste solution are administered in groups of three, with each group consisting of two water and one taste drop, applied successively in a random sequence (O'Mahony 1995). The first group contains the lowest concentration of the tastant, and taste concentrations are increased throughout the test. Patients are asked to identify the tasting drop within each group. Using the ascending method of limits (Cometto-Muñiz et al. 1998; Ahne et al. 2000) the test aims at assessing the taste threshold which is established as the concentration identified three times in a

Fig. 2 Taste sprays: suprathreshold taste solutions of the basic tastes sprayed onto the tongue, to be identified as sweet, sour, salty, bitter, or umami. This test provides information whether the patient is able to recognize and differentiate different taste qualities

row (for normative data see Gudziol and Hummel (2007)). A "quasi threshold" is obtained with the taste strips (Mueller et al. 2003; Landis et al. 2008) which are presented at four concentrations per taste quality. The final score (number of correct identifications) is used to gauge gustatory sensitivity.

Further taste test systems have been suggested, such as "taste tablets" dissolving in the oral cavity (Ahne et al. 2000), thus releasing taste substances, or wafers impregnated with taste substances (Hummel et al. 1997). However, these tests are not commercially available. Recent developments (Abarintos et al. 2019; Epstein et al. 2020) are based on dissolvable material impregnated with tastant.

Electrogustometry (EGM), first introduced in the nineteenth century, has since been established as a reliable procedure to assess gustatory function (Neumann 1864) (see also Krarup (1958)). In this test, perception thresholds are obtained for electrical stimulation (microampere currents) administered to target regions via small disk-shaped electrodes. The sensation is "electric," typically described as sour. The technique is appealing because it does not require extensive preparations, there are no solutions involved, and it is highly transportable, has no expiration date, and easily allows stimulation of places that are difficult to reach. For example, placing liquids on the hard palate may be problematic whereas stimulation of the palate is easy with electrogustometry. The technique does not require rinsing between stimulus presentations (Fons and Osterhammel 1966; Frank and Smith 1991; Murphy et al. 1995; Tomita and Ikeda 2002; Walliczek-Dworschak et al. 2017). It is highly practical in regional testing. The stimulated area is very small,

unlike liquids the electric stimulus does not spread, and lingering of the stimuli does not take place. Using EGM Tomita et al. (1986) found in patients whose chorda tympani nerve had been severed on one side during middle ear surgery that the region of taste loss always was congruent with the lesion of the chorda tympani nerve. This was in contrast to the subjective complaints about taste loss. This study supported the clinical usefulness of EGM in the evaluation of the ear surgery in individual patients (Tomita and Ikeda 2002; Saito et al. 2012). Using EGM Tomita et al. also provided a topography of gustatory innervation of the oral cavity by chorda tympani, glossopharyngeal or greater petrosal nerves (Tomita and Ikeda 2002). In addition, EGM can help to detect mild taste loss which does not cause major complaints (Tomita and Ikeda 2002). It has been used to evaluate gustatory changes in association with diabetes (Le Floch et al. 1992), radiochemotherapy, chemotherapy, or radiotherapy (Berteretche et al. 2004; Pavlidis et al. 2015).

However, there are several drawbacks: the method does not permit to differentiate between taste qualities in gustatory disorders, accompanying trigeminal sensations may occur at high stimulus intensities. Hence, EGM cannot replace chemical taste testing. When measuring taste threshold, the correlations between EGM results and results of other tests were not consistent among studies (Murphy et al. 1995; Stillman et al. 2003). It appears inappropriate to use EGM alone to track recovery in patients with taste disorders as their chief complaint (Tomita and Ikeda 2002). In short, the major clinical use of EGM focuses on evaluating the injury of taste nerves resulting from certain conditions (i.e., ENT-related surgery (Le Floch et al. 1992)). In most conditions, however, performing both chemical testing and EGM appears to be an optimal strategy (Tomita and Ikeda 2002).

5 Whole-Mouth Testing and Regional Testing

Whole-mouth testing, in which tastants are usually sipped and then expectorated or swallowed by participants, is aimed at an overall assessment of taste function which, in comparison to regional testing, may better reflect what is actually experienced during eating or drinking (Hummel et al. 2013). However, to detect discrete localized taste deficits, regional testing is irreplaceable, where the stimuli are placed on defined regions of the tongue, can provide information as to whether a given taste nerve is dysfunctional (for the topography of gustatory nerve innervation on the tongue, please refer to Tomita and Ikeda (2002) or whether there are lateralized differences (Shikata et al. 2000; McMahon et al. 2001; Nordin et al. 2007; Guder et al. 2012; Pellegrino and Hummel 2020). Among regional testing, the 2-alternative-forced-choice procedure is widely used (e.g., Hong et al. (2005); Cheled-Shoval et al. (2017)). Patients are asked to decide which of two stimuli presented, for example, on the left and right side elicit a taste sensation. This method may also be used to assess taste thresholds.

For whole-mouth testing frequently liquids are employed (Henkin et al. 1963). After swishing the drop in the closed mouth, the subject is asked to identify its taste quality. Four verbal gustatory descriptors are presented (sweet, sour, salty, and

bitter). A multiple forced-choice procedure is used (Gudziol and Hummel 2007). Alternative procedures are the use of tablets of four tastants instead of liquid solutions (Ahne et al. 2000) applied at different concentrations. Other systems like edible wavers have also been applied (Hummel et al. 1997).

For regional testing, most tests based on liquids are problematic because the test solution is likely to be distributed all over the tongue/mouth. For this reason, electrogustometry has clear advantages in regional testing. Chemical testing using adequate stimuli is also practical in regional testing. "Filter discs" (which are soaked in liquid) (Tomita et al. 1986) or "taste strips" impregnated with tastants are applied in many laboratories worldwide (Grungreiff et al. 1988; Mueller et al. 2003; Nordin et al. 2007; Landis et al. 2009; Konstantinidis et al. 2010; Weiland et al. 2011). The taste discs are 5 mm in diameter (0.8 cm^2), the size of the stimulating area with the taste strips is 2 cm^2.

Frequently taste identification thresholds of each gustatory area are assessed using increasing concentrations of tastants. For example, when testing the function of the facial nerve patients are instructed to keep their tongues immobile in a slightly protruded position and the stimulants are placed on the tip of tongue until subjects indicate the perceived taste. The tongue's extended position during testing appears to be important to avoid distribution of the tastant outside the tested regions; as soon as the tongue is moved into the mouth, tastants will spread throughout the oral cavity (Manzi and Hummel 2014). These tests are reliable, for example, with the taste strip method a test-retest reliability of $r = 0.68$ has been reported, for the 3-drop method this figure is $r = 0.69$ (Mueller et al. 2003). In addition, because taste thresholds are elevated with aging (Bartoshuk et al. 1986; Fukunaga et al. 2005) and women tend to show better taste acuity than men (Ahne et al. 2000; Gudziol and Hummel 2007; Barragán et al. 2018), age and gender-related normative data for the taste strip test have been established (Landis et al. 2008). Regional taste tests are also available with liquid tastants (Pingel et al. 2010). For lateralized assessment of gustatory sensitivity, about 20 μL of liquid tastants is administered using a pipette. The test-retest reliability of this test has been reported with $r = 0.77$, age and gender-related normative data are available (Pingel et al. 2010).

6 Taste Threshold Tests, Taste Identification Tests and Taste Intensity Ratings

6.1 Taste Threshold Tests

The "absolute" threshold (detection threshold) for a stimulus is the lowest concentration at which its presence can be detected. The recognition threshold is the lowest concentration at which the quality of a stimulus (e.g., sweetness) can be correctly identified. There are still other threshold measurements. For example, the *difference threshold* indicates the smallest increase in suprathreshold stimulus concentration that can be detected, the "just noticeable difference." Numerous techniques are employed (Harris and Kalmus 1949; Höchenberger and Ohla 2017).

Chemical threshold tests establish the lowest chemical concentration of a tastant that can be perceived or recognized as a quality (Doty 2018b). Both measures reflect taste sensitivity.

To obtain a threshold, numerous psychophysical procedures have been developed, including the ascending method of limits (AML), the descending method of limits (DML) procedure (Harris and Kalmus 1949), staircase procedures analogous to olfactory threshold tests (Ehrenstein and Ehrenstein 1999; Linschoten et al. 2001), or adaptive techniques like logistic regression (Höchenberger and Ohla 2019), single-interval adjustment matrix (SIAM; 50% of presented stimuli are blanks, limited number of 20 or 30 stimulus presentations) or "QUEST"(Watson and Pelli 1983; Höchenberger and Ohla 2017, 2019). SIAM and QUEST are adaptive yes-no methods based on Bayesian statistics for relatively fast estimation of taste sensitivity (time per threshold 6–10 min).

Numerous factors can impact taste thresholds, including water temperature (Hahn and Günther 1933), intertrial intervals (O'Mahony 1983), stimulus volume (Grzegorczyk et al. 1979), stimulus duration (Bagla et al. 1997), rinsing between trials (O'Mahony et al. 1974), or the tested area (Doty et al. 2016). With many variables possibly impacting on taste thresholds, experimenters are advised to (1) adopt validated tests, (2) adhere to standard presentation protocols of these tests, and (3) make sure that the parameters mentioned above are kept consistent among participants and sessions.

Electrical threshold means the lowest electrical stimuli one can perceive using the electrogustometry, which has been reported to correlate with chemical thresholds (Ellegard et al. 2007; Berling et al. 2011).They are typically determined using ascending series of stimuli until subjects indicate a percept. Typically, these measurements are repeated several times and then the average of these trials is used a threshold estimate. As mentioned above, threshold values can be influenced by stimulus duration, electrode size and generally, threshold values are higher as electrode size becomes smaller (Frank and Smith 1991; Miller et al. 2002; Nakazato et al. 2002; Nicolaescu et al. 2005).

6.2 Taste Identification Tests

In taste identification tests, the task is to report which taste quality (i.e. sweet, bitter, sour, and salty) is perceived on a given trial. This is often performed in a forced-choice fashion (Welge-Lussen et al. 2011; Hummel et al. 2013) (but see also Tomita et al. (1986); Mueller et al. (2003)). According to Doty et al. after presentation of the stimulus, the subject points to a chart indicating whether the taste sensation is sweet, sour, bitter, or salty (Doty 2018b). In order to bypass the issues that may arise from the forced choice of the taste qualities comparisons between a taste and a tasteless stimulus are used (Petty et al. 2020). Participants receive pairs of solutions, with one solution containing tastant, and the other containing solvent. Subjects have to decide which one contained the tastant. Using the staircase technique a threshold estimate is established based on the average of the last 4 turning points (Pribitkin et al. 2003).

To control for false positives there should be no more than two dilution steps between two successive reversals.

During testing, different taste qualities are presented in a counterbalanced order with a rinse of water between trials. These tests have been used successfully, for example, in identifying side effects of drugs (Doty and Haxel 2005). For example, terbinafine, an oral antimycotic, is reported to induce taste loss in 0.6–2.8% of people taking the drug (Doty and Bromley 2004). Most cases recovered spontaneously 4–6 weeks after discontinuing the drug (Veraldi et al. 2016) although persistent taste impairment also has been reported (Bong et al. 1998). Very few studies evaluated taste loss with quantitative psychophysical tests. Using 96-trial taste identification tests, Doty et al. found that patients with terbinafine-related taste complaints showed significantly worse taste identification abilities than healthy controls (Doty and Haxel 2005). In addition, for these taste complaints they also showed that olfactory dysfunction did not explain the symptoms because olfactory function was comparable between patients and controls. Because aging is also a risk factor of terbinafine-related taste loss (Stricker et al. 1996), Doty et al. suggested that "physicians should be particularly on the alert for taste dysfunction in elderly persons taking this medication who may become depressed or alter their food intake in response to decreased taste sensation." However, regarding drug-induced taste disorders, (1) most reports are anecdotal and few clinical studies are available, (2) disease conditions per se could also cause taste disorders, (3) the taste disorders occur only in a small portion of patients who may also have other risk factors for drug-related taste disorders (e.g., age $>$ 65, low body mass index (Stricker et al. 1996)). It is difficult to definitively establish in which specific way drug-induced taste disorders affect the quality of life of patients. One case report mentioned that a patient stopped taking terbinafine immediately when she experienced a complete loss of taste. "Unfortunately, recovery was incomplete and for 3 years she continued to have difficulty tasting sugar and salt in her food," as recorded, "the patient has accepted her disability and is keen to continue leading a normal life" and declined further investigation/treatment (Bong et al. 1998). (For taste disorders associated with other drugs, please see Doty and Bromley (2004)).

Taste complaints are reported by some patients with Parkinson's disease (PD) (Chaudhuri et al. 2006), which is interpreted by a hypothesis that the nucleus tractus solitaries (NTS, a brainstem region associated with gustatory processing) is close to brainstem regions where Lewy body pathology first appears (Beckstead et al. 1980; Morita and Finger 1985). Doty et al. found the scores of taste identification test scores were on average lower for PD patients in early stage than for controls (Doty et al. 2015). Hence, taste testing may be of value in detecting early stage PD (Melis et al. 2021). In this context it has to be noted that many subjects consistently confuse the quality of suprathreshold tastants (Landis et al. 2008; Soter et al. 2008; Correia et al. 2016).

6.3 Taste Intensity Tests

Generally, such tests require participants to rate the intensity of suprathreshold tastants on scales. Simple Likert-type scales have categories like "tasteless," "slightly bitter," "bitter," "very bitter," and "extremely bitter" (Delwiche et al. 2001). These techniques are often used in a clinical context (Lindley et al. 1999; Sicchieri et al. 2019; Hald et al. 2021) because they are intuitive, easy to use, and not time-consuming. However, results from these scales can be statistically problematic (Bishop and Herron 2015). Among the scales used are visual analogue scales, the general labeled magnitude scale (Bartoshuk et al. 2004; Baker et al. 2020), or cross-modal matching methods (Weiffenbach et al. 1986; Saluja and Stevenson 2018) (for review Snyder et al. (2015) and Doty (2018b)).

7 Tests Based on Electrophysiology, Brain Imaging or Morphological Changes

Electrophysiological Methods Oral gustatory stimulation induces EEG changes in the form of cerebral electrical potentials, so-called Gustatory Event-Related Potentials, GERP (Kobal 1985). With this method, Wallroth et al. showed that the human gustatory system detects a taste faster than it identifies a taste. Hedonic evaluations of the taste appear to run in parallel and facilitate taste identification (Wallroth and Ohla 2018). In addition to the recording of cerebral potentials, peripheral potentials evoked by oral taste stimuli could also be recorded directly with electrodes positioned on the surface of the tongue (Melis et al. 2020). Melis et al. showed that each taste quality can generate its own electrophysiological fingerprint on the tongue. Compared to psychophysical measures they are less biased by the subjects' beliefs and motives. Compared with olfactory ERP, GERP are only available in very few specialized centers and primarily used in experimental and medico-legal contexts (Kobal 1985; Genow et al. 1998; Hummel et al. 2010). More studies need to be done before these techniques could be applied in routine clinical practice. Occasionally, *functional MRI* in response to gustatory stimulation may be performed (Hummel et al. 2007), but its value as a diagnostic tool in individual cases remains as yet to be demonstrated (Mestre et al. 2017; Monteleone et al. 2017; Wistehube et al. 2018; Shi et al. 2019) (Fig. 3).

8 Assessment of Morphological Changes

Microscopy There are several techniques to assess morphological characteristics in patients' tongues in relation to taste function. Using a confocal laser scanning microscope contact endoscopy has been employed to examine fungiform papillae (FP), for example following dissection of the chorda tympani (Just et al. 2006) or to monitor the changes of single fungiform papillae on the human tongue (Just et al. 2009) over the course of weeks and to measure the volume of the taste buds over

Fig. 3 Stimulation for the recording of gustatory event-related potentials. (**a**) Stimulating device to obtain gustatory event-related potentials (GERP) (Gustometer GU001; Burghart instruments, Wedel, Germany). The fast syringes (top) are operated to produce precisely defined amounts of liquid which is drawn from the bottles. (**b**) Subject with extended tongue at gustometer outlet during GERP recording. The tastants are sprayed onto the tongue embedded in a constant sprays of tasteless liquid (artificial saliva)

time in vivo (Srur et al. 2010). Srur et al. found, in healthy subjects, the volumes of some taste buds increase or decrease, whereas the volumes of other taste buds remain unchanged over a 10-week period. They also reported, in a patient with taste disturbance, that taste buds exhibit volumetric changes while the morphology (shape, diameter) of fungiform papillae did not change over 8 weeks (Srur et al. 2011). However, this experimental technique is available in few sites only.

Papilla Counting Counting the number of papillae is technically less demanding than contact endoscopy. In this context the validated technique established by Nuessle et al. (2015) has been shown to produce reliable results on the number of fungiform papillae, using the "Denver Papillae Protocol Dichotomous Key." The video component of this article can be found at http://www.jove.com/video/52860/. The number of papillae has been shown to vary, for example, as a function of age, sex, smoking behavior, body weight, or disease including diabetes or ear surgery (Fischer et al. 2013; Pavlidis et al. 2013, 2014a, b; Saito et al. 2016; Piochi et al. 2018; Walliczek-Dworschak et al. 2018). However, the relationship between the number of fungiform papillae and taste sensitivity appears to be complex. In which way their density explains oral taste dysfunction is still discussed (for a review, please see Piochi et al. (2018)).

9 Biopsies

In taste disorders, especially in drug-induced taste disorders, cellular composition of taste buds might be changed (Wang et al. 2017). For example, in taste disorders, type II taste receptor cells, which express receptors for sweet, umami, and bitter tastes, may become reduced in number and abnormal in shape. GNAT3, the alpha-subunit of the heterotrimeric alpha-gustducin protein, is a marker of type II taste receptor cells. Hence, biopsies (e.g., Astbäck et al. 1999) with molecular localization tools can be utilized to assess morphological changes and define the cell type. Biopsies are invasive, but can be done relatively easily in humans (Ozdener et al. 2012; Archer et al. 2016; Walliczek-Dworschak et al. 2017). They reveal, for example, different gene expression levels in fungiform papillae between lean and obese. They identified a consistent reduction in the expression of taste-related genes (in particular reduced type II taste cell genes) in the obese compared to the lean group (Archer et al. 2019). As mentioned earlier, type II taste receptor cells express receptors for sweet, umami and bitter tastes. Their reduced expression might cause an altered taste in obese patients. A very practical video protocol for fungiform papillae biopsies is available through Spielman et al. (2010). Still, most research on taste buds and taste receptors is focused on animal studies (Wang et al. 2017).

10 Behavioral Methods

Behavioral methods (Wang et al. 2017) are used frequently in animals, for example for hedonic evaluation (i.e., acceptability) of a food and discrimination of food-related chemical stimuli. Such methods include the two-bottle preference, taste reactivity tests, or the brief-access taste test (Table 1). In humans behavioral taste tests are less frequently applied with the exception of the alteration of breathing patterns through taste stimuli (Bitter et al. 2010; Gudziol et al. 2010). In this method, nasal respiration was measured with a differential pressure transducer while suprathreshold gustatory stimuli were presented to the participants. Significant pressure differences in the nasal cavity were measured between presentations of tastants (citric acid or saline) and blanks (water). A possible application of this method could be in medical legal questions. For example, when a person claims taste loss following head trauma and seeks financial compensation, this method provides an objective measurement of a gustatory deficit.

11 Why Not Umami?

Umami is often described as the taste of chicken soup. It has long been claimed by the Asian literature to be a basic taste quality (Ikeda 1909, 2002), whereas the occidental scientific community estimated umami mainly as a “taste enhancer.” This controversy has been clarified when monosodium glutamate receptors were

Table 1 Behavioral methods for assessing taste function in rodents

Name of test	Goal of test	Expensive equipment required?	Animals require training?	Surgery required?	Responses confounded by post-ingestive actions of stimulus?	Time required to measure response to a stimulus
Two-bottle preference test (Cai et al. 2013; Gaillard and Stratford 2016)	Hedonically evaluate stimulus	No	No	No	Yes	48 h
Taste reactivity test (Bice and Kiefer 1990; Arias and Chotro 2005)	Hedonically evaluate stimulus	Yes (e.g., video equipment)	No	Yes	No	~10 s
Brief-access lick test (Treesukosol et al. 2011; Glendinning et al. 2015)	Hedonically evaluate stimuli	Yes (e.g., lickometer)	Yes (3 days)	No	No	~30 min
Threshold sensitivity test (Koh and Teitelbaum 1961; Eylam and Spector 2004)	Identify the lowest detectable concentration of a stimulus	Yes (e.g., gustometer)	Yes (weeks–months)	No	No	Several days
Taste discrimination test (Ninomiya and Funakoshi 1989; Dotson and Spector 2007)	Determine whether animal can discriminate the taste of 2 stimuli	Yes (e.g., gustometer)	Yes (weeks–months)	No	No	Several days
Taste quality assessment (Mukherjee and Delay 2011)	Determine whether a stimulus tastes similar to a reference stimulus	Yes (e.g., gustometer)	Yes (weeks–months)	No	No	Several days

found on the tongue surface acting as specific taste receptors (Li et al. 2002; Nelson et al. 2002). Still, in most clinical tests umami is not applied (but see also Mueller et al. (2011)). This is based largely on the fact that many people are not familiar with "umami." For example, a recent investigation in 105 Germans and 97 Norwegians reported that only 7% of them were familiar with umami taste (Singh et al. 2010). Umami is one of the five basic taste qualities in mammals (Kinnamon and Finger 2019) and should be evaluated to comprehensively explore individual taste function. However, the results are difficult to interpret if patients are not familiar with this taste. Hence, whether umami should be included in clinical evaluations should depend on whether the patient recognizes/identifies umami. Because of this uncertainty, interpretation of umami-based taste tests in patients is problematic.

12 Summary

Taste disorders, impacting mental and physical health, are related to many etiologies and are frequently caused by drugs. Recently, taste disturbance is also considered as one of the predominant symptoms of the corona virus disease 2019 (COVID-19) although the pathogenesis needs further research. The presence of discrete, localized taste disorders may be underestimated considering that whole-mouth taste perception is insured through several mechanisms. Individuals often fail to discern taste from flavor, and interviews/surveys are insufficient to properly assess taste function. Hence, various taste assessment methods have been developed. Among them, psychophysical methods are most widely applied in a clinical context. Less-biased electrophysiological, imaging, or morphological methods are used to a much lesser degree.

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What Does the Taste System Tell Us About the Nutritional Composition and Toxicity of Foods?

John I. Glendinning

Contents

Abstract

One of the distinctive features of the human taste system is that it categorizes food into a few taste qualities – e.g., sweet, salty, sour, bitter, and umami. Here, I examined the functional significance of these taste qualities by asking what they tell us about the nutritional composition and toxicity of foods. I collected published data on the composition of raw and unprocessed foods – i.e., fruits, endosperm tissues, starchy foods, mushrooms, and meats. Sweet taste is thought

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to help identify foods with a high caloric or micronutrient density. However, the sweetest foods (fruits) had a relatively modest caloric density and low micronutrient density, whereas the blandest foods (endosperm tissues and meats) had a relatively high caloric and high micronutrient density. Salty taste is thought to be a proxy for foods high in sodium. Sodium levels were higher in meats than in most plant materials, but raw meats lack a salient salty taste. Sour taste (a measure of acidity) is thought to signify dangerous or spoiled foods. While this may be the case, it is notable that most ripe fruits are acidic. Umami taste is thought to reflect the protein content of food. I found that free L-glutamate (the prototypical umami tastant) concentration varies independently of protein content in foods. Bitter taste is thought to help identify poisonous foods, but many nutritious plant materials taste bitter. Fat taste is thought to help identify triglyceride-rich foods, but the role of taste versus mouthfeel in the attraction to fatty foods is unresolved. These findings indicate that the taste system provides incomplete or, in some cases, misleading information about the nutritional content and toxicity of foods. This may explain why inputs from the taste system are merged with inputs from the other cephalic senses and intestinal nutrient-sensing systems. By doing so, we create a more complete sensory representation and nutritional evaluation of foods.

Keywords

Flavor integration · Flavor-nutrient conditioning · Food composition · Intestinal nutrient sensing · Taste qualities

Among hunter-gatherers, the search for food is one of the most time-consuming activities (Thomas 2006). It is generally assumed that the taste system evolved to facilitate this search process, permitting rapid identification of nutritious foods and avoidance of toxic ones (Breslin 2013; Chaudhari and Roper 2010; Garcia-Bailo et al. 2009; Prescott 2012; Scott 2011; Yarmolinsky et al. 2009). In this essay, I present evidence that input from the taste system alone provides rather limited information about the chemical composition of foods. To understand the functional significance of the taste system, I propose that it should be viewed as part of a more extensive food evaluation system.

To illustrate this point, I will discuss the functional properties of each taste quality and highlight their limitations at analyzing the nutritional composition and toxicity of foods. Then, I will describe how input from the taste system interacts with sensory inputs from (1) the visual, olfactory, somatosensory, and auditory systems to create a rich and nuanced representation of foods; and (2) the intestinal nutrient-sensing systems to promote intake of nutritious foods. While human food choices reflect a number of factors (e.g., culture, religious-based taboos, socioeconomic status), the flavor and nutritional attributes of food play a central role (Ertmans et al. 2001; Forde 2018; Sørensen et al. 2003; Stevenson and Prescott 2014).

1 Do Taste Qualities Provide Meaningful Information about the Nutritional Value and Toxicity of Foods?

The word "taste" is often used synonymously with the multi-modal sensory quality called flavor. Here, I limit use of "taste" to sensory input that arises from taste buds located in the epithelium of the tongue, palate, and throat. As teeth pulverize foods, chemicals are released, dissolve in saliva, and then interact with taste bud cells. Many of these cells express a single class of taste receptor (e.g., one that binds sugars), providing a peripheral basis for categorizing food-related chemicals into different taste qualities (Liman et al. 2014; Yarmolinsky et al. 2009). The apical membranes of these taste bud cells (i.e., microvilli) extend into pores in the oral epithelium. Receptors on the microvilli bind to food-derived chemicals in saliva and activate transduction pathways within taste bud cells. These cells in turn release neurotransmitters onto nearby taste afferent neurons, which relay signals to the nucleus of the solitary tract (NST) in the brainstem (Chaudhari and Roper 2010). Neurons in the NST transmit signals to higher processing areas, which identify and evaluate foods in part according to their taste quality, intensity, and pleasantness (Spector and Travers 2005).

The human taste system senses a large number of food-derived chemicals and categorizes them into at least 5 taste qualities – sweet, salty, sour, bitter, and umami (Beauchamp 2019). Some investigators have argued for the existence of additional taste qualities for sensing fats (Running et al. 2015) and calcium (Tordoff et al. 2012). Here, I examine the functional significance of these taste qualities by asking whether they provide reliable information about the nutritional composition and toxicity of foods, using previously published data. To this end, I critically evaluate the hypotheses that: (1) sweet taste helps identify foods with a high density of calories and micronutrients; (2) salty taste identifies foods with a high sodium content; (3) sour taste (a measure of acidity) signifies dangerous or spoiled foods; (4) umami taste reflects the protein content of food; (5) bitter taste signifies poisonous foods; and (6) fatty taste identifies triglyceride-rich foods.

1.1 Approach

I examined foods in their natural state – i.e., raw and unprocessed – for two reasons. First, I wanted to gain better insight into the sensory experiences that hunter-gatherers would have encountered while sampling plant foods in nature. Second, I wanted to facilitate comparisons across different types of food. These comparisons would have been confounded if I included both processed and unprocessed foods. This is because many processing techniques transform a food so dramatically that its final appearance, composition, and flavor bear no resemblance to the original substance. For example, to make foods more digestible and safe, they are ground, fermented, soaked, boiled, or roasted on a fire (Johns 1990). To make foods more palatable, they are treated with seasonings (to intensify natural flavors) and flavorings (to modify natural flavors).

At this point, there is only general information about the nutritional contents of the foods eaten by hunter-gatherers (Berbesque et al. 2011; Gallois et al. 2020; Hart and Hart 1986; Milton 1991, 2000; O'Dea 1991; Schaefer 1981; Sinnett and Whyte 1973). As a result, I relied on the extensive nutritional data available for commercial foods. The limitation of these data is that they do not necessarily reflect what hunter-gatherers encountered in the past. For example, the concentration of nutrients and allelochemicals in commercial plants has been manipulated by horticulturalists and plant breeders since the dawn of agriculture to increase sweetness and reduce toxicity and bitterness (Johns 1990). Further, as a by-product of artificial selection for increased yield over the past 70 years, the concentration of micronutrients in many plant crops in the USA has decreased (Davis et al. 2004; Klee and Tieman 2018).

I examined several different types of foods. The plant foods included endosperm tissues (beans, seeds, and nuts), underground storage organs or USOs (roots, rhizomes, and tubers), fruits, and vegetative structures (vegetables). The fungal foods included mushrooms. The animal foods included meat from mammals, birds, and fish.

1.2 Sweet Taste

This taste quality is elicited by foods that contain high concentrations of sugars – e.g., monosaccharides (e.g., glucose, fructose) and disaccharides (e.g., sucrose and maltose). Sweetness motivates consumption by most mammals (Ramirez 1990), and is perceived as pleasant by humans within hours of birth (Steiner 1973). The attraction to sweet foods is widely assumed to help animals including humans identify calorically-rich foods (Chaudhari and Roper 2010; Garcia-Bailo et al. 2009; Liman et al. 2014; Prescott 2012; Scott 2011; Yarmolinsky et al. 2009). It may also promote intake of essential micronutrients (Nocetti et al. 2020; Patocka et al. 2020; Slavin and Beate Lloyd 2012; van Duyn and Pivonka 2000).

There are several observations that contradict the hypothesis that sweet taste evolved as a mechanism for identifying and promoting intake of calorically and nutritionally dense foods. First, some plant tissues (e.g., *Stevia*, *Thaumatococcus daniellii* and Monk fruit) contain compounds that taste sweet to humans, but lack caloric value (Gong et al. 2019; Lewis 1992; van der Wel and Loeve 1972). Second, another class of naturally occurring sweetener – polyols – cause bloating and diarrhea when eaten in excess (Oku and Okazaki 1996). Third, the most abundant digestible carbohydrates in plant tissues – maltodextrins and starches – do not taste sweet to humans. Maltodextrins elicit a weak "starchy" taste (Lapis et al. 2016) and starches fail to elicit any taste at all (Lapis et al. 2017).

If sweet taste served as a reliable cue for calorically dense foods, then the foods with the sweetest taste should have the highest caloric density. To test this prediction, I compared the caloric density (kcal/100 g tissue) of seven different types of foods (Supplementary Table 1). Among these foods, the only ones that impart a

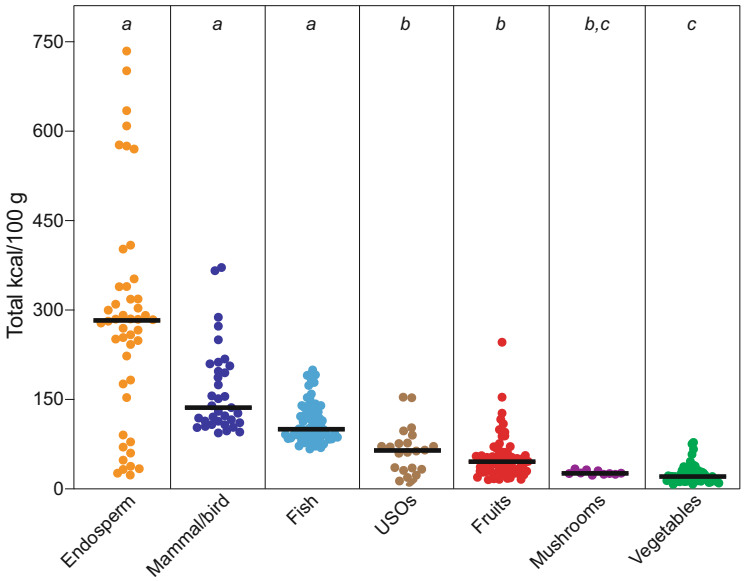

Fig. 1 Caloric density (kcal/100 g tissue) of foods commonly consumed by humans. The foods include endosperm tissues (i.e., beans, seeds and nuts, $n = 43$); meat from mammals and birds ($n = 35$); meat from fish ($n = 69$); underground storage organs, or USOs (i.e., roots, rhizomes, and tubers, $n = 22$); fruits ($n = 75$); mushrooms ($n = 9$); and vegetables ($n = 56$) (see Supplementary Table 1). To be included, the foods had to be raw and unprocessed. Within each panel, a circle corresponds to an individual food (e.g., avocado), and the horizontal line indicates the median value for a given food group. There was a significant difference in caloric density across the food groups (Kruskal-Wallis ANOVA statistic = 213.6; $P < 0.0001$). The food groups that differed from one another (Dunn's multiple comparison test, $P < 0.05$) are identified by unique letters (a, b, c) at the top of the panel

salient sweet taste are fruits, although it should be noted that some fruits lack a sweet taste (e.g., cucumbers, bell peppers, squashes and avocados).

Figure 1 illustrates the caloric density (kcal/100 g) of the different types of food. Endosperm tissues, mammal/bird meats, and fish meats had the highest caloric density; USOs and fruits had moderate caloric density; and mushrooms and vegetables had the lowest caloric density. Given that endosperm tissues and meats typically lack a sweet taste, these data refute the hypothesis that sweet taste serves as a reliable cue for calorically dense food.

Figure 2 shows the contribution of different macronutrients to the caloric content of different types of food. It is apparent that calories are derived primarily from starch and protein in endosperm tissues; protein and fat in animal tissues; starch in USOs; sugars in fruits; and a mixture of proteins, fats, starch, and sugars in mushrooms and vegetables. The predominance of sugar calories in fruits explains why so many of them have a sweet taste.

Is sweetness a cue for high micronutrient density? To test this hypothesis, I compared the concentration of 21 micronutrients in fruits with that in seven other

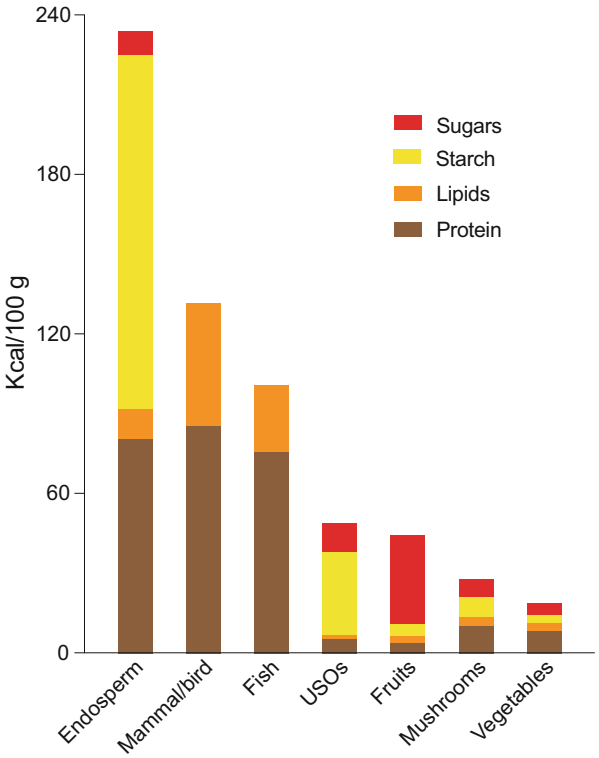

Fig. 2 Contribution of sugars, starch, lipids, and proteins to the calories present in seven different types food. The foods included endosperm tissues ($n = 43$); meat from mammals and birds ($n = 35$); meat from fish ($n = 69$); underground storage organs, or USOs ($n = 22$); fruits ($n = 75$); mushrooms ($n = 9$); and vegetables ($n = 56$) (see Supplementary Table 1). To be included, the foods had to be raw and unprocessed. The median caloric density (kcals/100 g) of each macronutrient is presented, separately for each food group

types of food (endosperm tissues, mammal/bird meats, fish meats, USOs, fruits, mushrooms, and vegetables). The results are presented as a heat map in Fig. 3. Endosperm tissue had a substantially higher concentration of most micronutrients than fruits, with the exception of niacin and vitamins A, C, and B12. Vegetables had micronutrient concentrations that resembled those in fruits. The other types of food – animal meats, USOs, and mushrooms – had micronutrient concentrations that were similar to or greater than those in fruits, with a few notable exceptions. They all had a relatively low concentration of vitamin C; the animal meats had relatively low concentrations of folate and manganese; and the mushrooms had relatively low concentrations of calcium and vitamin E.

Taken together, the data in Figs. 1, 2, and 3 reveal that the sweetest foods (fruits) have modest caloric and nutrient densities, while the blandest tasting foods (endosperm and animal tissues) have high caloric and nutrient densities. These findings, together with others (Mattes 2021; Ramirez 1990; Simko 2019), debunk the hypothesis that sweet taste is a reliable cue for identifying energy- and nutrient-rich foods in nature. They also help explain why hunter-gatherers and forager-horticulturalists relied on endosperm and animal tissues as their staple foods (Berbesque et al. 2011; Gallois et al. 2020; Hart and Hart 1986; Johns 1990; Laden and Wrangham 2005;

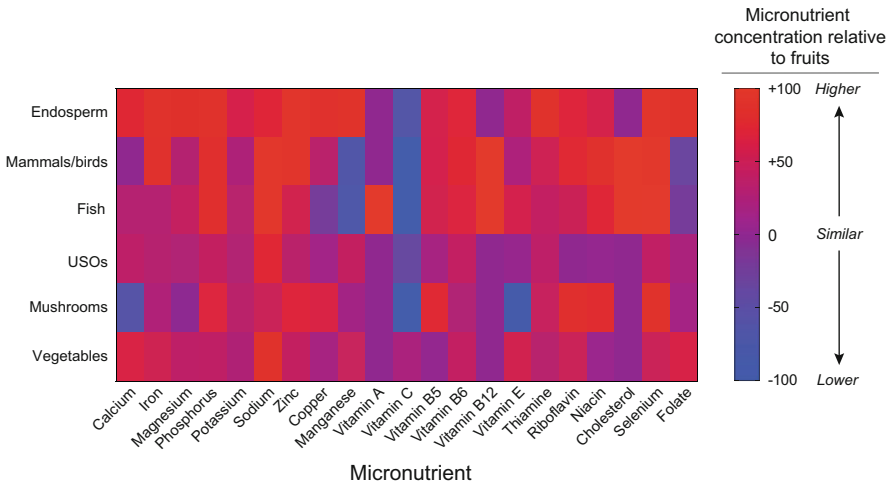

Fig. 3 Heat map comparing the concentration of 21 micronutrients in each food group (endosperm tissues, mammal and bird meats, fish meats, underground storage organs, mushrooms, and vegetables) to that in fruits. The heat map incorporates data from 43 endosperm tissues; 35 mammal and bird meats; 69 fish meats; 22 underground storage organs, or USOs; 75 fruits; 9 mushrooms; and 56 vegetables (see Supplementary Table 1). All foods were raw and unprocessed. For example, to normalize the concentration of calcium vegetables to that in fruits, I used the following equation: [(median mg calcium in vegetables/100 g – median mg calcium in fruits/100 g) / (median mg calcium in vegetables/100 g + median mg calcium in fruits/100 g)] x 100. A value near +100 (coded as red) indicates a substantially higher calcium content in vegetables relative to fruits, whereas a value approaching −100 (coded as blue) indicates a substantially lower calcium content in vegetables relative to fruits. Values that fall in between +100 and − 100 are coded as varying shades of purple

Milton 1991, 2000; Murray et al. 2001; O'Dea 1991; Schaefer 1981; Sinnett and Whyte 1973; Thomas 2006).

I do not mean to imply that the attraction to sweet foods is maladaptive. Human milk has a sweet taste (McDaniel et al. 1989) and thus likely stimulates feeding by infants. Fruits can provide a calorie bonanza when in abundance – e.g., at a fruiting tree. Likewise, honey is one of the most energy dense foods in nature (Murray et al. 2001) and has served as a critical food resource for hunter-gatherers who live in areas where it is locally abundant (Hart and Hart 1986; Huntingford 1955; Marlowe et al. 2014; Wrangham 2011). In several parts of Africa, hunter-gatherers even developed a symbiotic relationship with a species of bird, the greater honeyguide (*Indicator indicator* Sparrman). Trackers follow the honeyguides to bee hives and then reward the birds with honeycomb (Isack and Reyer 1989). Finally, cave paintings in central Sahara, Zimbabwe, and South Africa suggest that humans have been raiding bee hives from time immemorial (Pager 1973).

Another adaptive feature of sweet taste is that it promotes intake of nutritious foods that contain compounds which are distasteful but otherwise harmless (Beauchamp 2016). For example, lettuce varieties differ in their relative

concentration of sugars versus bitter-tasting sesquiterpenoid lactones (SLs). The varieties that were rated most palatable by human subjects were the ones with the highest ratio of glucose to SLs (Chadwick et al. 2016). In this case, the sweet taste of the glucose diminished the bitter taste of the SLs. Complementary findings have been reported in laboratory rodents (Formaker and Frank 1996; Vogt and Smith 1993) and humans (Kroeze and Bartoshuk 1985), using pure taste stimuli. In these studies, the sweet taste of sugars suppressed the bitter taste of quinine. A more recent study reported that the sweet taste of sucrose suppresses not only the bitter taste of quinine, but also the sour taste of citric acid and salty taste of NaCl in humans (Green et al. 2010).

1.3 Salty Taste

Salt (sodium chloride) is one of the oldest and most widely used seasonings and preservatives. It is also an essential mineral for maintaining osmotic homeostasis and cell membrane potentials (Michell 1989). Throughout history, however, humans have struggled to meet their sodium demands for three reasons. First, they include large amounts of plant material in their diet, which typically contains relatively low quantities of sodium (Fig. 4). There are exceptions to this pattern, as some vegetables have sodium concentrations that are comparable to those in many meats (Fig. 4). Second, humans lose sodium not only in urine and sweat, but also during pregnancy and lactation. This sodium loss can create physiological stress, particularly in hot and arid regions (Blair-West et al. 1968). Third, salt deposits are rare in nature, and the large ones are usually deep underground. While the oceans contain unlimited quantities of salt, it is time-consuming to remove salt from the water and many people live far away from the ocean.

When mammals are deficient in sodium, they exhibit a strong and specific hunger for sodium. This was illustrated vividly in a study of wild Australian rabbits that lived in areas with low-sodium vegetation (Blair-West et al. 1968). When different types of salt-soaked wooden stakes were placed in the ground, the rabbits gnawed avidly on stakes treated with sodium salts (NaCl and $NaHCO_3$) and largely ignored stakes treated with non-sodium salts (KCl and $MgCl_2$) or distilled water. There are also reports of wild and domesticated animals actively searching for sodium, particularly when sodium depleted (Shulkin 1991).

Even when humans are replete with sodium, they are attracted to sodium-containing foods (Beauchamp 1987). This is thought to reflect both direct and indirect actions of salt on taste. It can act directly by imparting a pleasant salty taste. Sodium may also act indirectly by blocking bitter taste, and thereby accentuate the more palatable flavor components in vegetables (Breslin and Beauchamp 1997) or enhancing the sweetness of some foods (Yasumatsu et al. 2020).

There are still many unanswered questions, however, about how salt enhances the flavor of foods. For instance, food manufacturers add salt to breads and cereals at concentrations that both enhance flavor (Lynch et al. 2009) and increase consumer preference (Antúnez et al. 2017), but nevertheless fail to elicit a salient salty taste.

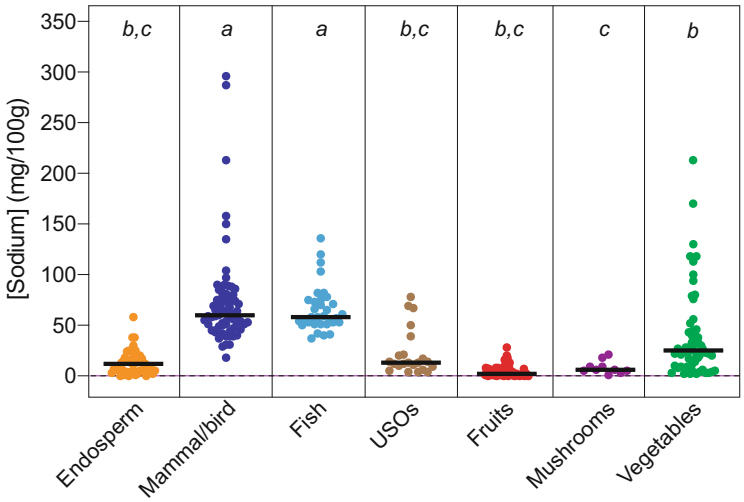

Fig. 4 Sodium concentration (mg/100 g) in foods commonly consumed by humans. For each food group, a circle corresponds to an individual food (e.g., tomato), and a horizontal line indicates the median value (see Supplementary Table 1). The foods included fruits ($n = 74$), vegetables ($n = 57$), endosperm (i.e., beans, seeds, and nuts, $n = 46$), underground storage organs, or USOs (i.e., roots, rhizomes and tubers, $n = 22$), mushrooms ($n = 10$), mammals and bird meat ($n = 33$), fish meat ($n = 69$). The medians differed significantly from one another (K-W statistic = 195.8, $P < 0.0001$), according to a Kruskal-Wallis nonparametric ANOVA. The food groups that differed from one another (Dunn's multiple comparison test, $P < 0.05$) are identified by unique letters (a, b, c) at the top of the panel

When the same foods are prepared without salt, they are described as bland and flavorless (Lynch et al. 2009). These observations reveal that even though salt is a critical component of the flavor of bread and cereals, it is not consciously perceived. This finding may be relevant to the attraction of humans to meats. This is because even though meats have a higher sodium content than most plant materials, they nevertheless lack a salient salty taste (in their raw and unprocessed form). It may be that there is enough sodium in meat to make its flavor attractive, but not enough to make it taste salty.

1.4 Sour Taste

Foods that elicit sourness contain organic acids. Humans (Liem and Mennella 2003) and other animals (Jacobs 1978; Laska et al. 2000; Shumake et al. 1971; Zhao et al. 2003) typically avoid eating foods with a high concentration of organic acids (e.g., raw lemons), although there are some exceptions, including a subset of children who enjoy extremely sour substances (Liem and Mennella 2003). It has been hypothesized that the avoidance of concentrated organic acids evolved as a

protective mechanism (Bushman et al. 2015; Valentová and Panovská 2003; Zhang et al. 2019). This hypothesis is based on the observations that acidic foods can cause dental erosion and tooth sensitivity (Saads Carvalho and Lussi 2020), and that the acidity (i.e., sourness) (Aubert et al. 2003; Hijaz et al. 2020; Hossain et al. 2014) and toxicity (Barceloux 2009; Shrivastava et al. 2017; Singh et al. 1992) of fruits tend to be highest when they are unripe. Thus, by avoiding foods with a sour taste, humans and other animals should reduce their chances of getting sick and dissolving their teeth.

Despite the potential dangers of sour foods, many humans (Rozin 1973) and other primates (Glaser and Hobi 1985; Laska et al. 2000) avidly consume foods and fluids with a sour taste. Indeed, humans have been purposefully fermenting foods (e.g., dairy products and vegetables) for many thousands of years as a method of food preservation. The fermentation imparts a sour taste because it increases the concentration of organic acids (e.g., lactic acid), which inhibit the growth of spoilage- and disease-causing microorganisms. That people actually enjoy the taste of these fermented foods is supported by the observation that even after the development of refrigeration, many societies continue to consume fermented foods like yogurts, cheeses, and pickled vegetables. They also continue to add acidic substances like vinegar, lemon, and lime juice to their foods. In fact, the sour taste of limes is one of the primary flavor principles of Mexican cuisine (Rozin 1973).

How can we explain the widespread consumption of sour foods? One way to approach this question is to compare the acidity (or pH) of different types of food. For example, most foods (i.e., mushrooms, USOs, vegetables, endosperm tissues, and fish) have relatively neutral pH values, ranging between 5 and 7 (Fig. 5a). This explains why these foods typically lack a sour taste. Fruits are the only group of foods that deviate from this pattern. Many of them have pH values that range between 2.5 and 4.5, making their tissues sour tasting. Their low pH reflects the presence of organic acids, including malate, citrate, quinate, and ascorbate (= vitamin C). The production of these acids helps preserve fruits by retarding growth of microorganisms.

Because humans cannot synthesize vitamin C, owing to the loss of a functional gluconolactone oxidase gene, they must obtain it from dietary sources to avoid developing scurvy (Sato and Undenfriend 1978). Based on this fact, it has been hypothesized that the attraction to sour foods represents an evolved mechanism for promoting consumption of foods containing vitamin C (Breslin 2013). While intriguing, this hypothesis is undermined by several observations.

1. The sourness of a fruit does not reliably predict its concentration of vitamin C. This is because two factors obscure any relationship between sourness and vitamin C concentration: (1) malate and citrate usually contribute more substantially to the pH of fruits than ascorbate (Colaric et al. 2005; Ikegaya et al. 2019; Kapur et al. 2018; Lim et al. 2017; Marsh et al. 2004; Woznicki et al. 2017), and (2) the sugars in fruits inhibit their sour taste to varying degrees (Colaric et al. 2005; Green et al. 2010).

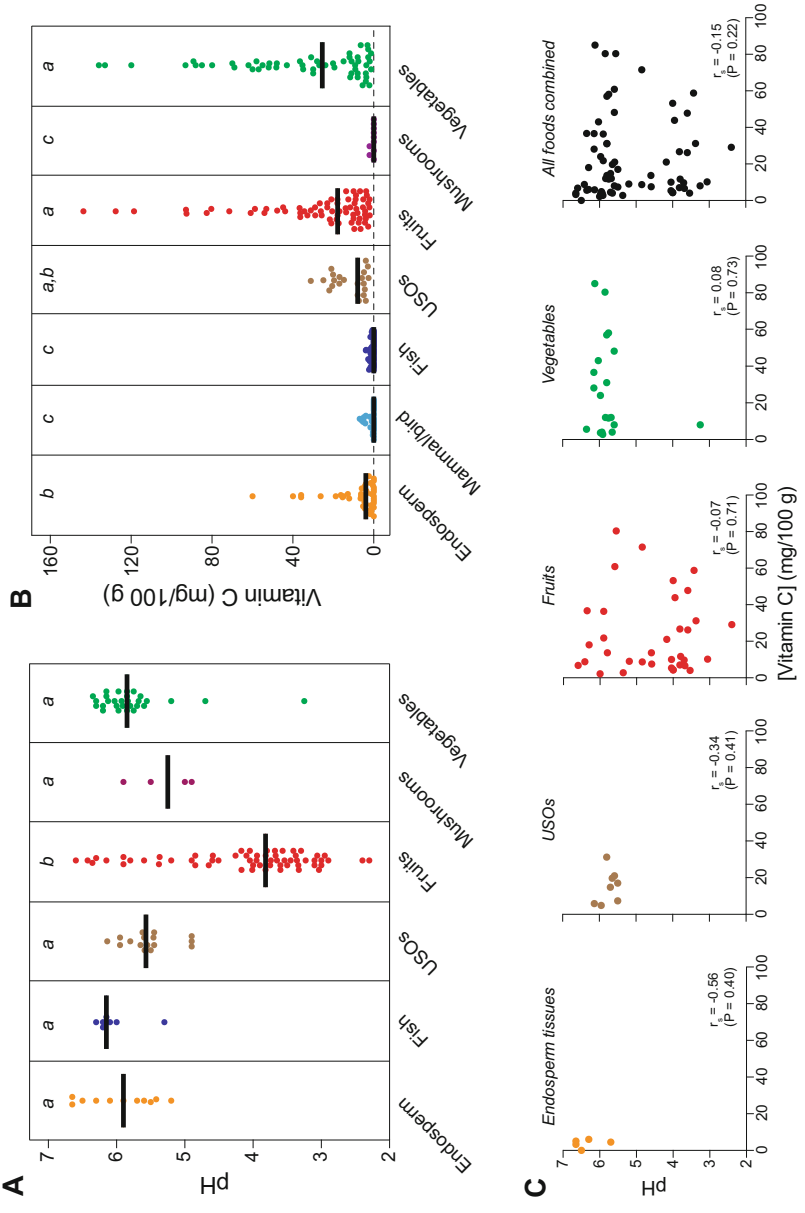

Fig. 5 Analysis of the (**a**) acidity and (**b**) vitamin C content of foods commonly consumed by humans. The acidity (pH) data are taken from Supplementary Table 2, and the vitamin C data from Supplementary Table 1. For each food group, a circle corresponds to an individual food (e.g., tomato), and a horizontal line

2. Fruits are not the only foods that contain vitamin C. Vegetables and USOs (to a lesser extent) contain vitamin C at concentrations that are comparable to those in fruits (Fig. 5b). The leaves of tropical trees also contain vitamin C at concentrations that exceed those in fruits (Milton and Jenness 1987). While the pH of tropical-tree leaves is unknown, the pH of most vegetables and USOs is relatively neutral (Fig. 5a), indicating that most would lack a sour taste altogether.
3. In Fig. 5c, I show scatterplots of pH x vitamin C concentration for individual foods. Each panel shows data from a different food group (i.e., endosperm tissues, USOs, fruits and vegetables). I analyzed data from the food groups both alone and in combination. There was no significant covariation between pH and vitamin C concentration for any food group, alone or in combination.

Taken together, these observations indicate that sour taste intensity is an unreliable proxy for vitamin C content of foods. So, what is the adaptive significance of the human attraction to sour foods? Given that vitamin C-deficient mammals do not appear to develop a specific hunger for vitamin C (Smith and Balagura 1975), it is unlikely that they can distinguish foods with high or low vitamin C content based on postoral mechanisms. Instead, humans may have evolved a simple heuristic for avoiding scurvy – eat anything that tastes mildly sour. This generalized attraction to mildly sour foods would increase the odds of ingesting foods containing vitamin C. It would also increase the range of available foods for a hunter-gatherer, given that sourness is a common taste among wild plants (Dénes et al. 2012; Dogan 2012; Nishida et al. 2000; Schaller 1963; Svanberg 2012; Svanberg and Ægisson 2012). Indeed, children that preferred sour tasting substances were found to be less fearful of novel foods and more willing to sample a greater variety of fruits (Liem and Mennella 2003). Another potential benefit of the attraction to sour foods is that ingesting sour (but not sweet, salty, bitter, or umami) stimuli induces cognitive changes in humans that promote risk-taking (Vi and Obrist 2018). Based on this result, the authors speculated that the regular ingestion of sour foods would help people acquire new skills and cope with novel situations.

1.5 Bitter Taste

Because virtually all naturally occurring poisons taste bitter to humans, this taste quality is thought to have evolved as a mechanism for avoiding poisonous foods (Glendinning 1994). Indeed, bitter substances typically elicit a suite of aversive responses (e.g., tongue retraction, gaping, increased latency to swallow, and nausea)

Fig. 5 (continued) indicates the median value. Within each panel, there were significance differences across the medians (Kruskal-Wallis nonparametric ANOVA, $P < 0.0001$). The medians that differed from one another (Dunn's multiple comparison test, $P < 0.05$) are identified by unique letters (**a**, **b**, **c**) at the top of each panel. (**c**) Bivariate scatterplots of pH x vitamin C concentration for those food groups (endosperm tissues, USOs, fruits, and vegetables) for which there were available data. A separate plot shows data from all foods combined. The results of a Spearman rank correlation are provided within each panel

(Brining et al. 1991; Grill and Norgren 1978; Hollingworth and Poffenberger Jr 1917; Peyrot des Gachons et al. 2011; Steiner 1973; Travers and Norgren 1986) and negative affective responses (Bartoshuk 1978) in humans and other animals. The intensity of the aversive responses has been reported to increase during the first trimester of pregnancy in humans (Duffy et al. 1998; Nordin et al. 2004), when the developing fetus is most vulnerable to ingested poisons.

If bitterness provides a reliable measure of toxicity, then all bitter-tasting substances should be poisonous. However, this is not the case (Glendinning 1994). In a comprehensive analysis of compounds that were either known to taste bitter or predicted to taste bitter based on chemical structure, the majority of the compounds had relatively low toxicity, as indicated by oral LD50 in rats (Nissim et al. 2017). Further, many wild (Dogan 2012; Ghirardini et al. 2007; Katz et al. 2012; Liu et al. 2012; Pieroni et al. 2002) and some commercial vegetables (Drewnowski and Gomez-Carneros 2000; Pieroni et al. 2002; Sameca et al. 2019; Sandell and Breslin 2006) are laden with phytonutrients that impart a bitter taste. It follows that if humans rejected all bitter-tasting vegetables, they would unduly restrict their dietary options (Glendinning 1994).

To permit consumption of bitter (but harmless) foods, humans and other animals appear to have evolved at least two mechanisms. First, infants and children generally avoid all foods with a bitter taste (Mennella and Beauchamp 1998), but as they develop into young adults, the blanket aversion to bitter-tasting foods can weaken. Second, repeated sampling of a harmless bitter substance can make its taste more acceptable to animals (London et al. 1979; Mura et al. 2018; Warren and Pfaffman 1959) and humans (Moskowitz et al. 1975; Nor et al. 2021; Rouseff 1990), particularly if the intake is associated with positive postingestive feedback (Falk et al. 1999; Sclafani et al. 1996; Zellner et al. 1985). This acquired preference for bitter substances may have permitted hunter-gatherers to exploit a wider range of nutritious plant tissues.

It is also notable that some mammals actually prefer dilute concentrations of quinine over water (Pieroni et al. 2002; Vitazkova et al. 2001). This unconditioned attraction to a "bitter" stimulus may be adaptive in some circumstances. For example, it has been reported to function as a feedforward mechanism for chemoprophylaxis against parasitic infections in mice (Vitazkova et al. 2001).

1.6 Umami Taste

The prototypical umami taste stimulus is monosodium glutamate (MSG). Glutamate is one of the most common amino acids in plant and animal tissues. For glutamate to elicit umami taste, however, it must be in the free form – i.e., unbound from proteins (Kurihara 2009). Free aspartate also elicits an umami taste, but its taste intensity is about 4 times less than that of MSG at equimolar concentrations (Kato et al. 1989).

The nature of umami taste in humans is complex. For example, there is an ongoing debate about whether umami represents a singular taste quality, or whether it is derivative of the other taste qualities (Beauchamp 2019). Further, the sensations

elicited by MSG vary depending on whether it is presented in water or food. On the one hand, when MSG is presented in water, it elicits an aversive flavor across a wide range of concentrations (Beauchamp and Pearson 1991; Yamaguchi and Takahashi 1984). This flavor is poorly defined, but consists of a brothy, meaty, or savory taste, and a tactile sensation that imparts a vague sense of oral "fullness" (Beauchamp 2009). On the other hand, MSG increases the palatability of soups, mashed potatoes, stews and meats; but not of fruits, fruit juices, sweet baked goods, or cooked cereals (Bellisle et al. 1989, 1991; Cairncross 1948; Maga 1994; Okiyama and Beauchamp 1998; Yamaguchi and Takahashi 1984).

One cannot explain the intensity of umami taste in foods based solely on free glutamate concentration. This is because many foods also contain free aspartate and different types of ribonucleotides (e.g., inosine-5′-monophosphate, guanosine-5-′-monophosphate and adenosine-5′-monophosphate) (see Supplementary Table 3). The ribonucleotides are particularly relevant because they synergize with free glutamate in ways that dramatically intensify umami taste (Rifkin and Bartoshuk 1980; Yamaguchi 1967, 1991; Zhang et al. 2008). Predicting umami taste is further complicated by the fact that the cooking process can activate enzymes in raw meats and mushrooms, which increase the concentration of free glutamate, free aspartate, and ribonucleotides (Rotola-Pukkila et al. 2015, 2019).

Kikunae Ikeda, who originally isolated MSG from seaweed in 1908, hypothesized that umami taste evolved as a mechanism to facilitate the detection and consumption of protein-rich foods (Kurihara 1987). This hypothesis is still the prevailing explanation for the evolution of umami taste (Chaudhari and Roper 2010; Töle et al. 2019; Yarmolinsky et al. 2009). It is undermined, however, by several observations:

1. If free glutamate concentration reliably predicts protein concentration, then the concentration of these two nutrients should covary across foods. To test this prediction, I plotted the available data on the concentration of free glutamate and protein in different types of food. Figure 6a shows that the concentration of free glutamate is highest in endosperm tissues; intermediate in animal meats, USOs, and fruits; and lowest in mushrooms and vegetables. Figure 6b illustrates that the concentration of protein is highest in endosperm tissues and animal meats; and lowest in USOs, fruits, mushrooms, and vegetables. If free glutamate concentration was a reliable proxy for protein concentration, then the concentration of free glutamate concentration should have been substantially higher in the meats.
2. In Fig. 6c, I show scatterplots of the concentration of free glutamate and protein in individual foods. Each panel shows results from a different type of food (i.e., endosperm tissues, mammal/bird meats, fish meats, USOs, fruits, mushrooms and vegetables). I analyzed data from the food groups both alone and in combination. There were no significant correlations between the concentration of free glutamate and protein in any food group, except mushrooms. The concentration of protein increased significantly with that of free glutamate in mushrooms. The functional significance of the latter finding to flavor is unclear, however, given the low concentration of free glutamate and protein in mushrooms.

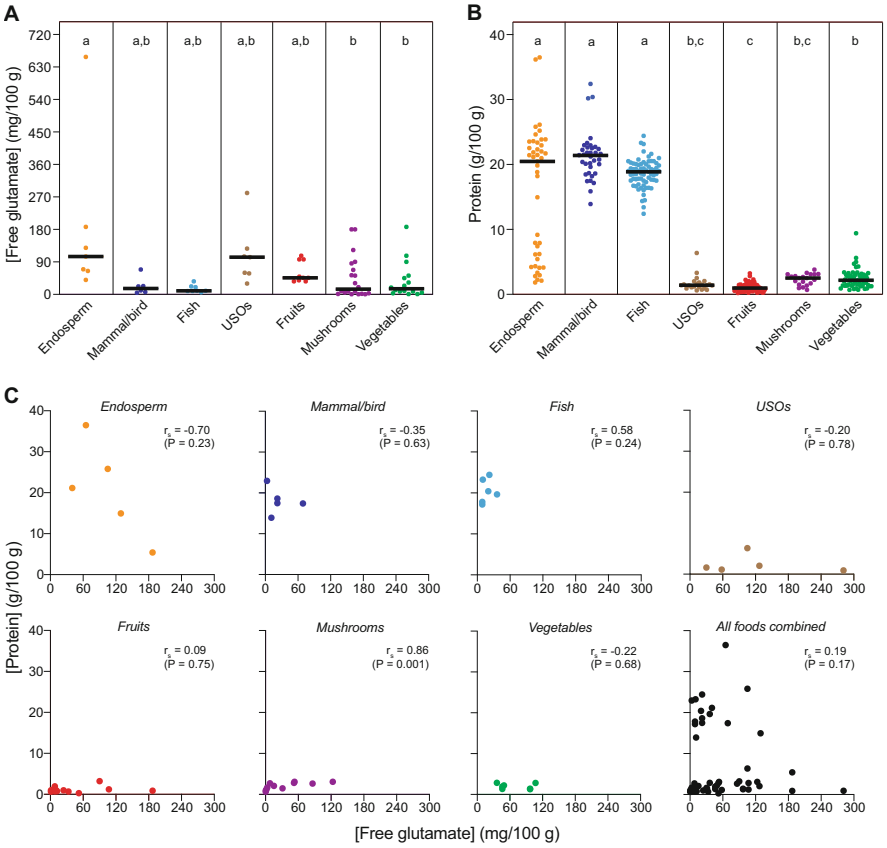

Fig. 6 Analysis of the (**a**) free glutamate and (**b**) protein content (mg/100 g fresh weight) of foods commonly consumed by humans. The food groups included endosperm tissues, mammal and bird meats, fish meats, underground storage organs (USOs), fruits, mushrooms, and vegetables. See Supplementary Table 3 for raw data and sample sizes. For each food group, a circle corresponds to an individual food (e.g., tomato), and a horizontal line indicates the median value. Within each panel, there were significant differences across the medians (Kruskal-Wallis nonparametric ANOVA, $P < 0.002$). The medians that differed from one another (Dunn's multiple comparison test, $P < 0.05$) are identified by unique letters (a, b, c) at the top of each panel. (**c**) Bivariate scatterplots of protein x free L-glutamate concentration for those food groups (endosperm tissues, mammal/bird mean, fish meat, USOs, fruits, mushrooms, and vegetables) for which there were available data. A separate plot shows data from all foods combined. The results of a Spearman rank correlation are provided within each panel

3. The umami taste intensity evoked by a variety of processed foods in human subjects does not reliably predict differences in protein content of the same foods (Buckley et al. 2018).
4. During the cooking process, Maillard reactions between amino acids and sugars create the dominant "meaty" flavor of cooked meats and the oxidation of lipids creates the distinctive odors of different types of meats (Shahidi 1994; Suleman

et al. 2020). These findings suggest that it is the Maillard and oxidation products, and not free glutamate and ribonucleotides, that give cooked meat its distinctive flavor.

These observations, together with another study (Hartley et al. 2019), contradict the claim that free glutamate taste provides a measure of the protein content of foods.

If umami taste is an unreliable proxy for the protein content of foods, then why did this taste quality evolve? And, why have humans been flavoring their foods with sauces containing high concentrations of MSG and ribonucleotides over the last two millennia (Curtis 2009; Mouritsen and Styrbaek 2014)? One hypothesis stems from the observation that the concentration of umami tastants increases as foods spoil (Ninomiya 1998). Accordingly, it is possible that the attraction to umami initially evolved as a mechanism for promoting consumption of partially spoiled foods (Breslin 2013). Throughout recorded history, humans have been subjecting foods to controlled spoilage as a method of preservation – e.g., curing and smoking meats; fermenting fruits, vegetables, beans, and dairy products. There is also evidence that hunter-gatherers regularly scavenged meat from carcasses (Thomas 2006) and that this partially spoiled meat represented a significant source of nutrients for them (Pobiner 2015). Spoilage not only makes foods easier to digest (Ranciaro et al. 2014), but it also increases the concentration of nutrients and probiotic bacteria (Chang et al. 2010; Won et al. 2011). Eating carcasses would also lower the cost and risk of meat procurement relative to hunting.

Umami taste differs from the other major taste qualities in one important respect – it lacks a clear and definable taste quality of its own. Given that its primary impact is to enhance existing flavors, perhaps umami taste does not actually function as a stand-alone taste quality. Instead, it may function as a generalized palatability enhancer, adding depth and hedonic appeal to the flavor of many foods (Breslin 2013).

1.7 Fat Taste

Edible fats and oils consist predominantly of triglycerides, but they also contain small quantities of mono- and diglycerides, essential fatty acids (linolenic acid and linoleic acid), phosphatides, sterols, fat-soluble vitamins, tocopherols, and fatty alcohols. Among the macronutrients, fats are the most calorically dense. According to the Atwater general factor system, fats contain 9 kcal/g, while carbohydrates and proteins contain only 4 kcal/g (http://www.fao.org/uploads/media/FAO_2003_Food_Energy_02.pdf). Accordingly, it is not surprising that humans have evolved the ability to distinguish foods with varying concentrations of fat (Mela 1988) and feed preferentially on the ones with the highest fat concentration (Drenowski and Greenwood 1983; Sobek et al. 2020). What remains unclear is how fats are sensed.

Most work on fat taste has focused on free fatty acids (FFAs), primarily linolenic and linoleic acid (Mattes 2009). A recent publication argued that the taste quality of FFAs is unique and called it “oleogustus “(Running et al. 2015). While humans

(Chalé-Rush et al. 2007; Running et al. 2015) and rodents (Mattes 2009) can both detect FFAs based on taste input alone, their hedonic responses to them are diametrically opposed. Humans are repelled by FFAs (Running et al. 2015) and rodents are attracted to them (Cartoni et al. 2010; Laugerette et al. 2005). Human subjects have described the oral sensations of linolenic acid and linoleic acid as "a complex, decidedly unpleasant, smooth, fatty tasting liquid which is rated strangling, nauseous, and slowly developing" (Schiffman and Dackis 1975). In light of these sensory characterizations, it is likely that FFAs occur at subthreshold concentrations in preferred fatty foods.

Edible fats and oils reliably alter the odor, appearance, and tactile properties of foods (Mattes 2009), but they do not elicit a salient taste quality in humans. This observation does not exclude the possibility, however, that fats still activate the human taste system. They may elicit a more limited type of taste input, which enhances the hedonic appeal of foods without actually eliciting a salient taste sensation (Spector and Glendinning 2009). In mice, there is evidence that the attraction to triglycerides is mediated at least in part by taste (Sclafani and Ackroff 2018). See chapter 13 in this book for more information on fat taste.

1.8 Calcium Taste

Calcium is critical for cell signaling and neurotransmitter release; and in vertebrates, it is the major component of bone. Natural sources of calcium include seafood, dark leafy greens, legumes, seeds, rhubarb, and the bone of vertebrate prey. Captive rodents and other wild animals show preferences for low but not high concentrations of calcium and regulate its intake according to physiological need (Tordoff 2001). In humans, calcium elicits a unique taste quality, consisting of bitter and sour taste components, which is reported to be mediated in part by the human sweet taste receptor (T1R3) (Tordoff et al. 2012). More work is needed to explain why the taste of calcium requires a functional T1R3 receptor, but nevertheless lacks any apparent sweetness.

In sum, the forgoing discussion indicates that the taste system contributes to the flavor of foods and offers some insight into their nutritiousness and toxicity. The evidence presented above, however, indicates that taste quality alone provides incomplete and, in some cases, misleading information about the nutritional value and toxicity of foods.

2 Taste Is Just One Component of Flavor

Food does not merely stimulate the taste system. It also stimulates the four other sensory systems in the head – vision, olfaction, oral/nasal somatosensation, and audition – resulting in multi-modal flavor percepts. The creation of flavor percepts provides a more detailed sensory representation of foods, and thus increases our ability to determine the chemical composition of foods. Below, I discuss how vision,

olfaction, oral/nasal somatosensation, and audition each contribute to the eating process.

Visual input helps us locate and procure foods in the environment. It also helps us evaluate the physical characteristics of food (size, shape, surface features, and color) and the extent to which a food's quality has deteriorated, owing to attack by microorganisms, insects, or larger animals (Köster 2002). Olfactory input largely complements the visual system by helping us discriminate foods and evaluate their quality and degree of spoilage (Bushdid et al. 2014; Köster 2002).

Input from the oral/nasal somatosensory system helps us evaluate both the tactile (shape, firmness, tenderness, consistency, succulence, juiciness) and chemesthetic (temperature, irritancy, pungency) properties of food (Green 2012; Simons et al. 2019; Szczesniak 1991; Viana 2011). These sensations are mediated by distinct branches of the trigeminal nerve, which innervate the oral epithelia (Roper 2014). Inputs from these distinct branches provide foods with some of their most intriguing qualities – e.g., the heat of chili peppers, coolness of menthol, tingle of Szechuan peppers and carbonation, velvety smoothness of chocolate, crispness of a potato chip, astringency (i.e., rough, "sandpapery" or dry sensation) of unripe fruits and red wines, and sharp stinging sensations of radishes, lemons, and pickles (Komai and Bryant 1993; Viana 2011).

Trigeminal inputs complement taste inputs in several ways. First, many naturally occurring compounds in plant tissues elicit rejection by activating T2R bitter taste receptors in taste bud cells and transient receptor potential (TRP) channels in trigeminal neurons (Startek et al. 2019). Second, human psychophysical (Mela 1988) and electrophysiological (Rolls et al. 2018) studies indicate that the dominant sensory component of fat flavor is the oily mouthfeel generated by triglycerides. For example, the responsiveness of neurons in the orbitofrontal cortex and amygdala of macaques to macaques vary as a function of the coefficient of sliding friction (CSF)[1] of triglyceride solutions (Rolls et al. 2018). Thus, given that edible fats and oils would decrease the CSF between the tongue and palate, it is possible that humans use this tactile property of triglycerides to assess the fat content of foods.

Sound also contributes to the flavor of foods, particularly if they are crisp or crunchy – e.g., celery and carrots (Spence 2015a). As foods are pulverized by the teeth, sound waves are transmitted through the jaw bone and skull via conduction and stimulate the cochlea directly (Vickers and Bourne 1976). The preference for "noisy" vegetables and fruits (Cliff et al. 2015) may have evolved as a simple heuristic for helping humans select the freshest and hence most nutritious foods (Szczesniak and Kahn 1971).

In sum, the integration of taste, vision, olfaction, oral/nasal somatosensation, and audition dramatically increases the ability of humans to accurately identify and discriminate foods. While this multi-modal sensory integration can alter the salience of taste input (Bartoshuk and Klee 2013; Spence 2015b; Verhagen and Engelen

[1]CSF is a measure of the force that opposes the motion of two surfaces sliding against each other (e.g., between the tongue and palate or the cheek and teeth).

2006), it is notable that the flavor of many natural foods is dominated by one or two taste qualities. For example, the dominant flavor component of many fruits is a sweet and sour taste (Klee and Tieman 2018; Ma et al. 2015; Mikulic-Petkovsek et al. 2012) while that of many wild vegetables is a bitter taste (Dogan 2012; Ghirardini et al. 2007; Katz et al. 2012; Liu et al. 2012; Pieroni et al. 2002).

3 Some Foods Promote Intake by Activating Post-Oral Nutrient Sensors

After food has been swallowed, its chemical composition is evaluated by an intestinal chemosensory system. This system consists of nutrient- and poison-responsive epithelial sensory cells, which convey information about chemicals in the gut to vagal and spinal sensory neurons (Kaelberer et al. 2018; Sclafani et al. 2016; Tan et al. 2020) and ultimately to the striatum (Han et al. 2018). There is evidence that this intestinal chemosensory system exists in both mice and humans (Buchanan et al. n.d.).

I provide an example of how intestinal nutrient-sensing modulates feeding in Fig. 7. In this case, mice were presented with two solutions – one contained a low-calorie sweetener (saccharin) and the other a sugar (glucose). The saccharin solution was substantially sweeter than the sugar (glucose) solution, as indicated by higher peripheral taste nerve responses (Fig. 7a) and higher initial rates of licking (Fig. 7b). However, when the mice were offered both solutions during a 24-h preference test, the mice consumed 3 times more of the glucose solution (Fig. 7c) (Glendinning et al. 2010). The higher glucose intake stems from the fact that glucose activated nutrient sensors in the small intestine, which stimulated robust consumption. This type of flavor-nutrient learning can help rodents learn to discriminate between flavored solutions that differ in nutritional value and feed selectively on the most nutritious ones (Sclafani et al. 2015).

To provide direct evidence that the flavor-nutrient learning is mediated by intestinal chemosensory mechanisms, investigators have fitted rats or mice with an intragastric (IG) catheter. In this experimental paradigm, an animal is offered two flavored solutions (e.g., grape and cherry), each during different training sessions. In a typical experiment, whenever the animal consumes one of the flavored solutions (e.g., grape), an equivalent volume of 8% sucrose solution is co-infused into its stomach. Whenever the same animal consumes the other flavored solution (e.g., cherry), an equivalent volume of water is infused into its stomach. After several training sessions, the rodent learns to associate the grape flavor with the post-oral actions of the sucrose solution. It subsequently consumes more of the grape solution and prefers the grape over the cherry solution. Using this approach, it has been established that IG infusions of sucrose (Sclafani and Glendinning 2005), fats (Sclafani and Glendinning 2005), proteins (Perez et al. 1996), and MSG (Ackroff and Sclafani 2011) can condition a preference for flavored solutions and that IG infusions of a bitter-tasting stimulus (Glendinning et al. 2008) can condition an aversion to flavored solutions. Indeed, this flavor-learning mechanism is so potent

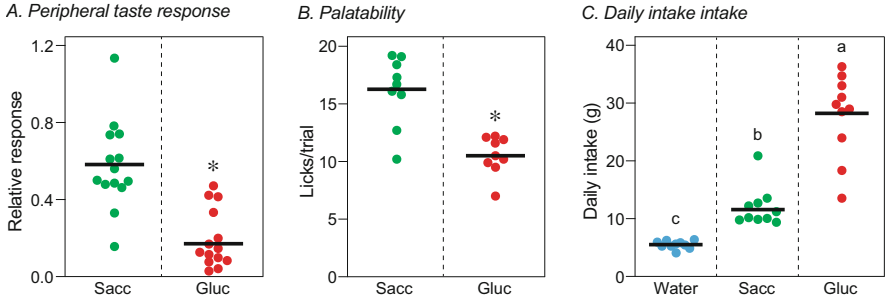

Fig. 7 Illustration of how the post-oral nutritive actions of sweeteners can alter the initial taste-mediated responses of C57Bl/6 mice. The sweeteners included 38 mM saccharin (Sacc) and 333 mM glucose (Gluc). In panel (**a**), I show peripheral taste responses to lingual stimulation with Sacc and Gluc. The peripheral taste responses are indicated by responses of the whole chorda tympani (CT) taste nerve, which innervates taste buds in the anterior tongue. CT nerve responses to each sweetener solution were normalized to the response to 100 mM NH_4Cl. I compare the relative response to each sweetener solution with paired t-test ($^*P < 0.0001$). In panel (**b**), I compare palatability of the Sacc and Gluc solutions to the mice. Licking responses of the mice to the sweetener solutions were obtained during brief-access lick tests. I compare lick rates for each sweetener solution with paired t-test ($^*P < 0.0001$). In panel (**c**), I show daily intakes of water, Sacc and Gluc during a two-bottle preference test, during which mice had a choice between a sweetener solution and water over 2 days. Because water intake during the preference tests with sweeteners was virtually nonexistent, it is not presented. These long-term preference tests are thought to incorporate any post-oral nutritive actions of the sweeteners. Different letters above bars (a, b, c) indicate means that differ significantly from one another (Tukey post hoc test; $P \leq 0.05$). Within panels (**a**–**c**), a circle corresponds to an individual mouse, and a horizontal line indicates the mean response. The data in panels (**a**–**c**) are taken from Glendinning et al. (2010)

that IG infusions of sugars can even condition preferences for inherently aversive solutions – e.g., ones containing sucrose octa-acetate, citric acid, or ethanol (Ackroff and Sclafani 2002; Myers and Sclafani 2003; Perez et al. 1998; Sclafani et al. 1996).

While it is possible to condition a preference for inherently aversive flavors (e.g., bitter tasting solutions), it is much easier to do so with inherently preferred flavors (e.g., sweet tasting solutions). This is because rodents ingest greater quantities of the inherently preferred solutions during training. The higher intake would increase the number of flavor-nutrient pairings, resulting in stronger stimulation of the post-oral nutrient sensors and hence more rapid flavor-nutrient conditioning. This observation reveals an underappreciated role of the taste system. It promotes intake of palatable and nutritious substances, thereby enhancing flavor-nutrient learning (Sclafani and Glendinning 2005).

The evidence for flavor-nutrient learning in humans is less convincing than in rodents. Despite several reports of successful flavor-nutrient learning in humans, these findings have been difficult to replicate in other laboratories (Yeomans 2012). The lack of replication most likely reflects the challenges of controlling for critical design features in experiments – e.g., the novelty of the conditioning stimulus, the quantity of nutrients ingested during training, the appetitive state, age, and prior dietary experience (Yeomans 2012).

4 Conclusions

This essay examined whether taste input provides accurate and reliable information about the nutritional composition and toxicity of foods. The available evidence indicates that taste input alone provides ambiguous information about both attributes of foods. To overcome this ambiguity, humans and other mammals integrate input from the taste system with that from other cephalic sensory systems to create a more nuanced and complete sensory representation of foods. There is also evidence that rodents (and perhaps humans) integrate input from the taste system with intestinal nutrient sensors to help identify and promote intake of nutritious foods.

We still do not understand how proteins and complex carbohydrates are sensed. This is because most research on the taste system has focused on the building blocks of these macromolecules – e.g., mono- and disaccharides, amino acids, and fatty acids. Despite this dearth of knowledge, we know that human societies have relied throughout history on endosperm tissues and USOs as staple foods since the late Paleolithic (Liu et al. 2013; Wadley et al. 2020). What makes this observation remarkable is that endosperm tissues and USOs typically have a bland flavor and thus would not be expected to stimulate intake. Further, many of them have to be processed and cooked extensively before they are edible and digestible. Given these constraints, it is unclear what motivated early humans to consume them initially. The most parsimonious explanation would invoke some type of flavor-nutrient learning, but we lack consistent evidence for this type of learning in humans. Notwithstanding this uncertainty, there is no question that once early humans discovered the nutritional value of cooked endosperm tissues and USOs, there was no turning back. They embraced these energy-rich foods and learned how to improve their flavor with seasonings and flavorings. Indeed, there is evidence for the use of flavorings dating back at least 6,000 years (Saul et al. 2013).

Little is known about whether humans and other mammals regulate intake of micronutrients – e.g., free amino acids, vitamins, minerals, and free fatty acids. Given that many micronutrients have an inherently aversive flavor (Schiffman and Dackis 1975), it is unlikely that their presence in foods motivates intake. In fact, it is more likely that the aversive flavor of micronutrients is masked by the more abundant macronutrients. So, how did early humans obtain adequate micronutrients in their diet? The simplest way to do so would have been to eat a diverse diet, consisting of meats, vegetables, and fruits (Krebs-Smith et al. 1987; Nicklaus 2009). In support of this explanation, populations of hunter-gathers have been found to select highly varied diets (Berbesque et al. 2011; Hart and Hart 1986; O'Dea 1991), and consume foods that contain higher concentrations of micronutrients than modern foods (Eaton et al. 1996).

In closing, there is widespread evidence that our hunter-gatherer ancestors were able to avoid many of the metabolic and cardiovascular diseases that plague modern society (e.g., type 2 diabetes and hypertension) (Lindeberg et al. 2003; Lindeberg and Lundh 1993; O'Dea 1991). Indeed, studies of relict populations of extant hunter-gatherers, living the "old way," indicate that many of these people were able to select a healthy diet and live a relatively long life (Hart and Hart 1986; Marlowe et al. 2014;

Thomas 2006). Understanding how they did so is a highly topical issue. This is because the modern diet is low in natural foods (fruits, vegetables, and endosperm tissues) and high in processed foods, which are typically enriched with sugars and *trans* fats (Liem and Russell 2019). The problem with processed foods is that they are highly palatable and obesogenic, and people cannot reliably determine their caloric content (Brunstrom et al. 2018). This may explain why a recent epidemiological study (US-Burden-of-Disease-Collaborators 2013) concluded that the modern diet constitutes the greatest risk to a long and healthy life.

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