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A Pharmacological Perspective on the Study of Taste

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Abstract——The study of taste has been guided throughout much of its history by the conceptual framework of psychophysics, where the focus was on quantification of the subjective experience of the taste sensations. By the mid-20th century, data from physiologic studies had accumulated sufficiently to assemble a model for the function of receptors that must mediate the initial stimulus of tastant molecules in contact with the tongue. But the study of taste as a receptor-mediated event did not gain momentum until decades later when the actual receptor proteins and attendant signaling mechanisms were identified and localized to the highly

specialized taste-responsive cells of the tongue. With those discoveries a new opportunity to examine taste as a function of receptor activity has come into focus. Pharmacology is the science designed specifically for the experimental interrogation and quantitative characterization of receptor function at all levels of inquiry from molecules to behavior.

This review covers the history of some of the major concepts that have shaped thinking and experimental approaches to taste, the seminal discoveries that have led to elucidation of receptors for taste, and how applying principles of receptor pharmacology can enhance understanding of the mechanisms of taste physiology and perception.

I. Introduction

Taste traditionally has been regarded as one of the sensory modalities and, like vision and hearing, a perceptual phenomenon to be studied using the techniques developed in the field of sensory perception and interpreted through a conceptual framework provided by psychology. As a perception, taste is an internal experience of part of the external world, a means of abstracting information from the environment, but also a component of awareness and consciousness (Gallo, 2016). Visual, auditory, and tactile sensory systems each have evolved to capitalize on the information transmitted by forms of energy—electromagnetic energy, waves of air pressure, force of mass against the surface of an organism, respectively. Olfaction and gustation (taste), however, have developed to extract the information that can be provided by chemicals, rather than energy, in the environment. Whereas the other sensory modalities largely provide the means by which an organism can safely navigate, locate, and identify objects in the environment, taste has developed for a very specific teleonomic endpoint—a decision on whether a substance should be ingested (Breslin and Spector, 2008; Breslin, 2013).

Over the course of the many years in which taste has been systematically studied, the objective of its scientific interrogation predominantly has been to describe quantitatively the qualitative sensations evoked by chemical stimuli impinging on the tongue. Procedures and analytical methods developed to this end owe much to the influence of the paradigm of psychophysics (Bartoshuk, 1978; Snyder et al., 2006), a discipline that, in the mid-1800s, arose out of a philosophical struggle to link events in the physical world with private sentience or mental experiences (Heidelberger, 2003). In the middle of the 20th century, another scientific perspective began to gain entry to the study of taste when physiologists began to examine, with a very different set of methodological and analytical tools and conceptual framework, peripheral mechanisms that mediate taste sensations (Oakley and Benjamin, 1966). The focus for these scientists was how chemical signals originate on the surface of the tongue and how they are transmitted

through the nervous system. Questions on the nature of the interface between lingual tissues and the chemical stimuli for taste began to unfold and gain momentum. Receptors that were anticipated to serve as that interface inevitably were discovered expressed on specialized cells within the taste bud.

The recognition of cell surface receptors as the mediators of the tastant stimulus presents an opening for yet another scientific discipline to contribute and shape the direction of the discovery in the field of taste. Pharmacology is the science of receptors and their function, and it grew from concerted efforts to understand how exogenously applied chemicals impact and control the actions of any biologic system (Limbird, 2005). The experimental and analytical methods, as well as the underlying principles of pharmacology, would therefore seem ideally suited to the study of taste (Palmer, 2007). A central goal of pharmacology is the rigorous quantification of a lawful relationship between concentration of chemical stimuli and the biologic changes they evoke—the concentration-response function. All of the biology, including behavior, which results from the interaction between receptors and their chemical ligands, must abide by the same lawfulness manifest in the concentration-response function (Kenakin, 2001). Principles of pharmacology, developed over the course of more than a century, provide the explanatory reach for an accounting of receptor-mediated phenomena from the molecular level to that of whole organisms, and taste, clearly receptor-mediated, should prove no exception.

This review will cover the conceptual contributions that inspired the search for receptors of taste stimuli, early attempts that led to dead ends, and the elucidation of molecular components of signaling pathways that ultimately revealed the identity of the receptors. Special focus will be placed on the role that concentration-response data have played in the development of a "taste receptor" concept. The value of applying the methods and conceptual framework of pharmacology to the study of taste will be emphasized. To this end, the discussion will be centered upon the receptors and cells for which the clearest associations with specific taste responses have been established, those mediating the taste qualities of "sweet" and "bitter." As a consequence, little will be said

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ABBREVIATIONS: 2-AFC, two alternative forced choice; CALHM1, calcium homeostasis modulator 1; CS, conditional stimulus; CTA, conditioned taste aversion; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; IP$_3$, inositol-1,4,5-trisphosphate; JND, just noticeable difference; LMS, labeled magnitude scale; PDE, phosphodiesterase; PLC, phospholipase C; PROP, propylthiouracil; PTC, propylthiocarbamide; SNAP25, synaptosomal-associated protein 25; TRPM5, transient receptor potential melastatin-5; US, unconditional stimulus; VFTD, Venus flytrap domain.

herein about mechanisms of "salty" and "sour" tastes, which are assumed, but not yet proven, to result from modulation of ion channels expressed in a subset of taste cells (reviewed in Munger, 2016). Although a G protein-coupled receptor (GPCR) mediating "umami" taste has been identified, the relationship between stimulus and response appears to be complicated by the likely involvement of multiple receptors (reviewed by Chaudhari et al., 2009), and therefore will be addressed only briefly here.

II. Taste Quality

The most prominent and commonly familiar of taste-related phenomena is the sensory experience of taste quality, a property of recognition. The concept of a taste quality owes its origins to the notion of qualia, an often-debated cognitive state said to result from the processing of sensory input, argued to be a foundational component of consciousness and awareness (Keeley, 2009; John, 2010; Kanai and Tsuchiya, 2012). Qualia are private events that are experienced as unique sensations caused by specific stimuli and are accessible only to the individual experiencing the "quale" (singular of qualia), and which cannot be directly observed by, or communicated to, others (Dennett, 2002). For example, the subjective experience of "redness" is communicated to others only indirectly through the descriptive label of "red" (Kanai and Tsuchiya, 2012). Qualia are considered to be monotonic, unchanging mental entities that are not directly modifiable by other related variables, such as amplitude, of the stimulus generating a particular perceptual quale (Dennett, 2002).

The notion of qualia extends to the concept of taste quality, a fundamental sensory perception identifying a particular taste stimulus. Potentially there could be an infinite number of taste qualities, considering the broad variety and range in complexity of substances that are taken into the oral cavity, but a limited set generally has been settled upon comprising a relatively few irreducible "basic tastes." The predominant consensus holds that there are five basic tastes—sweet, bitter, salty, sour, and "umami," the last of which is described as the savory taste common to a variety of foods including meat, cheese, tomatoes, mushrooms, and others food substances (Kurihara, 2015). As perceptual qualia, taste qualities also are thought to be monotonic and unaffected by variations in the related variable of taste "intensity." In support of this assertion, fructose, glucose, and sucrose have been shown to be indistinguishable when their respective concentrations were adjusted to produce the same level of stimulus "intensity," a phenomenon referred to as "monogeusia" (Breslin et al., 1996). Similarly, rats were unable to discriminate between the tastes of quinine and denatonium (both recognized as bitter by humans) when these two tastants also were adjusted to equivalent "intensities," suggesting "that there is only one qualitative type of bitterness"

(Spector and Kopka, 2002). The basic taste concept has gained support in recent years through studies of genetically engineered mice, which have provided compelling evidence for the existence of dedicated taste quality-specific Type II "receptor cells" in the taste buds (see *section IX.C*), faithfully communicating their taste signals by means of a "labeled line" (Yarmolinsky et al., 2009) to segregated topographic representation in the gustatory cortex (Chen et al., 2011).

However, others have demonstrated the existence of receptor cells that respond to more than one basic taste stimulus (Caicedo et al., 2002), and individual Type III "presynaptic" cells can respond to humoral communications from multiple tastant-specific receptor cells (Tomchik et al., 2007). Furthermore, extensive lesioning of rat gustatory cortex impairs some, but not all, ability to discriminate among different quality categories of taste stimuli (Bales et al., 2015). Thus, not all of peripheral or central taste physiology supports the basic taste concept.

A major problem for the basic taste concept is that the number and identity of taste qualities has varied across time and cultures (Erickson, 2008). In Western scientific literature, only sweet, bitter, salty, and sour had been recognized as basic tastes for many decades, and there was little mention of umami until the TAS1R1/R3, a heterodimeric class C GPCR, associated with its taste was identified in 2002 (Li et al., 2002; Nelson et al., 2002). In contrast, umami had long been accepted as a basic taste in Asian cultures (Lindemann et al., 2002; Nakamura, 2011). But umami itself does not follow the expected pattern of a basic taste. Several lines of evidence indicate the involvement of additional receptors other than TAS1R1/R3, expressed in different subsets of receptor cells, in the mediation of umami taste responses (Pal Choudhuri et al., 2015). Additionally, although the taste of umami 5′-ribonucleotides is detected at concentrations as low as 0.05 mM in human taste tests (Rifkin and Bartoshuk, 1980), concentrations as high as 1 mM have been shown to be inactive in cell-based assays of the TAS1R1/R3 receptor in the absence of glutamic acid (Li et al., 2002). Furthermore, a basic taste should be irreducible and therefore analogous to a primary color, and like primary colors, combinations of basic taste stimuli should account for all other taste sensations outside of the basic set of five. However, there is little empirical support for this notion, as has been pointed out by others (Delwiche, 1996; Erickson, 2008). Indeed, precisely what information is conveyed by the concept of a basic taste is unclear.

The basic taste concept currently remains as a linguistically convenient categorization scheme, so much so that its use is difficult to avoid in discussions of tastant agonists and their receptors, taste cells, and taste responses. But its pervasive influence has been argued to lead to an unintended consequence of favoring experimental designs and interpretations of empirical

results that tend to confirm the paradigm at the cost of those which could in principle refute it (Schiffman and Erickson, 1980; Erickson, 2008). For example, in taste discrimination experiments (see *section X.A.2*), it is common practice to train multiple concentrations of a single tastant, representing what is considered to be a quintessential basic taste stimulus, to control against the possibility that "intensity" would serve as the discriminatory cue. According to the basic taste concept and in keeping with the properties supposed of qualia, taste quality is independent of concentration. However, training multiple concentrations within the taste active range as a single discriminatory cue guarantees that result. Thus, despite any apparent conveniences afforded by the basic taste concept, it is a paradigm of limited explanatory power and might even tend to guide scientific thought toward mired tautologies.

There is no question that all organisms with chemosensory systems are capable of discriminating among different classes of chemicals and even among some of the members within a single chemical class. This is known by directly observing overt behavior. A concept of taste quality need not rely on the basic taste concept if operationalized in terms of discrimination from, or generalization to, a tastant standard. Thus, taste quality parsimoniously can be defined as the stimulus properties of a tastant agonist that serve as a discriminatory cue, without reference to basic tastes.

III. A Very Brief History of the Receptor Concept in Pharmacology

Many excellent reviews of the development of the receptor concept are available (Maehle et al., 2002; Limbird, 2004, 2005; Colquhoun, 2006; Maehle, 2009; Pruell et al., 2009). For the purposes of this review, some of the highlights in the progression of observation and thought are herein summarized.

The receptor concept largely evolved from 1) observations of the biologic responses to a range of agonist concentrations and 2) the impact that a second chemical, an antagonist, added to the preparation could have on that concentration-response relationship. As concentration of agonist increased, so also did the magnitude of the response, but only up to point—at higher concentrations the relationship broke down so that further increases in agonist concentration could evoke little if any further increase in response magnitude. In other words, the concentration-response function had a maximum and therefore was saturable. An antagonist that could translate the concentration-response function to a higher range (a rightward shift) strengthened the notion that there was a finite site at which ligands competed for occupancy. These observations led to postulation of the existence of a "receptive substance" on the surface of tissues that was the mediator of the responses to exogenously applied chemicals (Langley, 1906).

The concentration-response relationship and the behavior of the biologic system in the presence of the ligands inform considerably on the nature of the receptor. Among the more important and generalizable properties of receptors are that they are expressed in limited numbers, must have at least one and perhaps multiple ligand binding sites (Cui et al., 2006; Vafabakhsh et al., 2015), can assume multiple confirmations (Kenakin, 1996, 1997a), and are capable of transducing an external chemical signal into biologically relevant information (Kenakin, 2014). All of these characteristics are evident from the analysis of concentration-response functions (see *section V*) and were recognized to be defining properties of receptors well before the actual receptor entities were isolated and identified as proteins (Limbird, 2005).

Eventually, receptors were separated from their biologic tissues and studied independently, confirming and expanding on the knowledge gained through functional pharmacology (Lefkowitz et al., 1972; Mukherjee et al., 1975; Williams and Lefkowitz, 1976), and finally in 1986, the gene for the $\beta 1$ adrenergic receptor, a GPCR that mediates contraction of cardiac tissue, was identified (Dixon et al., 1986; Frielle et al., 1987). Isolation of the gene and transferring it to a "null cell" then allowed the development of recombinant cell lines, giving direct control over the expression of the receptors and enabling robust pharmacologic analysis (Kenakin, 1997b).

Importantly, now that the amino acid sequence was available from the isolated gene, the relationship of the concentration-response function to the structure of the protein could be explored. Mutations, either experimentally induced or naturally occurring through genetic variation, illuminated the nature of the agonist binding site by correlating the molecular changes with right-left shifts in the concentration-response function (Ohta et al., 1994; Jiang et al., 1997; Malherbe et al., 2003; Mao et al., 2010). Regions of receptors involved in G protein coupling also could be defined (Wess et al., 1997; Flock et al., 2017), building the groundwork for a structural basis of agonist efficacy (Kenakin, 2002; Deupi and Kobilka, 2007).

IV. Development of the "Taste Receptor" Concept

Recognition of the presence of sets of enzymes occurring in the lingual epithelium provided the basis for the earliest proposals of a molecular mechanism underlying the initiation of taste signaling. The assumed ability to detect and discriminate potentially thousands of tastants was conjectured to result from a combinatorial association between tastant control over operational states (active, inhibited, and neutral) of six enzyme types localized to specific regions appearing in and near the taste buds (Baradi and Bourne, 1951). Sensory neurons innervating these regions presumably then would be differentially modulated across the constellations of enzyme activities.

By the middle of the 20th century, evidence from physiologic studies, in which neural responses to increasing concentrations of tastant appeared to fit logistic functions, mounted in favor of a receptor-based mechanism. Beidler (1954) postulated the existence of a "receptive substance," the term that first was used by Langley decades before in the development of the general receptor concept to account for the competitive relationship between pilocarpine and atropine in smooth muscle tissue preparations. Possibly, Beidler came to a similar conclusion independently without knowledge of the advances that had taken place in the field of pharmacology; there are no references to Langley or other key contributors to the developing receptor concept in Beidler's publications. Beidler reasoned that the logistic fit of concentration-response data from gustatory nerve fiber recordings suggested an initial, reversible binding of the tastant molecule (or ion) to the receptive substance in a way quantitatively similar to that described for ligand-protein binding by Scatchard (1949) and Klotz (1953) and the mass action law model accredited to Langmuir (1918). Tastant-protein binding then was expected to be followed by an enzymatic reaction that followed Michaelis-Mentin kinetics. In accordance with these assumptions, Beidler applied the mass action principle to quantify the relationship between tastant concentration and sensory response. The resulting equation, now often referred to as the "Beidler equation," is essentially equivalent to the Langmuir adsorption isotherm (see *section V*), which previously was applied by Hill (1909) and later Clark (1927) to describe concentration-response functions generalizable to any agonist-receptor association [as such, the function does not accommodate a variable for agonist efficacy, introduced by Stephenson (1956) subsequent to Beidler's work]. Beidler deserves full credit for being the first to articulate that sensory responses to taste stimuli behave as though they were mediated by receptors.

Beidler's compelling rationale for a receptor-mediation of taste responses inspired subsequent attempts to identify and isolate the receptor proteins from taste tissue using biochemical techniques. Among the earliest efforts were studies in which papillae (structures in the tongue containing taste buds) dissected from bovine (Dastoli and Price, 1966; Dastoli et al., 1968) and rat (Hiji et al., 1971) tongue were homogenized in phosphate buffer and pelleted by centrifugation at 75,000 g. Apparently, the receptors were assumed to have been released into the supernatant instead of remaining in the membrane pellet. Therefore, the supernatant was subsequently fractionated and then tested for interactions with sweet mono- and disaccharides and saccharine as measured by refractometry and UV-difference spectrometry. A peak of activity was detected in one of the resulting fractions and evaluated across concentration ranges of the different sweeteners. Data obtained were fit by the linear form of the Beidler equation and parameters derived from resulting plots,

appearing to define a sweet tastant binding site. However, others found equivalent results when the isolated protein fraction was obtained from tongue epithelium devoid of taste buds (Koyama and Kurihara, 1971) or when the protein isolate was replaced by immunoglobulin (Nofre and Sabadie, 1972), and thus the putative sweet tastant binding site was dismissed as an artifact.

The supposition that tastant molecules were ligands for receptors later led to attempts to characterize binding sites for sweeteners in lingual tissue using radioligand binding methods (Cagan, 1971; Cagan and Morris, 1979; Zelson and Cagan, 1979). These early efforts do not appear to have been guided by contemporaneous standards of receptor pharmacology and therefore have carried forward little useful information. For example, affinities of radioligands used in these studies, such as ^{14}C-labeled sucrose (Cagan, 1971), and even ^{3}H-labeled monellin (Cagan and Morris, 1979), would not have been sufficiently high for ligand-receptor complexes to remain stable during washes to separate free from bound radioligand (Limbird, 2005; Kenakin, 2014). The relatively low specific radioactivity of ^{14}C-labeled ligands also brings into question the sensitivity of those assays, complicating interpretation of the data they produced. The report of a ^{3}H-monellin binding site in bovine taste tissue, implied to be a receptor for sweet tastants (Cagan and Morris, 1979), was essentially devoid of rigorous pharmacologic characterization. Later nerve recordings of calf glossopharyngeal and chorda tympani nerves, afferents that carry taste signals, found no responses to monellin, casting further doubt on the validity of the earlier radioligand binding studies.

Identification of the actual receptors mediating the taste responses observed by Beidler awaited interrogation through the techniques of molecular biology and consequently occurred at the turn of the century.

V. Basic Principles of Receptor Function as They Relate to the Study of Taste

A. Affinity

The affinity of a molecule (ligand) in solution for a receptor is defined by the concentration (in units of molarity) of that ligand that occupies 50% of the available receptors at equilibrium. The attraction between a ligand and a receptor is a chemical reaction resulting from weak, usually reversible, attractive forces. When equilibrium is reached, the forward rate of receptor occupancy is equal to the reverse rate of dissociation of the ligand from its receptor. Although there are exceptions, generally on-rate is diffusion limited and dependent on the concentration of the ligand. The off-rate is dependent only on the rate of dissociation, not concentration. Affinity therefore is most impacted by the off-rate—a ligand that remains bound to a receptor longer (i.e., with a slower off-rate) will have a higher affinity. With a labeled detectable ligand of sufficient affinity and specificity, an

assay can be carried out that provides a mathematical function that quantitatively describes the relationship between ligand concentration and fractional receptor occupancy (Limbird, 2005; Kenakin, 2014).

To date, there have been no direct measures of tastant affinity for an intact TAS1R (heterodimeric GPCRs that mediate sweet and "umami" tastes, see *section VIII.B*) or TAS2R (GPCRs that mediate bitter taste, see *section VIII.A*). Early attempts using radioligands with low specific activity and low affinity were unproductive, even misleading. More recently, Nie et al. (2005) used fluorescence circular dichroism to obtain affinity constants for three sweet tastant molecules—glucose, sucrose, and sucralose—binding to the N-terminal domains isolated from murine T1R2 and T1R3 (protomers of the heterodimeric GPCR that mediates sweet taste, see *section VIII.B.*) The resulting K_D values of 7.3, 2.9, and 0.9 mM, respectively, were acknowledged to be somewhat lower than expected from cell-based assays of intact functional receptors (see *section IX.E*) and in vivo behavioral assays (see *section X.A*), but the results have helped to define the structural requirements and likely sites of interaction between tastant ligand and receptor domains. The closest estimates of tastant affinities for functional receptors are obtainable, under some assumptions that will be made clear below, from potencies resulting from concentration-response analysis of cell-based assays. Affinity is related to the potency of an agonist through the empirical proportionality factor of intrinsic efficacy (Black et al., 1985; Leff, 1988).

B. Intrinsic Efficacy

Intrinsic efficacy is the ability of a ligand to stabilize a receptor conformation that favors coupling to a signaling system that could evoke a biologic response. Intrinsic efficacy can be thought of as the amount of stimulus a ligand delivers to a biologic system through a receptor, ranging along a continuum from positive, through neutral, to negative efficacy (Kenakin, 2002). Absolute values for the magnitude of efficacy have not yet been determinable, and therefore efficacy is empirically determined as a relative value among known ligands for a specific receptor (Kenakin, 1994).

A ligand with positive intrinsic efficacy, i.e., one that can initiate receptor-mediated signaling, is an agonist; any tastant that elicits a taste quality response therefore is an agonist. Intrinsic efficacy among agonists can vary—the highest efficacy agonists, or *full agonists*, are those that elicit the maximum response mediated by occupying 100% or less of the receptors available and specific to that agonist. Agonists with relatively low positive intrinsic efficacy, referred to as *partial agonists*, fail to elicit maximal responses from a biologic system even when receptor occupancy is at 100% (Kenakin, 1987). Evidence consistent with partial agonism by some bitter ligands has been reported in recombinant murine TAS2R cell-based assays (Lossow et al., 2016). Ligands with negative intrinsic efficacy,

or *inverse agonists*, stabilize conformations that lower the probability of receptor signaling. Their effect is evident in biologic systems that exhibit a basal tonicity that results from constitutive activity of receptors. In some cases, the tone of a particular system was not revealed until inverse agonist effects were observed. Lactisole, known for its ability to block taste responses elicited by sucrose and other sweeteners, is an example of an inverse agonist (negative intrinsic efficacy ligand) for the TAS1R2/R3 receptor expressed in sweet-committed Type II receptor cells (Galindo-Cuspinera et al., 2006). A neutral efficacy ligand, more commonly referred to as an *antagonist*, binds to and occupies a receptor site, but does not alter the function of the receptor other than to prevent access by other efficacious ligands. At least 13 molecules have been shown in the scientific literature to antagonize the activity of bitter tastants on select hTAS2R receptors in recombinant cell-based assays (reviewed in Jaggupilli et al., 2016). For example, Givaudan 3727 (4-(2,2,3-trimethylcyclopentyl)butanoic acid) is a noncompetitive inhibitor of TAS2R43 and TAS2R44/31 (Slack et al., 2010), receptors implicated in the bitter off-tastes of saccharin and acesulfame potassium (Pronin et al., 2007; Roudnitzky et al., 2011). The multidrug resistance protein 1 (MRP-1) inhibitor probenecid also has been shown to non-competitively antagonize hTAS2R38, hTAS2R43, and hTAS2R16 in recombinant cell-based assays as well as reduce the bitter taste intensity of salicin (Greene et al., 2011).

C. Potency and Response Magnitude

Potency of an agonist is defined as the concentration at which 50% of the maximal response for that agonist is achieved. Since taste responses can be interpreted as a close approximation of receptor pharmacodynamics unobscured by the impact of pharmacokinetics, expressing tastant potencies in units of molarity is justifiable and strengthens the conceptual link between the observed behavioral responses and receptor occupancy. The numeric value of potency, or EC_{50}, is a convenient locator of the active concentration range and a handy comparator for ranking agonists of a given receptor by their potencies. Agonist affinity, a function of receptor occupancy, is the first determinant of the ordinal location of potency in an active range of concentrations (Kenakin, 2001). Tissue-dependent variables that enhance the efficiency of coupling agonist-occupied receptors with the response, such as receptor density, the numbers and types of G proteins present, as well as other morphologic and physiologic characteristics specific to a tissue, work in conjunction with positive intrinsic efficacy to shift EC_{50} values to lower concentration ranges (Kenakin, 2014). In this context it is important to note that recombinant expression of GPCRs often results in relatively high receptor densities, an artificial condition that potentially could shift concentration-response functions to the left (i.e., lower concentrations ranges) of those

obtained from natural biologic systems. However, concentration-response functions generated by recombinant cell lines expressing TAS1 or TAS2 receptors are similar in range to those reported for in vivo assays. So far, there is little evidence indicating that intrinsic efficacies of tastant agonists influence observed potencies of tastants in either cell-based or in vivo assays.

The magnitude of agonist response is a function of both intrinsic efficacy of the agonist and tissue-dependent variables, but is independent of agonist affinity (Kenakin, 2014). As alluded to above, differences in maximal responses among some bitter tastants acting at the same receptor in recombinant cell-based assays suggest differences in efficacies. Nevertheless, tissue-dependent variables probably better explain magnitudes of tastant responses in vivo. For example, measures of the subjective experience taste intensity (the equivalent of tastant response magnitude) have been shown to correlate with density of fungiform papillae (Miller and Reedy, 1990; Delwiche et al., 2001b).

D. Quantification of Pharmacological Functions

A few elegant mathematical models that provide a quantitative description of receptor occupancy and its relationship to concentration-response functions have proven sufficient to explain the majority of receptor-mediated biology.

1. The Langmuir Adsorption Isotherm. The basic equation that relates ligand concentration to receptor occupancy is given by

$$Y = \frac{[A]}{K_D + [A]}$$

where Y = fractional receptor occupancy, the number of receptors occupied by agonist (A) relative to the total number of receptors, $[A]$ = the concentration of the agonist, and K_D = the affinity constant of the agonist for the receptor.

The equation assumes a simple bimolecular interaction and describes a rectangular hyperbolic function. Derivation of the Langmuir equation can be found in Limbird (2005).

2. The Beidler Equation. Beidler reasoned from the appearance of concentration-response functions obtained in gustatory nerve recordings that tastant molecules must be imparting their stimulus by interacting with cell-surface proteins in accordance with mass action law and derived the following equation (Beidler, 1954):

$$Kc = \frac{R}{Rm - R}$$

Rm = maximum response, R = response magnitude observed at a given tastant concentration, K = tastant binding equilibrium constant, and c = tastant concentration.

The Beidler equation can be rearranged to

$$\frac{R}{Rm} = \frac{c}{K + c}$$

and if it is assumed that R is directly related to receptor occupancy as Beidler did, then R/Rm is equivalent to Y, the fractional receptor occupancy, and then the Beidler equation is formally equivalent to the Langmuir equation. According to the equation, a tastant with an affinity (K_D) for its receptor of 10 mM will occupy half of the available receptors at a concentration of 10 mM. If the concentration of tastant is raised 10-fold to 100 mM, then approximately 91% of the receptors will be occupied. Continuing to raise the tastant concentration will have proportionally less impact on receptor occupancy as saturation is approached. A concentration of the hypothetical agonist at 1 mM will occupy 9.1% of the receptors, a value that reasonably can be assumed to be in the range of threshold detection. Thus, the entire concentration-response function for a tastant, like any other agonist, would be expected to be contained within two orders of magnitude.

VI. A G Protein for Taste

By the 1990s, evidence of signal transduction mechanisms for taste was beginning to appear in the scientific literature. Salty (Heck et al., 1984) and sour (Kinnamon et al., 1988) tastes had been associated with ion conductances, whereas bitter and sweet tastants were shown by several reports to stimulate G protein-dependent generation of cAMP in lingual tissue (Avenet et al., 1988; Naim et al., 1991; McLaughlin et al., 1993). Mobilization of intracellular calcium also resulted from the application of some bitter tastants to taste buds (Akabas et al., 1988; Hwang et al., 1990; Bernhardt et al., 1996). The apparent involvement of second messenger cascades for at least some taste signaling pathways prompted the search for possible taste-specific G proteins.

By screening a lingual tissue cDNA library with degenerate primers based on conserved regions of known G protein α subunits, a new G protein with expression limited to taste buds was discovered (McLaughlin et al., 1992). The localization of the G protein to taste sensory tissue and its absence from non-sensory lingual epithelium strongly suggested its role in taste signaling. The complete sequence was found to be approximately 80% identical to that of transducin, the G protein already known at the time to be critical in the signal transduction of vision. Because of its close structural relatedness to transducin and its likely taste-signaling role, the new G protein was named "gustducin." Antibodies raised against an epitope of α-gustducin were used to immunohistochemically visualize its localization to the apical microvilli structures (discussed below) at the pore of human taste buds (Takami et al., 1994; Bernhardt et al., 1996).

A few years later, gustducin was proven critical for detection of sweet and bitter tastants, but not sour and salty tastants, in a knockout mouse model (Wong et al., 1996).

The signal transduction coupling of α-gustducin was somewhat of an enigma and, to some degree, still is. Because of its close sequence relatedness to α-transducin, it was suspected that gustducin might similarly interact with a phosphodiesterase (PDE) to decrease cellular concentrations of a cyclic nucleotide, as is the case in visual signal transduction (Arshavsky et al., 2002). Both gustducin and transducin belong to the $G_{i/o}$ family of G proteins, which signal through inhibition of adenylyl cyclase, an activity that is sensitive to ADP-ribosylation by pertussis toxin (West et al., 1985; Hoon et al., 1995). Supporting this notion, taste cells were found to express high amounts of PDE3 (McLaughlin et al., 1993, 1994), and in contrast to some earlier studies, cAMP (and cGMP) decreased in response to bitter tastants (Ruiz-Avila et al., 1995). Activation of cGMP-dependent PDE by α-gustducin was directly demonstrated in a cell-free system when the signaling proteins were purified and reconstituted with the GPCR rhodopsin (Hoon et al., 1995; at the time, receptors that mediate taste signals had not been identified). Furthermore, in the same study, gustducin was shown to be a substrate for ADP-ribosylation that could be enhanced by the presence of a $\beta\gamma$ subunit. Thus α-gustducin seemed to be functionally equivalent to α-transducin, and thereby suggested that the signal transduction of taste was similar to that of visual systems.

But taste cells also were found to express several other G protein α subunits (McLaughlin et al., 1992, 1994; Kusakabe et al., 1998) including α-transducin—the first evidence of this G protein subunit occurring outside the visual system (Takami et al., 1994). Further beclouding the picture were multiple reports of tastant-activation of an inositol phosphate-dependent signaling cascade and consequent mobilization of intracellular calcium in taste buds or cells (Akabas et al., 1988; Hwang et al., 1990; Spielman et al., 1994, 1996; Bernhardt et al., 1996).

Binding sites specific for inositol-1,4,5-trisphosphate (IP_3) had been visualized by autoradiography to be localized to the apical pore of rat taste buds, providing direct evidence of the existence of protein components of the inositol phosphate second messenger cascade (Hwang et al., 1990). Administering denatonium, which is bitter tasting to humans (Delwiche et al., 2001a) and aversive to rats (Brasser et al., 2005), at concentrations as low as 10 μM to the lingual tissue stimulated increases in IP_3 (Hwang et al., 1990; Rössler et al., 1998; Rossler et al., 2000). These observations clearly implicated phospholipase C as the effector enzyme that would be activated by receptors mediating a taste signal through a G protein.

VII. Discovery of Phospholipase C β2

By using degenerate primers based on conserved sequences from isoforms of both rat and human phospholipase C (PLC) β, a novel rat PLCβ gene with a sequence identity closely resembling that of human PLCβ2 was detected (Rössler et al., 1998). In situ hybridization showed that the expression of the phospholipase was exclusive to taste cells (now known to be expressed also in hematopoetic cells and platelets, e.g., Adamiak et al., 2016). Antibodies directed against an epitope of the human isoform were able to visualize the location of the rat PLCβ2 to a specific subset of cells within the taste bud, intensely labeling the microvillus region of the taste bud apical pore. Addition of these antibodies to a functional assay greatly diminished the ability of 100 μM denatonium to stimulate production of IP_3 in rat circumvallate tissue homogenate (Rössler et al., 1998). The same effect was achieved with U73122, a potent inhibitor of PLC (Jin et al., 1994). Interestingly, simultaneous measures did not indicate any effect of denatonium on cAMP.

Since gustducin is a member of the pertussis-sensitive $G_{i/o}$ family of G proteins, it was speculated that the $\beta\gamma$ units associated with α-gustducin could be the important mediator of PLCβ activation. A transgenic mouse model was created in which green fluorescent protein (GFP) was expressed under control of the promotor for gustducin, enabling the visual identification of gustducin-positive subsets of taste receptor cells (Wong et al., 1999), thereby facilitating the discovery of other proteins that could be involved in taste signal transduction. A novel Gγ subunit, Gγ13, was identified from a cDNA library made from GFP-positive cells (i.e., taste cells that expressed gustducin) isolated from the tongues of these transgenic mice (Huang et al., 1999). Although Northern blots indicated the presence of Gγ13 in a few other tissues, it was absent from taste cells that did not express gustducin. Colocalization of Gγ13 and gustducin in the GPF-positive cells was further supported by immunocytochemistry. Gγ13 thus was a good candidate for having a role in taste transduction, and indeed, antibodies directed against γ13 prevented increases in IP_3 stimulated by denatonium. In the same study, two β subunits, β1 and β3, also were found to be expressed in GFP-gustducin cells. But β3 exclusively colocalized with gustducin, whereas β1 was found to occur in cells with no visible gustducin marker. Results from a trypsin digest assay indicated that β1γ13 could enhance gustducin activity stimulated by addition of denatonium, but results were inconclusive with the β3γ13 dimer. A subsequent study (Rossler et al., 2000) confirmed expression of β3, but not β1, in taste cells by immunohistochemistry, with staining concentrated in the microvillus region. Also in that study, antibodies raised against β3 blocked the ability of denatonium (100 μM) to stimulate IP_3 generation in rat circumvallate tissue.

All the components were in place favoring a major role for the PLC-inositol phosphate signal transduction cascade, stimulated by the $\beta\gamma$ subunits associated with α-gustducin, as a primary pathway at least for bitter

taste. But with the potential for signaling via other G protein-dependent mechanisms and the open question of the nature of signaling by other non-bitter tastes, compelling confirmation of the central role of PLC in transduction of GPCR-dependent taste still was needed. The confirming evidence was provided by genetically engineered mice deficient of PLCβ2 (Zhang et al., 2003) and the IP3III receptor (Hisatsune et al., 2007) that exhibited severe impairment of sensitivity not only to bitter but also to sweet and "umami" tastants, while their responsiveness to salty and sour tastants remained intact.

The finding of a G protein required for taste signaling indicated that at least some of the receptors mediating taste had to be GPCRs. Within the next 10 years, genes were identified that encoded the GPCRs which, when activated by tastant agonists, mediate bitter, sweet, and "umami" tastes.

VIII. Receptors that Mediate Taste Signaling

Examination of regions of the human genome that are syntenic with murine genes associated with sensitivities toward aversive tastants (Adler et al., 2000; Matsunami et al., 2000) and sweet tastants (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001) led to the discovery of two families of GPCRs that also were selectively coexpressed with gustducin in rodent tongue. Shortly thereafter, these two families, designated TAS1R and TAS2R (originally T1R and T2R, respectively), were confirmed by recombinant expression in HEK293 cells and by genetic knockout to be mediators of sweet and bitter tastes.

A. TAS2R Receptors

The TAS2Rs were the first of these receptors to be directly associated with taste responses, such as the aversive taste of cycloheximide in mice (Chandrashekar et al., 2000; Mueller et al., 2005), and bitter tasting glucosides (Bufe et al., 2002; Mueller et al., 2005), propylthiouracil (PROP) and propylthiocarbamide (PTC) in humans (Bufe et al., 2005; Mueller et al., 2005). There are approximately 25 members of this family in humans and 35 in mice; the relatively large number possibly a requirement for detecting potentially thousands of bitter tastants (reviewed in Behrens and Meyerhof, 2006, 2009).

TAS2R receptors do not fit neatly into a structural classification within the GPCR superfamily. Some structural analyses associate the TAS2Rs with the Frizzled group (Fredriksson et al., 2003), but others (Cvicek et al., 2016) still report that TAS2Rs resemble class A receptors more closely than when compared with other categories. Although perhaps distantly related to class A rhodopsin-like receptors (Di Pizio et al., 2016), characteristic motifs conserved among class A receptors are absent in TAS2Rs (Singh et al., 2011). Within the

TAS2R family, amino acid identity varies between approximately 30% and 70% (Adler et al., 2000).

Whereas a few TAS2Rs appear to exhibit highly selective agonist association, several TAS2Rs are said to be "broadly tuned," as they have been shown in cell-based assays to be activated by multiple agonists known to impart bitter tastes (Behrens et al., 2004; Brockhoff et al., 2007). Additionally, it has been noted that there are very few antagonists relative to the number of agonists unequivocally associated with specific TAS2Rs, an observation that has been contrasted to agonist: antagonist ratios identified through database searches of GPCRs that have been the targets of drug discovery efforts (Di Pizio and Niv, 2015). These observations have been postulated to reflect underlying structural features of TAS2Rs that would promote a "broad tuning" toward a conceivably very large set of bitter agonists, a capacity that could provide an adaptive advantage against ingestion of potentially many toxic compounds that might be encountered by foraging animals (Brockhoff et al., 2010).

However, libraries reported to have been screened for agonist activity are relatively small, with only 104 compounds for human TAS2Rs (Meyerhof et al., 2010) and 210 compounds for murine TAS2Rs (Lossow et al., 2016). These libraries were composed of compounds known to be bitter tasting (and were therefore biased focused libraries), and the majority of hits demonstrated low apparent affinity toward TAS2Rs. On the other hand, random libraries composed of hundreds of thousands of compounds typically are screened against GPCRs that are considered targets for pharmacotherapeutic intervention. The likelihood of finding more antagonists and agonists of TAS2Rs, displaying ranges of selectivity and affinity, should grow with continued screening efforts. For example, a library of 15,854 compounds screened in a high throughput campaign aimed at discovering antagonists for hTAS2R31, a commercially relevant target given its mediation of bitter tastes imparted by some non-nutritive sweeteners, yielded 139 candidates (Slack et al., 2010). One of the hits, GIV3727 [4-(2,2,3-trimethylcyclopentyl)butanoic acid], was singled out "as a robust inhibitor of hTAS2R31" and subsequently pharmacologically characterized as noncompetitive with IC_{50} values against cognate agonists in the low micromolar range. At a higher concentration of 25 μM, antagonist activity also was observed at additional TAS2Rs. Although not reported, it would be reasonable to assume that the remaining 138 candidates from the screen were still less potent and less selective.

As larger sets of precision pharmacological tools become available for probing structure-activity relationships, any properties unique to TAS2Rs will become increasingly clarified. The lack of potent, selective ligands furthermore has hindered the effort to associate in vivo bitter taste responses to specific TAS2Rs. To date, there only are

few potent, selective agonists of TAS2Rs that have been useful for unambiguously associating a receptor with bitter taste.

B. TAS1R Receptors

TAS1Rs are class C GPCRs that form heterodimers to make functional receptors (reviewed in Bachmanov and Beauchamp, 2007; Li, 2009). A single receptor is known to mediate the taste of sucrose and other sweeteners (Nelson et al., 2001), and one for the taste of glutamic acid and other amino acids (Nelson et al., 2002). These two receptors differ by one of the protomers in the pair, TAS1R2 and TAS1R1, respectively, while the TAS1R3 is common to both receptors (Zhao et al., 2003).

If the term "broadly tuned" were to be used as a generalization to describe the ligand binding capacity of a GPCR, it might best be applied to the TAS1R2/R3 (heterodimer of TAS1R2 and TAS1R3) receptor, for which many agonists, representing many different chemical classes, are known, including mono- and disaccharides, amino acids, peptides, proteins (Morini et al., 2005), small molecules (Masuda et al., 2012), diterpene glycosides (Hellfritsch et al., 2012), dihydrochalcone glycosides (Winnig et al., 2007), and presumably even lead salts, which were used by ancient romans as sweeteners (Reddy and Braun, 2010). In addition to agonists, an inhibitor of sweet taste in humans, lactisole, has been characterized as an inverse agonist (Galindo-Cuspinera et al., 2006). Clofibrate and structurally related phenoxy herbicides also have been shown to potently antagonize TAS1R2/R3 receptor activity in cell-based assays (Maillet et al., 2009), but much higher concentrations of clofibrate were needed to achieve modest inhibition in human tests of sweet taste (Kochem and Breslin, 2017). Positive allosteric modulators of sucrose and sucralose activation of TAS1R2/R3 also have been discovered (Servant et al., 2010; Zhang et al., 2010).

As with other members of the class C family of GPCRs, the TAS1R2/R3 receptor has a large extracellular N-terminus Venus Flytrap domain (VFTD). The VFTD of the TAS1R2/R3 dimer has been identified through domain isolation experiments as the binding site for glucose, sucrose, and sucralose (Nie et al., 2005). Positive allosteric modulators of sucrose and sucralose (Zhang et al., 2010), the dipeptide-like sweeteners aspartame and neotame (Xu et al., 2004), and the 10.7 kDa dimeric protein, monellin (Jiang et al., 2004), also bind to the VFTD, but their sites appear to be localized to the TAS1R2 protomer. A cysteine-rich domain in the TAS1R3 protomer, connecting the VFTD to the 7-transmembrane spanning (7TM) domain, was determined through site-directed mutagenesis (Jiang et al., 2004) and by saturation difference NMR spectroscopy (Assadi-Porter et al., 2010) to be critical for the agonist activity of the sweet protein brazzein, as well as the potently sweet protein thaumatin (Ohta et al., 2011). Finally, the 7TM domain of the

TAS1R also has been shown to contain the binding site for the sweet agonist cyclamate (Jiang et al., 2005b) and also for the inverse agonist lactisole (Jiang et al., 2005a).

Another closely related class C heterodimer GPCR, TASR1/R3 (heterodimer of TAS1R1 and TAS1R3), was found to be activated by select amino acids. The amino acid-sensitive TAS1R1/R3 was expressed in an exclusive pattern on the tongue not shared with other receptors mediating sweet and bitter responses. Since glutamate and other amino acids that in humans are reported to generate a "savory" taste acted as agonists of this receptor, TAS1R1/R3 was designated the "umami" receptor. However, a substantial body of evidence suggests that multiple receptors could mediate the tastes of amino acids, suggesting that "umami" taste results from more than one taste signaling pathway (Chaudhari et al., 2009; Yasumatsu et al., 2009, 2012).

IX. Native and Recombinant Taste Cells

The sensory organelle is the taste bud (Fig. 1), a heterogeneous cluster of specialized cells that are distinguishable morphologically and functionally (reviewed in Roper and Chaudhari, 2017). In mammals, taste buds are located in papillae, cushion-like structures that project
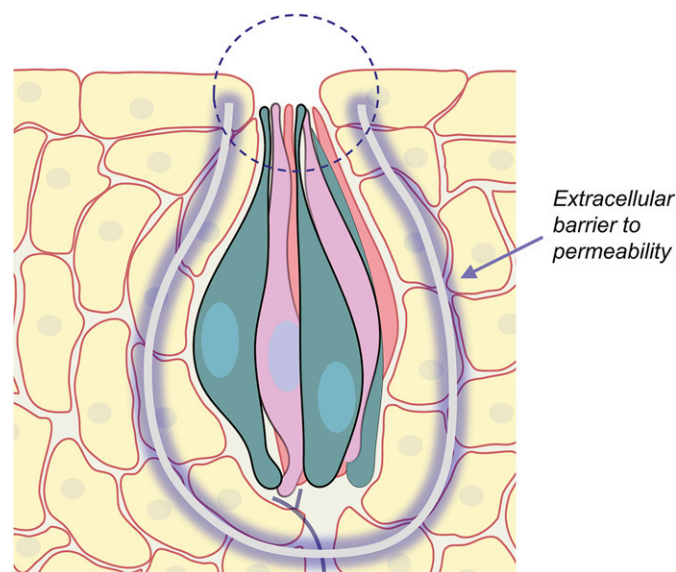

Extracellular barrier to permeability

Fig. 1. The taste bud is the sensory organelle for taste. The taste bud comprises a cluster of specialized cells designated Type I, Type II receptor cells, and Type III presynaptic cells. The functions of Type I cells are poorly understood, but the Type II receptor cells and Type III presynaptic cells are known to generate taste signals. The Type II receptor cells express GPCRs that mediate the taste responses to bitter and sweet stimuli, as well as the taste of glutamic acid (umami). Expression of the tastant GPCRs is concentrated in microvilli structures that extend into a pore that is formed by an opening in the surface layer of lingual epithelial cells (highlighted by the dashed circle). The epithelial cells form tight junctions so that only the apical microvilli are exposed to solutions bathing the tongue's surface. Some chemicals in solution are able to partition across cell membranes and penetrate the epithelial layer, but the entire taste bud is encapsulated by a permeability barrier composed of claudin proteins and extracellular glycoprotein matrix.

from or are surrounded by invaginations in the epithelial surface. The numbers and pattern of the papilla distribution are species dependent. Circumvallate papilla, localized to the posterior third of the tongue, are relatively larger and less numerous than the fungiform papillae, which are distributed in punctate loci across the anterior two thirds of the tongue. In humans, hundreds of taste buds typically are found in the circumvallate papillae, whereas fungiform papillae contain only two to four taste buds (Arvidson and Friberg, 1980; Miller and Reedy, 1990).

The taste bud resembles a head of garlic, with the cloves being the different cell types enclosed within (Fig. 1). The entire structure is surrounded by epithelial cells and extracellular matrices except for a small pore, about 10 μm in diameter that permits limited access by materials in the mucosa of the tongue's surface to the taste cells (Miller and Reedy, 1990; Royer and Kinnamon, 1991). The epithelial cells are conjoined by tight junctions (Gao et al., 2009), which minimize exposure of taste cells. Claudin proteins and glycoproteins form layers of extracellular matrix that are a formidable barrier (Michlig et al., 2007; Dando et al., 2015), which has been shown to resist penetration by compounds presented in solution to the taste bud. Thus, the entire structure is designed to seal off and protect the taste cells from substances in the oral cavity, while exposing only the very apical ends.

Unlike all other sensory receptor cells, taste cells are not of neural origin, but instead are derived from epithelial cells. Taste cells rapidly turnover, and depending on the type of taste cell, post-mitotic populations are eliminated with half-lives of between 8 and 24 days and are replenished from local progenitor cells in the surrounding epithelium (Perea-Martinez et al., 2013).

There are several different types of taste cells, distinct in morphology and function, within the taste bud. Type 1 cells have been characterized as glia-like, wrapping lamellae around other cells within the taste bud (Pumplin et al., 1997), and expressing glial markers such as the glutamate aspartate transporter (Lawton et al., 2000). Reminiscent of oligodendrocyte function, Type 1 cells have been proposed to regulate the interstitial milieu surrounding taste cells, possibly maintaining ionic gradients in the extracellular space (Dvoryanchikov et al., 2009). Type 1 cells also express nucleoside triphosphate dihydrolase-2 and are thus enabled to clear secreted ATP, a major humoral factor for cell-to-cell communication within the taste bud.

More is known about Type II (now often referred to as "receptor cells") and Type III cells (also called "presynaptic cells"), which are responsible for generating receptor-mediated signals that ultimately are detected as taste. These cells are fusiform in shape and extend an apical microvillus into the pore of the taste bud, maximizing cell-surface interface with the mucosa of the tongue and the tastant molecules carried therein

(Fig. 1). It is in these apical microvilli where proteins that initialize taste signals are densely expressed, as evinced by immunohistochemical staining (Yang et al., 2000; Tizzano et al., 2015). The Type II receptor cells express the GPCRs and all the proteins associated with second messenger cascades described above and therefore can be functionally identified by their responsiveness to GPCR tastant agonists, such as saccharin and cycloheximide. Type III cells, on the other hand, do not respond to GPCR tastant agonists, but can be depolarized by application of 50 mM KCl and thus functionally distinguished from Type II cells (DeFazio et al., 2006). Type III cells additionally have been shown to respond to high, aversive tasting concentrations of NaCl (Oka et al., 2013) and to organic acids that are regarded as sour by humans (Huang et al., 2008b). The mechanisms by which high NaCl concentrations could elicit aversive tastes currently are unknown. Organic acids probably activate sour taste responses by decreasing intracellular pH subsequent to diffusion across the plasma membranes of taste cells (Roper, 2007; Munger, 2016).

Expression of the synaptosomal-associated protein 25 (SNAP25) was detected by single-cell reverse transcriptase polymerase chain reaction from KCl-responsive cells but not in GPCR tastant-responsive cells, which exclusively expressed messages for PLCβ2. Further analysis of the KCl-responsive cells revealed the presence of neural cell adhesion molecule, synapsin 1, and the α1a subunit of P/Q-type voltage-gated calcium channels. None of these markers were found in the single-cell transcripts of GPCR tastant-sensitive, PLCβ2-expressing cells (DeFazio et al., 2006). Electron micrographs show Type III cells (identified morphologically and by the presence of SNAP25) in forming synapses with sensory neurons at the base of the taste bud (Yang et al., 2000). Thus, Type III cells are distinguished from Type II receptor cells by their ability to function as classic excitable presynaptic cells. In addition to these neural, presynaptic characteristics, Type III cells contain vesicles in which neurotransmitters, such as serotonin (Kaya et al., 2004), norepinephrine (Huang et al., 2008a), and GABA (Huang et al., 2011), are thought to be sequestered, as well as enzymes needed for neurotransmitter synthesis (Dvoryanchikov et al., 2007).

A more fundamental difference between the two cell types is evident in the ways in which they propagate their taste signaling information to other neighboring cells. Type II receptor cells do not form conventional synapses (Clapp et al., 2004) and do not have the molecular machinery for exocytosis (DeFazio et al., 2006); although they are capable of generating action potentials by means of voltage-gated sodium and potassium channels (Chen et al., 1996; Romanov et al., 2008), they do not express voltage-gated calcium channels that are critical for the process of exocytosis (Clapp et al., 2006). Instead, the TRPM5 (transient receptor potential melastatin 5)

channel, by virtue of its gating by intracellular calcium, plays a central role at the terminus of the signal transduction cascade.

A. Transient Receptor Potential Melastatin 5

The TRPM5 channel is an important component of the taste signaling cascade of GPCRs for taste. Ablating the gene for TRPM5 has been shown to severely impair (Damak et al., 2006) or eliminate (Zhang et al., 2003) taste responses mediated by TAS1R and TAS2R receptors in mice. Originally mischaracterized as a calcium conducting channel (Pérez et al., 2002; Zhang et al., 2003), TRPM5 is a homo-tetrameric channel with a monovalent cation conductance (Hofmann et al., 2003; Liu and Liman, 2003; Prawitt et al., 2003). Each unit of its structure spans the plasma membrane six times with binding sites for calcium on the intracellular side (reviewed in Clapham, 2003; Zheng, 2013). When measured in whole cell patch clamp of recombinant HEK-293 cells stably expressing TRPM5 and perfused with buffered solutions of calcium, the conductance was demonstrated to be activated by intracellular calcium in a concentration-dependent manner, with an apparent EC_{50} of 840 nM, and a half-time to peak of approximately 3 seconds (Prawitt et al., 2003). In other whole cell patch clamp experiments using recombinant TRPM5-expressing HEK-293 cells, dialysis with 200 nM calcium was sufficient to evoke a TRPM5 conductance (Palmer et al., 2010). The close accord of intracellular calcium mobilization and TRPM5 activity was demonstrated by incubating the TRPM5-expressing HEK-293 cells with both calcium-sensitive and membrane potential-sensitive fluorescent dyes and simultaneously recording membrane potential and calcium responses to ATP activation of native P2Y receptors in a FLIPR (fluorescence imaging plate reader) assay (Bryant et al., 2008). ATP concentration-response functions for TRPM5-mediated membrane potential and calcium responses were identical and the kinetic traces from both measures were superimposable. Thus, it would be expected that TRPM5 activity in taste cells would follow faithfully the concentration-response functions of tastant receptor activation. The functional characteristics of TRPM5 conductances are consistent with the kinetics of intracellular calcium mobilization that occurs as a consequence of receptor activation of the phospholipase C second messenger signaling cascade, and this has been confirmed in native taste cells expressing a GFP-labeled construct of TRPM5 (Zhang et al., 2007).

Despite the demonstrated significance of TRPM5 in GPCR-mediated taste signaling, mice genetically deficient of TRPM5 are not completely devoid of responsiveness to bitter, sweet, and "umami" tastants (Damak et al., 2006; Devantier et al., 2008). Recently another member of the TRP channel family, TRPM4, also has been demonstrated to be involved in GPCR-mediated taste signaling (Dutta Banik et al., 2018). TRPM4 was found to be coexpressed in 90% of TRPM5-expressing mouse taste cells, and ablation of the gene encoding TRPM4 impaired responses of isolated taste cells to bitter, sweet, and "umami" tastants. In behavioral assays, TRPM4 knockout mice also were less sensitive to the tastants, and double knockout of both TRPM4 and TRPM5 completely eliminated taste responses. Thus, it appears that TRPM4 might work in tandem with TRPM5 to propagate fully the taste signals generated by tastants acting at GPCRs.

B. ATP Propagates the Taste Signal Cell-To-Cell

The combination of increased intracellular calcium and membrane depolarization is the final stimulus for the release of ATP, which is the humoral intercellular messenger that propagates the taste signal (Finger et al., 2005). The identity of ATP as the messenger has been established by multiple observations. The first indication came from immunohistochemical evidence of intense staining of labeled antibodies directed against P2X3 and P2X2 ionotropic purinergic receptors for ATP (reviewed in Jarvis and Khakh, 2009) on nerve fibers innervating rat taste buds (Bo et al., 1999). The two proteins can combine to form a functional heterodimer ion channel (Lewis et al., 1995). Ablation of the genes for either P2X2 or P2X3 in mice resulted in taste impairments, but loss of both genes in a double knockout created a mouse model that has been characterized as "taste blind" (Ohkuri et al., 2012; Sclafani and Ackroff, 2014). These findings strongly implicated ATP as an important humoral factor in the communication between taste cells and sensory afferents in the taste bud. Release of ATP in response to tastant stimulation has been directly observed in both taste tissue (Finger et al., 2005) and isolated taste cells (Huang et al., 2007).

The mechanism by which ATP is released from taste cells originally was thought to occur through hemichannels —either connexins (Romanov et al., 2007) or pannexin 1 (Huang et al., 2007; Dando and Roper, 2009), both of which are known to conduct ATP as an intercellular signaling molecule for a variety of cell types (reviewed in Wang et al., 2013). However, ATP release from taste cells was found to be unimpaired in mice devoid of functional pannexin 1 (Romanov et al., 2012), which furthermore were normal in physiologic and behavioral measures of taste responsiveness (Vandenbeuch et al., 2015). Another candidate channel, calcium homeostasis modulator 1 (CALHM1), also found to be selectively expressed in mouse taste receptor cells, now is thought to be the more likely conduit of ATP, as loss of CALHM1 by genetic ablation results in a taste-impaired phenotype (Taruno et al., 2013). Indeed, recent evidence has been reported indicating that CALHM1 interacts with CALHM3 to form a hetero-hexameric functional unit that serves as the channel for communication of ATP between taste cells (Ma et al., 2018).

C. Type II Receptor Taste Cells Each are Dedicated to Signal a Specific Taste Quality

Several different models of neural coding have been, and continue to be, debated to account for the ability of an organism to discriminate among different tastes. According to the "labeled line" hypothesis (Yarmolinsky et al., 2009), Type II receptor cells are committed to transmitting a signal that remains faithful all along the afferent pathway to regions in the brain that are topographically separate (Chen et al., 2011), independently processing incoming information about the different taste qualities (see *section II* for discussion of taste quality). An alternative, perhaps more nuanced model posits a combinatorial processing of multiple potential sources of taste signals from either or both "specialist" Type II cells and "generalist" Type III cells, with the potential of further coding occurring among specialist and generalist afferent nerve fibers (Wu et al., 2015). Although a strong consensus on the mechanism of neural coding for taste has not yet been reached, abundant evidence supports the notion of "specialized" Type II cells, dedicated to convey signals for a specific taste quality, and "generalized" Type III cells, potentially capable of carrying information about multiple qualities.

Type II taste cells can be categorized according to whether they exclusively express TAS1R2/R3, TAS1R1/R3, or any of the TAS2R receptors. The categorization is supported by non-overlapping patterns of specific receptor expression. In the earliest studies of tastant receptor identification, in situ hybridization of anti-sense RNA probes indicated that TAS1R1 and TAS1R2 occurred in separate subpopulations of cells from mouse taste tissue, whereas TAS1R3 was coexpressed in both sets of cells. TAS2R probes appeared in yet another subpopulation that did not overlap with any TAS1R receptor-expressing cells (Nelson et al., 2001). More recently, sequences for GFP-labels have been inserted into the genes encoding TAS1R and TAS2R receptors to create murine models in which receptor location can easily be visualized (Damak et al., 2008; Voigt et al., 2012). These studies have confirmed that receptors for tastant agonists, which impart specific taste qualities, are expressed exclusively in independent subpopulations of Type II cells. Moreover, fluorescence video microscopy of Type II receptor cells from mice engineered to express GFP-labeled PLCβ2 (and thus unequivocally identifying receptor cells) were demonstrated to be narrowly tuned to tastant activation, responding only to agonists each representing independent taste qualities (Tomchik et al., 2007). Interestingly, also in that study, Type III presynaptic taste cells responded to multiple tastant agonists regardless of taste quality category, a finding that appears to be inconsistent with expectations of the labeled line hypothesis of neural taste coding.

Although the receptors expressed in Type II cells are selective for tastant agonists each of which is associated with a taste quality, the stimulus qualities of sweet, bitter, and "umami" are properties that originate with the cell. This has compellingly been shown to be the case by experiments in which the expression of a κ opioid receptor was directed to sweet-specific cells by placing its coding sequence downstream of the promotor region for the TAS1R2 protomer. When expression was induced in vivo, the genetically engineered mice responded to solutions of spiradoline (a selective κ opioid agonist) as though they were solutions of a sweet tastant. Conversely, when the receptor was expressed in bitter cells, spiradoline solutions were avoided (Zhao et al., 2003). The concentration-response functions obtained in the in vivo brief access assays (see *section X.A.1*) of these experiments were consistent with the affinity values established for spiradoline at κ receptors (Cheng et al., 1992).

A preponderance of evidence indicates that it is the specialized characteristics and resulting functional distinctions of Type II receptor taste cells that are the source for independent taste qualities. The receptors expressed in these cells are selective for the ligands that are associated with the taste qualities. Taste cells therefore convey two pieces of information: 1) taste quality, determined by the specific cells stimulated, and 2) tastant concentration, by way of concentration-dependence of receptor activity.

D. Recombinant Cell-Based Assays for TAS2R Receptors

Identification of the genes for TAS1Rs and TAS2Rs has enabled the creation of recombinant cell lines so that the pharmacology of the agonist-receptor function can be systematically and conveniently investigated in vitro. Unlike native taste cells, where α-gustducin is thought to be critical for coupling the receptors for tastant agonists to the phospholipase C signaling cascade, recombinant cells generally require coexpression of a promiscuous Gα subunit for efficient measurement of tastant receptor responses. The murine G protein α15 subunit first was used to generate recombinant HEK293 cells, useful for measuring responses of transfected tastant receptors by recording intracellular calcium mobilization with fluorescence imaging microscopy. For example, coexpression of Gα15 enabled the first functional characterizations of the murine TAS2R5 (Chandrashekar et al., 2000), a receptor mediating the aversive taste response of cycloheximide in rodents (Mueller et al., 2005), and the human TAS2R16 receptor, which responds to the bitter tastant salicin (Bufe et al., 2002).

Both murine Gα15 and its human ortholog Gα16 are capable of coupling many different GPCRs to the phospholipase signaling pathway (Offermanns and Simon, 1995) and therefore have proven very useful in the development of assays based on fluorescence measurement of intracellular calcium mobilization (Zhu et al., 2008). Ueda et al. (2003) replaced the α16 C

terminus with different-length portions of the gustducin C terminus to optimize the coupling of tastant receptors to calcium signaling. In the process, the last 44 residues, which include β sheet and α helix structures as well as the extreme amino terminus, were determined to be critical for efficient tastant receptor coupling to calcium signaling. The resulting chimeric G protein α subunit, designated $G_{16}/gust_{44}$, was coexpressed with mTAS2R5 and hTAS2R16 in HEK293 cells and concentration-response functions for cycloheximide and salicin were established by calcium imaging microscopy. The functions for mTAS2R5 and hTAS2R16 yielded EC_{50} values of 0.5 μM and 2 mM, respectively, in close agreement with previously reported results (Chandrashekar et al., 2000; Bufe et al., 2002). The β sheet region of $G\alpha_{16}/gust_{44}$ apparently was required for coupling the tastant receptors to the calcium response, as calcium responses to salicin were not detected when hTAS2R16 was coexpressed with shorter α subunit chimeras that did not contain the structure (Ueda et al., 2003). The $G\alpha_{16}/gust_{44}$ chimera has since been incorporated into most recombinant cell-based assays for functional characterization of tastant receptors.

Now recombinant cell-based assays have been developed for all known human and mouse TAS2R receptors, and nearly all have been reported to demonstrate agonist activation by ligands characterized as bitter tasting to humans (Meyerhof et al., 2010) or aversive to mice (Lossow et al., 2016). Detailed concentration-response analyses of TAS2R agonists have been carried out in many of the recombinant cell-based assays. However, there are relatively few comparable concentration-response analyses in human or rodent in vivo studies to support the evidence that what is observed in the cell-based assay is in fact the equivalent of a bitter taste response. There are a few notable exceptions that convincingly associate TAS2R activity with specific taste responses in vivo.

Ablation of the gene that encodes for TAS2R5 in mice eliminated the aversive taste response to cycloheximide (Mueller et al., 2005), confirming the association between receptor, ligand, and behavior. As mentioned above, expression of the mTAS2R5 receptor in a null cell line created for the first time a recombinant taste cell in which the pharmacology of the receptor could be studied in vitro by a calcium fluorescence imaging assay. The recombinant cells responded to cycloheximide with a concentration dependence that varied between two alleles of the gene encoding the receptor—years before, strain-dependent sensitivities to cycloheximide in a taste-related behavioral assay had been documented and localized to the Cyx gene locus (Lush and Holland, 1988). Concentration-response functions now obtained from cells expressing the receptor from the allele found in "taster" strains were shifted to the left of those obtained from cells expressing the receptor found in less sensitive "non-taster" strains (e.g., CB57Bl/6J). The shift between the concentration-response functions of the two recombinant cell lines was consistent with the differences in ranges of sensitivities observed previously in the behavior of the mouse strains (Chandrashekar et al., 2000). Murine TAS2R5 was therefore regarded as the first example of a taste receptor isolated and unequivocally associated with a cognate tastant agonist.

The first evidence for a genetic basis of human taste was provided by tests of sensitivity to the bitterness of phenylthiocarbaminde (PTC) and propylthiouracil (PROP), by which distinct "taster" and "non-taster" phenotypes have been established (Fox, 1932; reviewed in Bartoshuk, 2000). The responses have been found to be critically dependent on the hTAS2R38 receptor (Kim et al., 2003). The phenotypes have been shown to result from amino acid variations at position 49 (alanine to proline), 262 (valine to alanine), and 296 (isoleucine to valine) of the TAS2R38 receptor sequence. Alleles identified as PAV and AVI, in reference to the substitutions at positions 49, 262, and 296, underlie the two most common phenotypes of "taster" and "non-taster," respectively (Kim et al., 2003; Bufe et al., 2005).

The genes encoding the PAV and AVI alleles were cloned from two individuals who were homozygous for each and used to create allele-specific recombinant hTAS2R38-HEK293 cell lines in which calcium responses to PTC and PROP could be rapidly assessed in a calcium fluorescence imaging assay (Bufe et al., 2005). Concentration-response analysis of the PAV allele of hTAS2R38 yielded EC_{50} values of 1.1 and 2.1 μM, respectively, for PTC and PROP. In contrast, cells expressing the AVI form of hTAS2R38 were unresponsive to PTC and PROP tested up to concentrations of 1 mM. Point mutation of the PAV allele sequence then was used to create additional hTAS2R38 variants representing less common haplotypes of PVI, AAI, and AAV, as well as three others (PAI, AVV, and PVV) that are not known to occur in human populations. By comparing the concentration-response functions for PTC and PROP obtained from all eight hTAS2R38 variant cell lines it was determined that substitution at positions 49 and 262 were most important for receptor activity, whereas those occurring at 296 had no measurable impact. Interestingly the EC_{50} values obtained for all responsive mutants were approximately equivalent, but response maxima were substantially reduced as a consequence of the substitutions. Antibody detection of the C-terminal HSV tag present on all of the hTAS2R38 variants indicated some differences in receptor expression. However, equivalent levels of hTAS2R38 expression were apparent for the AVI, AAI, and PAV constructs, suggesting that positions 49 and 262 probably are not involved with receptor-ligand interactions, but instead might be important for coupling the receptor to signal transduction (Bufe et al., 2005).

Another human receptor, hTAS2R16, was transfected into HEK293 cells to generate a recombinant cell line

responsive to β-glucopyranosides (Bufe et al., 2002). The concentration-response analysis for the bitter glucoside salicin yielded an EC_{50} of 1.4 mM, essentially equivalent to the results obtained from triangle tests (a taste discrimination assay, see *section X.B.1*) performed with human subjects (EC_{50} of 1.1 mM). Several other related glucosides generated similar concentration-response functions in both the cell line and in the human taste discrimination test.

Aloin, a substance from *Aloe spp* used as a bitter flavoring agent for some alcoholic beverages, has been shown selectively to stimulate human hTAS2R43 with an in vitro potency of 1.2 μM (Pronin et al., 2007). The in vitro potency of aloin was allele dependent, with a tryptophan residue at position 35 (located in the first intracellular loop) being critical for high potency. Human subjects with this allele also were reported to detect aloin bitterness with a concentration threshold of 1 μM. At least 10- to 20-fold higher concentrations were required to achieve the same activity both in vitro and in vivo for hTAS2R43 alleles in which serine replaced tryptophan at position 35. This receptor appears to be involved in the bitter taste imparted by high concentrations of the non-nutritive sweeteners saccharin and acesulfame potassium (Kuhn et al., 2004). As with hTAS2R43, saccharin and acesulfame potassium at high concentrations also are agonists for the closely related hTAS2R31 (formerly hTAS2R44), and its activation might contribute substantially more to the bitter off tastes of non-nutritive sweeteners (Roudnitzky et al., 2011; Allen et al., 2013).

Aristolochic acid, isolated from *Aristolochia spp* often used in traditional Chinese herbal remedies (Wang and Chan, 2014), is a potent agonist of both hTAS2R43 and hTAS2R31. Tryptophan at position 35 of both receptors also was a determinant of aristolochic acid potency, with EC_{50} values of 8 and 240 nM, respectively, for hTAS2R43 and hTAS2R31, and lower potencies when tryptophan was substituted by other amino acids (Pronin et al., 2007). Only one other receptor, hTAS2R14, is known to be activated by aristolochic acid in a cell-based assay and then only at much higher concentrations (Pronin et al., 2007).

A comprehensive analysis of concentration-response functions for agonists of recombinantly expressed human (Meyerhof et al., 2010) and mouse (Lossow et al., 2016) TAS2Rs has been carried out, with the majority of the EC_{50} values reported in supplemental material for each publication. With the few exceptions noted above, most of the known TAS2R receptor agonists are neither potent nor selective. Perhaps the greatest obstacle to unequivocal assignment of an in vivo tastant agonist response to any particular receptor is the promiscuity of many bitter ligands. For example, quinine, the quintessential bitter tastant standard, appears to activate at least nine different human TAS2Rs in a recombinant cell-based assay (Meyerhof et al., 2010).

To date, very few ligands other than agonists have been reported for the TAS2R receptors. A noncompetitive antagonist of hTAS2R43 and hTAS2R31, GIV3727 (mentioned above), inhibited responses to aristolochic acid, saccharin, and acesulfame potassium in cell-based assays and also was effective in mitigating the bitter off tastes of saccharin and acesulfame potassium in human taste tests (Slack et al., 2010). In addition to GIV3727, probenecid has been reported to antagonize hTAS2R16 in vitro, but with only modest inhibition of the bitterness of salicin in human taste tests (Greene et al., 2011). Additional molecules have appeared in the patent literature that show promise as selective high affinity antagonists for receptors that mediate bitter taste (see for example Karanewsky et al., 2011). As research continues, the discovery of new potent, highly selective antagonists, as well as more information on genetics underlying bitter taste phenotypes, is likely to clarify the uncertain relationships between most bitter tastant agonists and the receptors through which they impart their taste properties.

E. Recombinant Cell-Based Assays for TAS1R2/R3 Receptors

Serial selective expression of the protomer units TAS1R2 and TAS1R3 for rat (Nelson et al., 2001) and human (Li et al., 2002; Servant et al., 2010) receptors in HEK293 cells resulted in the generation of recombinant cell lines useful for pharmacologic analysis of agonists that are detected as sweet in behavioral assays. The heterodimeric functional receptor was responsive to a broad variety of compounds, across multiple chemical classes, reflective of the great variety of compounds that are detected as sweet tasting. Those cell lines expressing rat versions of the receptor were not responsive to the human sweeteners aspartame, cyclamate, and the protein thaumatin, consistent with the lack of sensitivity to these compounds by rodents in behavioral assays (Bachmanov et al., 2001; Palmer et al., 2013). This species-dependence of responsiveness to sweeteners has been exploited to identify likely agonist binding sites within the structure of the receptor (Jiang et al., 2004, 2005a,b; Zhang et al., 2010).

Concentration-response functions for several sweet-tasting agonists have been generated in the hTAS1R2/R3-expressing cell lines, showing characteristic logistic functions from which EC_{50} values have been derived, or can be estimated by visual inspection, so that agonist potency profiles of sweeteners can be established (see Table 1). With few exceptions, the potencies explicitly reported or that are apparent from the functions of each sweetener obtained by these cell-based assays are relatively stable across experiments and laboratories. For example, hTAS1R2/R3 cell-based assay EC_{50} values explicitly reported for sucrose of 54 and 62 mM (Servant et al., 2010) generally are in agreement with concentration-response functions graphically presented in other studies (which

TABLE 1

In vitro potency values for sweet-tasting compounds

Potencies (EC$_{50}$ values) of tastant agonists of human TAS1R2/R3 obtained from concentration-response functions in fluorescence calcium imaging cell-based assay. In many cases, potency values were not explicitly stated in the literature cited. When not explicitly stated, EC$_{50}$ values have been approximated by visual inspection of concentration-response curves shown in the figures from those papers (italicized in the table).

Tastant	EC$_{50}$	Reference
Sucrose	*~60 mM (Graph, Fig. 2C), without Gα15*	Li et al. (2002)
	~30 mM (Graph, Fig. 2D), with Gα15	
	~60 mM (Graph, Fig. 4b)	Zhang et al. (2010)
	62 mM	Servant et al. (2010)
	54 mM	
	~30 mM (Graph, Fig. 2B)	Xu et al. (2004)
	19.4 mM (in Supporting Information, caption to Fig. 6)	
Sucralose	27 μM	Shimizu et al. (2014)
	~100 μM (Graph, Fig. 2a)	Zhang et al. (2010)
	~100 μM (Graph, Fig. 2c)	
	~200 μM (Graph, Supporting Information, Fig. S2A)	
	60 μM	Servant et al. (2010)
	120 μM	
	59 μM	
	74 μM	
	39 μM	
	36 μM	
	31 μM	
	36 μM	
	62 μM	
	62 μM	
Saccharin	*~200 μM (Graph, Fig. 2C), without Gα15*	Li et al. (2002)
	~60 μM (Graph, Fig. 2D), with Gα15	
	300 μM	Sanematsu et al. (2014)
	370 μM	Imada et al. (2010)
	42 μM (in Supporting Information, caption to Fig. 6)	Xu et al. (2004)
ACE K	120 μM	Winnig et al. (2007)
	940 μM	Imada et al. (2010)
Aspartame	*~2 mM (Graph, Fig. 2C), without Gα15*	Li et al. (2002)
	~0.5 mM (Graph, Fig. 2D), with Gα15	
	500 μM	Winnig et al. (2007)
	1.3 mM	
	1.2 mM	Imada et al. (2010)
	5 mM	Liu et al. (2011)
	~0.3 mM (Graph, Fig. 2B)	Xu et al. (2004)
	245 μM (in Supplemental Table 1)	Maillet et al. (2015)
D-Tryptophan	3.3 mM	Jiang et al. (2005b)
	4.3 mM	Jiang et al. (2005a)
	~3 mM (Graph, Fig. 2C), without Gα15	Li et al. (2002)
	~0.8 mM (Graph, Fig. 2D), with Gα15	
	~7 mM (Graph, Fig. 4b)	Zhang et al. (2010)
	370 μM (in Supporting Information, caption to Fig. 6)	Xu et al. (2004)
Cyclamate	*~2 mM (Graph, Figs. 4C and 5E)*	Shimizu et al. (2014)
	3.1 mM	Jiang et al. (2005b)
	1.86 mM (in Supplemental Table 1)	Maillet et al. (2015)
	~3 mM (Graph, Fig. 4b)	Zhang et al. (2010)
	2.2 mM	Winnig et al. (2007)
	4.6 mM	Imada et al. (2010)
	~0.5 mM (Graph, Fig. 2B)	Xu et al. (2004)
Monellin	11.3 μM	Jiang et al. (2004)
Brazein	86.6 μM	Jiang et al. (2004)
Neotame	*~500 nM (Graph, S1A)*	Servant et al. (2010)
	14 μM	Liu et al. (2011)
	~2 μM (Graph, Fig. 2B)	Xu et al. (2004)

have not explicitly stated potency values). The lowest EC$_{50}$ reported is 19.2 mM (given in the supporting information of Xu et al., 2004). The non-nutritive sweetener sucralose is most often reported to be approximately 1000 times more potent than sucrose in cell-based assays. The concentration-response range of sucralose was intensively examined by Servant et al. (2010) for the purpose of demonstrating the effectiveness of a positive allosteric modulator of the hTAS1R2/R3 receptor; EC$_{50}$ values were reported to range between 31 and 120 μM, with a median value of 61 μM. Shimizu et al. (2014) also reported a slightly lower EC$_{50}$ for

sucralose of 27 μM, and graphic representation of concentration-response functions presented by others appear to indicate potencies of approximately 100–200 μM (with one example of a range 10-fold shifted to the right, evidently an outlier compared with all other sets of data found in the literature; see Table 1). Relatively few studies of concentration-response data generated using rodent TAS1R2/R3 cell-based assays have been published. The potency of sucralose in a recombinant murine TAS1R2/R3 cell-based assay graphically places its potency at about 10-fold to the right of human (Shimizu et al., 2014). Although EC$_{50}$ values were not

stated, graphic representation of the concentration-response functions for several sweeteners in a rTAS1R2/R3 assay (Nelson et al., 2001) suggests sucrose potency (~70 mM) to be similar to that of hTAS1R2/R3, whereas acesulfame potassium (~5 mM) and saccharin (~2 mM) appear as lower potency than has been shown for hTAS1R2/R3.

Concentration-response analyses from recombinant cell-based assays for TAS1 and TAS2 receptors clearly indicate the same adherence to pharmacological principles observed for any receptor-agonist pairing—nothing out of the ordinary has been revealed with respect to tastant agonist pharmacodynamics. A lawful expression of the receptor-mediated events thus should be expected to translate to the behavioral output of in vivo studies of taste. Whereas pharmacological studies of receptors mediating drug action on behavior must take into account the substantial impact of route of administration, distribution, metabolism, and elimination processes on observed dependent variables, taste studies have little in the way of pharmacokinetic complications to impede a direct interpretation of receptor functionality. Therefore in vivo taste studies should more closely resemble the pharmacodynamic results from tastant receptor cell-based assays.

X. Concentration-Response Relationships from In Vivo Taste Studies

A. Animal Models

In vivo assays, both animal and human models, have been extensively used to investigate the sensory aspects of taste. For animal models, the taste sensory measurements must be operationalized to overt behavioral responses that can be objectively observed and recorded. These assays can be classified as either measurement of consumption or discrimination. In the former, the volume of a tastant solution that is consumed is taken as an indication of its taste, but only indirectly. Palatability, or the probability that a substance in the oral cavity will be ingested or rejected (Palmer, 2007; Long et al., 2010), is thereby quantified in consumption assays and at the level of the data can only be taken as a direct measure of aversiveness or appetitiveness.

1. Consumption Assays. The simplest measure of animal taste is that obtained by two-bottle preference, where the volume consumed from two bottles in the home cage, one bottle containing water and the other a tastant solution, is compared after a specified period of time (usually 24–48 hours). The ratio of the two volumes then is taken as an indication of the appetitiveness or aversiveness of the tastant relative to water. Without being able to resolve taste quality and because of the potential for postingestive effects (Sclafani, 2001; Sclafani and Ackroff, 2012; Myers et al., 2013), the value of this assay mostly is limited to a minimal indication of the presence or absence of a functional receptor mediating a

taste response. For example, the two-bottle preference test has been used to support the notion of genetically determined differences in taste sensitivity among different mouse strains (Boughter and Whitney, 1997; Inoue et al., 2007) and also to rapidly demonstrate the effects of disruption of genes thought to encode receptors mediating taste responses (Nelson et al., 2001; Damak et al., 2003). Nevertheless, the two-bottle preference test has been used to observe concentration dependence of consumption. With the caveat of the potential for postingestive effects, the concentration ranges for some tastant stimuli are reflective of those obtained in cell-based assays. For example, Bachmanov et al. (2001) reported an active range for sucralose preference in a two-bottle assay for B6 mice of between 0.25 and 25 mM, roughly consistent with the murine receptor cell-based concentration-response data of Shimizu et al. (2014).

The brief access assay, another consumption-based taste model, represents an improvement over the two-bottle method in that it can be used to rapidly establish concentration-response functions for a tastant while minimizing the impact of postingestive effects. In the brief access assay, rodents lick tastant solutions through spouts presented singly from an array of bottles mounted on a linear actuator (Smith, 2001; Devantier et al., 2008; Long et al., 2010). The array is moved so that a single spout is positioned for access through a port. A shutter over the port limits duration of spout access to a short period of time, usually 5 seconds per stimulus presentation. A convenient measure of taste stimulus cue is achieved through the rate at which the animal licks solutions from the spout, a dependent variable consistently shown to vary as a function of tastant concentration. As a consumption model for taste, the brief access assay can directly provide information only on aversiveness and appetiveness of a tastant solution, but taste quality often is inferred from the results, and the behavior therefore is referred to as "taste-guided." Given the short time courses involved in the measurements, concentration-response functions for taste obtained through the brief access assay should present a relatively close representation of the underlying activity of the receptors mediating taste signals in the tongue.

Full concentration-response functions often have been reported in studies using the brief access assay for both aversive and appetitive tastants. The results appear to match well those of the few cases in which recombinant cell-based assays of rat or mouse receptors have been used to generate concentration-response functions for tastants.

Cycloheximide is a eukaryotic protein synthesis inhibitor produced by *Streptomyces sp* (Leach et al., 1947) common in the rhizosphere where burrowing herbivorous rodents are likely to ingest it while foraging. The compound is toxic to mice, with an LD_{50} of 133 mg/kg (Lewis, 1996), and probably as an adaptive consequence, solutions of cycloheximide are aversive to mice

(Lush and Holland, 1988). Cycloheximide potencies reported for brief access assays using CB57bl/J6 mice (Adler et al., 2000; Boughter et al., 2005; Devantier et al., 2008), identified as a "non-taster" strain (Lush and Holland, 1988), are in close agreement with the concentration-response function obtained from the cell-based assay mentioned above for mTAS2R5 isolated from "non-taster" strains (Chandrashekar et al., 2000).

The brief access assay also has been used to assess in vivo pharmacology of tastant agonists in "humanized" mice that have been engineered to express the human ortholog of receptors, such as hTAS2R16 and hTAS2R38. Mueller et al. (2005) directed the expression of these receptors into bitter taste cells by inserting them under the control of a TAS2R promoter. Whereas control mice were indifferent to the aversive tastes of phenyl-β-D-glucopyranoside (an agonist at hTAS2R16; Bufe et al., 2002) and PTC, lick rates to these respective tastants were suppressed in mice expressing the hTAS16 and hTAS2R38 receptors. The resulting concentration-response functions were essentially equivalent to those reported for cell-based assays of these receptors (Bufe et al., 2002, 2005).

For other bitter tastants, the relationship between in vivo and in vitro measurements is not so straightforward. As mentioned above, many of the receptors thought to mediate bitter taste signals have been characterized by agonists that are not selective, and therefore any measurement of taste potency in intact organisms will be complicated by the likelihood of taste responses that result from activities of multiple receptors.

The brief access assay also has been used to establish concentration-response functions for appetitive tastants such as sweeteners. Sucrose potencies determined from concentration-response functions for sucrose using Sprague-Dawley rats have been shown to range narrowly between 40 and approximately 150 mM (Grobe and Spector, 2008; Palmer et al., 2013; Hashimoto and Spector, 2014). This range is consistent with the only published concentration-response function for rTAS1R2/3 (Nelson et al., 2001) found for this review.

The similarity between potencies obtained from concentration-response functions in cell-based assays and the brief access assay described here suggests 1) that the receptor is readily accessible to the tastant molecule in solution on the tongue, and 2) the behavioral measure taken in the brief access assay can directly reveal the activity of the agonist-occupied receptor without the complexities of pharmacokinetics that can otherwise obscure interpretations of pharmacodynamics in other in vivo systems. Potency data from brief access assays that do not match those of recombinant receptor cell-based assays point the way to further experimentation for investigating additional processes that are likely to impact the concentration of the tastant at the receptor compartment, or other factors that could impede agonist-receptor interactions, and thus greatly add to the knowledge base for taste processes. For example, Long et al. (2010) reported a pharmacologic analysis of taste-guided behavior mediated by the transient receptor potential vanilloid-1 ion channel receptor in which it was noted that the agonist potency profile and effectiveness of selective antagonists did not match in vitro potencies, but apparently were impacted by properties of ligand lipophilicity that influenced access to the receptor compartment.

2. Taste Discrimination. Taste discrimination is a behavioral paradigm that provides an objective determination of the taste quality detected by animals and humans. In animal models, the two main taste discrimination assays are operant taste discrimination and conditioned taste aversion (CTA), also referred to as conditioned avoidance.

In operant taste discrimination, the animal reports the taste quality it detects through operation of manipulanda. The procedure usually requires first training the animal to perform an operant task such as food-reinforced lever presses (Morrison and Norrison, 1966; Morrison, 1969; Palmer et al., 2013) or water-reinforced spout licking (Swartzwelder et al., 1980; St John and Spector, 1998; Grobe and Spector, 2008). Then through additional training, an antecedent, specific taste cue becomes associated with reinforcement on one of two or more operant choices. Different taste stimuli then are similarly trained to be exclusively associated with responses on another available manipulandum. Once animals are fully trained, the distribution of responses from one operant choice to the other following a taste stimulus is taken as a direct indication of its similarity or disparity with the sensory qualities of the training cues. The paradigm can be used to test the organism's ability to discriminate one taste quality from another (Stapleton et al., 2002; Grobe and Spector, 2008; Palmer et al., 2013) or a taste stimulus from water (Mariotti and Fiore, 1980; Slotnick, 1982; Delay et al., 2004). In the latter case, the lowest concentration of a tastant that reliably can be discriminated from water is defined as the taste detection threshold. For example, the sucrose detection threshold for Sprague-Dawley rats was determined by this method to be between 2 and 4.5 mM (Stapleton et al., 2002). In this case, the threshold was defined as the concentration that was discriminated from water statistically above 50% of trials. In other studies, taste threshold has been operationally defined as the inflection point of a sigmoidal function of the probability of correctly identifying a taste stimulus after multiple trials. Using this technique, Bales et al. (2015) reported a threshold of 12 mM for Sprague-Dawley rats (in the control, sham-lesioned rats of that study). With accommodation to the different statistical methods for their criterion, these threshold values appear consistent with the low ends of concentration-response function data obtained for sucrose from rat brief access assays and the cell-based rTAS1R2/R3 assay described above.

In another taste discrimination method (Palmer et al., 2013), rats were trained to discriminate a single concentration of sucrose (100 mM) from water, NaCl, quinine, and citric acid randomly presented in a 96-well plate with at least 90% accuracy. Rather than licking from spouts, rats in this study sampled stimulus solutions by licking from individual wells of the 96-well plate. Concentration-response functions obtained under these conditions yielded an EC_{50} for sucrose-appropriate lever presses of 28 mM (95% confidence interval 13–61 mM). In this study, the lick rates for the sucrose solutions were simultaneously recorded (Fig. 2), and concentration-response analysis of these yielded a similar EC_{50} for sucrose of 53 mM (95% confidence interval 23–123 mM). Thus, the studies from these different laboratories using different methods corroborate and are consistent with the in vitro results from the rTAS1R2/R3 cell-based assay described above.

CTA as used for the study of taste essentially is a Pavlovian taste generalization assay (Reilly, 1999; Heyer et al., 2003; Spector and Glendinning, 2009; Lin et al., 2014). An animal, usually a rodent, is given an injection of a drug that causes an aversive interoceptive unconditional stimulus (US) that is temporally paired with ingestion of a tastant solution [the conditional stimulus (CS)]. The association between US and CS is established (one pairing usually is sufficient) when subsequent presentations of the tastants are avoided. The animal's responses to a variety of other tastant solutions, or different concentrations of a single tastant, are recorded, and the presence of the relevant stimulus cue can be determined by the degree of avoidance observed. Although CTA can switch the valence of sucrose from appetitive to aversive, it does not affect its concentration-response relationship. When sucrose CTA has been combined with the brief access assay (Heyer et al., 2004), EC_{50} values for lick suppression are essentially the same as those of sucrose-dependent increases in licking without CTA. Thus nothing is changed with respect to the input of the stimulus mediated by receptor signaling; it is the animal's reaction to the stimulus that has changed.

B. Human Taste Tests

A broad variety of confounding variables from testing conditions have been shown to influence humans taste tests of even simple solutions of basic taste stimuli, such as color of the samples (Johnson and Clydesdale, 1982), instructions given to the participants (Frijters, 1979), characteristics of the immediate testing environment (Shepherd et al., 2008), interpretations of labels used to define dependent variables (Bartoshuk, 2000), among others (Meilgaard et al., 2016). The inherent complexities of humans as experimental subjects add further challenges in controlling variables that can influence experimental outcome. Factors such as age (Weiffenbach et al., 1982; Schwartz et al., 2009), ethnicity (Sable et al., 2012; Williams et al., 2016), gender (Bartoshuk et al., 1994; Yoshinaka et al., 2016), obesity (Pepino et al., 2010; Overberg et al., 2012), acute (Al'absi et al., 2012; Ileri-Gurel et al., 2013) and chronic (Small and Apkarian, 2006) stress, and mood (Nakagawa et al., 1996; Platte et al., 2013) are among many that have been reported to impact taste sensitivities. Thus, identifying extraneous variables and controlling for their potential impact on the results of human studies of taste present greater challenges than is the case for animal models where the subjects and conditions are more tractable. As might be expected, human studies often require larger n sizes and more trials per subject than is the case for animal studies to manage the variability and its effects on statistical power (Meilgaard et al., 2016).

Measurement of human taste is conducted by means of assays that generally fall into two methodological categories—discrimination and scaling (Bartoshuk, 1978; Snyder et al., 2006; Meilgaard et al., 2016). As in animal models of discrimination, human subjects also must perform the task of detecting differences between at least two tastants. But rather than operate a manipulandum to indicate a detected difference as in animal models, samples are directly distinguished one
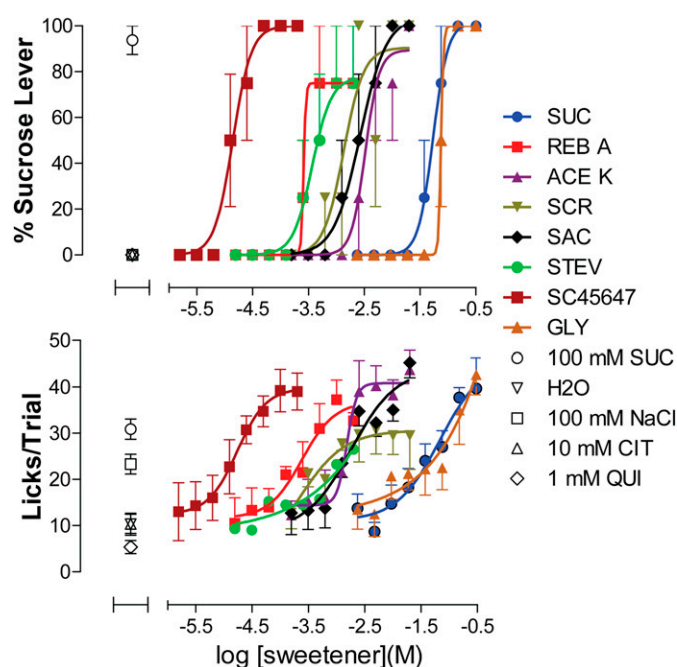

Fig. 2. Simultaneous measurement of taste quality and palatability as a function of tastant concentration. Rats were trained in an operant procedure to lick solutions from a 96-well plate and then press one of two available taste-associated levers for food. One lever was associated with the taste of sucrose, whereas the alternative lever was associated with the tastes of quinine, sodium chloride, citric acid, and water. The number of licks from a well on a given trial was taken as an indication of the solution's palatability and the distribution of lever presses as a measure of taste quality. Along with sucrose (SUC), the concentration dependence of taste-related behavior was tested for rebaudioside A (REB A), acesulfame potassium (ACE K), sucralose (SCR), saccharin (SAC), stevioside (STEV), SC45647, and glycine (GLY). Palatability and taste quality operate in the same concentration ranges. This figure is from Palmer et al. (2013) and its use here is permitted through the Creative Commons License.

from another in human taste discrimination studies. With scaling methods, subjects are instructed on how to use a scale that is designed by the investigator to indicate regular intervals of a taste property. In both discrimination and scaling, subjects commonly are presented with samples of test solutions dispensed in cups. Volumes of test sample typically range between 5 ml (e.g., Mennella et al., 2014, 2011) and 30 ml (e.g., McBride, 1986). In some cases, however, sample is applied to specific locations on the subject's tongue by the investigator using a cotton swab soaked with specific concentrations of tastant (McMahon et al., 2001; Heath et al., 2006). Presentation of samples containing different concentrations usually is randomized, but the "up-down" presentation procedure, in which the subject's response on one trial determines whether a higher or lower concentration is presented on the next, is sometimes used to arrive at the threshold value with fewer trials (Bartoshuk, 1978; Linschoten et al., 2001). Water is commonly offered as a rinse, and occasionally subjects additionally are given a bland cracker in between trials.

1. Taste Discrimination. Taste discrimination tests frequently have been used to establish the thresholds at which tastants elicit a taste response in humans. The variability inherent in human testing is concentrated on the single value determined for the threshold concentration, and therefore precise quantitation is allusive. Nevertheless, threshold values should approximate the beginning of the concentration-response function, thus providing an indication of the entire function's domain location.

The simplest version of the discrimination task is a monadic design in which subjects are presented with a tastant and asked to report whether they can detect the presence of the taste stimulus. "Yes-no" responses recorded over many trials of a randomized series of concentrations then can be used to construct a "psychometric function" where the threshold value is operationally defined as the concentration of tastant that is correctly identified on at least 75% of trials (i.e., midway between "guessing" at 50% and "certainty" at 100%; Linschoten et al., 2001). Using this procedure, Heath et al. (2006) studied the effects of monoamine reuptake inhibitors paroxetine and reboxetine in healthy subjects on thresholds for the basic taste stimuli sucrose, quinine, NaCl, and HCl. Average thresholds when measured prior to administration of inhibitors were determined to range between 24 and 26 mM for sucrose. Following administration of the SSRI paroxetine, sucrose thresholds obtained in the same individuals decreased to a value of 17 mM, suggesting that increases in serotonin resulting from paroxetine administration might have enhanced sweet taste sensitivity. Curiously, when measured before and after a placebo condition (administration of lactose), thresholds of 44 and 41 mM were reported, clearly higher than the 24–26 mM values recorded for the baseline pretreatment condition. The authors offer the

speculation that unaccounted environmental variables must have had some noticeable influence on the experimental outcome across sessions (Heath et al., 2006).

Another commonly used taste discrimination method is the two-alternative forced choice (2-AFC), in which the subject identifies which of two samples presented is the one that imparts a declared stimulus property, such as "sweetness" or "sweeter than" (Bartoshuk, 1978; McClure and Lawless, 2010). A study using a 2-AFC procedure (Joseph et al., 2016) reported sucrose threshold measurements for 216 children (ages of 7–14 years) to range between 0.23 and 153.8 mM, with an overall average of 12 mM. Further analyses found that sucrose taste thresholds correlated with subject-dependent variables such as sex, where girls had lower thresholds than boys on average (10.5 vs. 13.9 mM, respectively).

Also often used is the triangle test, a slightly more complicated discrimination task in which a subject attempts to distinguish one test sample from two "blanks" (two samples that are identical; Lau et al., 2004; McClure and Lawless, 2010). Many trials are conducted until the concentration that is distinguishable from the relevant comparator blanks is determined to statistical confidence. For example, a triangle test was used to investigate the relationship between sucrose thresholds and density of fungiform papillae in a group of 170 young adult men (Zhang et al., 2009). A mean threshold of 10.83 mM was reported for the group overall, ranging among individuals (each tested five times) from 5.85 to 19.88 mM. Sucrose detection thresholds were inversely correlated with density of fungiform papillae.

In some cases of threshold determination, a distinction is made between "detection threshold" and "recognitions threshold," where the former is the concentration at which a subject identifies some unspecified taste stimulus in a sample, and the latter is that which the taste quality of interest is recognizable. For example, Chang et al. (2006) found taste detection thresholds for sucrose in adult men and women of between 1.8 and 2.2 mM, with higher recognition thresholds of between 13.5 and 17.9 mM. Slightly different thresholds resulted from sorting the subjects according to their sensitivities to the bitter taste of PROP, with "super tasters," "tasters," and "non-tasters" having sucrose detection thresholds of 1.5, 1.8, and 3.7 mM, respectively, and recognition thresholds of 14.3, 15.5, and 18.6 mM, respectively.

Although the variability in threshold measurements can be substantial due to the subject- and condition-dependent variables mentioned above, a tendency for sucrose values to occur in the low 10s of millimolar is evident in the literature. Threshold concentrations in this range correspond to the low end of the concentration-response functions obtained in recombinant TAS1R2/R3 cell-based assays (i.e., below EC_{50}, see Table 1), as would be expected at the lowest fractions of receptor occupancy. Indeed, Li et al. (2002) reported that human detection thresholds (determined by a version of the triangle test)

for sucrose, D-tryptophan, aspartame, and saccharin equated with the lowest of the concentrations that stimulated responses in HEK293 cells expressing hTAS1R2/R3.

Pharmacological interpretation of threshold measurements conducted for tastant agonists that impart bitterness, however, is complicated by the multiplicity of receptors potentially mediating the response. Nevertheless, in the relatively few cases where a single tastant agonist unambiguously has been associated with a specific TAS2R receptor, threshold measurements obtained by discrimination tests can provide some insight into ligand-receptor functionality.

As mentioned earlier, it is clear that PROP and PTC are highly selective and potent agonists for the TAS2R38 receptor in recombinant cell-based assays. Bufe et al. (2005) identified subjects carrying PAV and AIV alleles and tested them in a 2-AFC discrimination test for threshold measurement of both PTC and PROP. Average PTC and PROP detection threshold values obtained from the resulting psychometric functions gave values of 3.28 and 10.7 μM, respectively, for subjects that were heterozygous for PAV. In contrast, subjects heterozygous for AIV required much higher concentrations, with detection thresholds measured at 97.4 and 327 μM for PTC and PROP, respectively. The investigators noted that the detection threshold values were comparable to EC_{50} values obtained from concentration-response analyses in their cell-based assay of the same haplotypes cloned out of two subjects and expressed in HEK293 cells (Bufe et al., 2005). A similar observation had previously been made of a correspondence between EC_{50} values obtained from salicin concentration-response functions generated in recombinant hTAS2R16 cell-based assays and a triangle discrimination test in humans.

The establishment of sensory thresholds often has been, and remains, a central experimental objective of human discrimination tests, not only because of the significance ascribed to thresholds by the psychophysics paradigm (see *section XI*), but also as a matter of practicality. Determining discriminability across the entire range of taste active concentrations in humans is a labor-, time-, and resource-intensive operation, requiring many subjects, trials, and test sessions. However, to arrive at a full accounting of tastant agonist behavior, taste responses must be examined across the full expanse of agonist-active concentrations, as is done for other receptor-mediated biology. Although there is an abundance of reports from which to draw inference relating taste responses to the lowest levels of receptor occupancy, it is equally, if not more, important to define the linear range and saturation region of the tastant concentration-response relationship. In the relatively few examples of studies in which discriminability is examined as a function of tastant concentration, some observations emerge that are puzzling in the context of what would be expected of receptor-mediated behavior.

One such example is given by McBride (1983) in a test of the Weber-Fechner psychophysical model (see *section XI*) applied to sucrose taste. "Just noticeable differences" (JNDs) were determined in 20 adults across 60 sessions using a variant of 2-AFC for six concentrations of sucrose, ranging from 25 to 500 mM. The JNDs obtained were directly proportional to the increases in sucrose concentration (except, as is often the case, near threshold), thereby largely confirming the Weber-Fechner model. Of particular significance for the current discussion, the JND remained relatively intact even at 500 mM, where discriminability from higher concentrations should be reaching a limit if receptors mediating the sucrose taste response also are simultaneously approaching the limits of occupancy. At what point does discriminability saturate? McBride (1983) states that "The viscosity of sucrose solutions increases markedly above .5000 M: the confounding of viscosity and sweetness would leave the investigator uncertain as to which cue serves in the discrimination." It was thus implied that saturation of the discriminability of sucrose might not be observable, because physical properties independent of taste could continue to change and be detected by the subject as the concentration increased beyond full receptor occupancy.

2. Scaling of Taste Intensity. Studies focusing on threshold values are acknowledged to fall short of providing a full account of the sensory experience obtainable from the entire range of active concentrations of a tastant stimulus (Bartoshuk and Snyder, 2004; Snyder et al., 2006), and measuring discriminability over the entire tastant concentration range is an arduous task. To address these perceived deficiencies, scaling methods have been developed with the intention of efficiently arriving at a comprehensive assessment of taste across the suprathreshold concentration range while relying on fewer subjects, trials, and test sessions (Stevens, 1969; Bartoshuk, 2000). Stevens (1957, 1961), strongly arguing the need for an alternative to Fechner's original model (which he considered "a failure"), laid much of the theoretical groundwork for a methodology by which humans could translate the magnitude of their subjective sensory experience into units on a scale.

According to Stevens, psychologic sensory magnitude and physical stimulus output are related by a power function (see *section XI*). By this relationship, the sensation experienced is proportional to the change in stimulus raised to an exponent. That ratio would be seen to hold over a wide range of stimuli, although not expected to continue infinitely—the limits, as well as the exponent, would be empirically determined (Stevens, 1957).

Under these assumptions, Stevens (1969) developed a method for scaling the subjective intensity of tastant stimuli. Subjects were instructed to assign a numerical value indicating their subjective experience of stimulus intensity to each of a series of tastant solutions. At the start of the experiment, subjects were given a single

concentration of tastant serving as a standard that was arbitrarily predesignated to represent an intensity value of 10. Then, to each subsequent tastant solution, subjects were explicitly instructed to assign a number reflecting their subjective intensity as a proportion of that experienced through contact with the standard of 10. "Thus, if a taste seems seven times as strong as the standard, call it 70; if half as strong, call it 5; if a 20th as strong, call it 0.5, etc. There is no upper or lower limit to the numbers you may use. The task is to make the numbers proportional to the taste intensity."

A straight line with a slope of 1.3 was fit to a log-log plot of the concentration of sucrose on the abscissa and perceived sweetness intensity on the ordinate, satisfying the linear version of Steven's equation (see *section XI*). In other words, the subjective experience of sweetness intensity increased exponentially as the 1.3 power of sucrose concentration. Thus, as noted by Stevens, the resulting concentration-response function for the sweetness of sucrose was positively accelerating. This positive acceleration continued through the highest concentration tested, 50% (by weight) or 1.46 M, and thus sweetness intensity ratings did not evince saturation. The exponents reported for several other early studies of sweetness intensity ratings varied considerably, indicating either positive or negative acceleration in the concentration-response functions (tabulated in Meiselman, 1971).

Using slightly different methodology, Bartoshuk et al. (1982) presented evidence that the exponents obtained from sweetness intensity power functions for sucrose could vary as temperatures of the solutions applied to the tongue ranged from 4 to 36°C. On the other hand, Calviño (1986) noted that sucrose intensity ratings often could be observed to depart from the linearity of the log-log plots used for representing data fit to power functions, implying more than one function. A series of sucrose intensity ratings confirmed that subjects' sweetness responses could be described by two power functions, each applied to data obtained across concentrations ranging below and above 12.5% (~365 mM). Calviño found that a better fit of the data could be achieved by using the Beidler equation (*section V*), and thereby appears to be the first to recognize that the characteristics of human taste intensity functions should follow those defined by receptor occupancy theory.

Scaling methods have continued to develop (Bartoshuk et al., 2004), but the introduction of each new scale is motivated by an objective to arrive at a better quantification of the subjective experience rather than bringing intensity rating data into alignment with receptor theory. For example, the "labeled magnitude scale (LMS)," in which subjects are instructed to scale their taste sensations in references to internal standards labeled by the verbal descriptors "barely detectable, weak, moderate, strong, very strong, and strongest imaginable" with respect to other oral sensations, was derived with the aim of obtaining valid across-group comparisons of taste

intensity (Green et al., 1993). A recent modification of the LMS, the "generalized labeled magnitude scale (gLMS; Bartoshuk et al., 2004), instructs the subject to generalize taste intensity ratings to the "strongest imaginable sensation of any kind." The gLMS is posited to normalize subjective taste sensations across individuals that are thought to experience vast differences in taste intensities (Bartoshuk et al., 2004) and now is in common use. Regardless of whether scaling methods accurately translate private events of sensory experience, the question of whether the data they generate can be related back to receptor function should be addressed. Although several earlier psychophysical studies of human sweet taste intensity appear to be at odds with the limits set by receptor function, a few more recent reports seem to be more in line with receptor theory.

A study examining the effects of obesity on sweet and umami taste among a group of 57 women used the gLMS to characterize the concentration dependence of sucrose sweetness intensity (Pepino et al., 2010). Rectangular hyperbolic concentration-response relationships were evident from graphs in which the square roots of sweetness intensity ratings were plotted against 20 mM increments of sucrose concentration, approaching saturation at 360 mM and beyond. These results are consistent with those of cell-based concentration-response analyses of sucrose activation of TAS2R2/R3 receptor-mediated response.

The gLMS also recently was used with a large group of 401 subjects to evaluate the concentration dependence of sweetness intensity for non-nutritive sweeteners acesulfame potassium, sucralose, aspartame, and rebaudioside A, in addition to sucrose (Antenucci and Hayes, 2015). Curve-fitting and data analyses were performed using nonlinear regression, yielding sigmoidal functions (plotted on semi-logarithmic scales), indicative of saturability, for all but acesulfame potassium, which generated an inverted-U function (suggestive of the occurrence of aversive tastes at higher concentrations). The maximal intensities for each of the non-nutritive sweeteners, indicated by R_{max} values from the curve fit, were below that obtained for sucrose. Potencies also were calculated, with reported EC_{50} values for aspartame, sucralose, rebaudioside A, and sucrose of 2.33, 1.99, 0.21, and 400.6 mM, respectively. Although the EC_{50} value determined for aspartame sweetness intensity falls within the range of those reported for cell-based TAS1R2/R3 assays, sucralose and sucrose were markedly less potent. Specifically, sucralose potency for sweet taste intensity differed by more than 10-fold from the median EC_{50} value reported in Servant et al. (2010) (see Table 1). An EC_{50} of 400.6 mM was determined for sucrose, placing its potency at approximately 40-fold to the right of most human threshold determinations, raising questions about the relationship between sweetness intensity, threshold detection, and receptor occupancy. Also, a sucrose EC_{50} of 400.6 mM (approximately

13.7 g/100 ml) implies that the sweetness intensity of a typical serving of cola, containing 11 g/100 ml of sugar (whether sucrose or high fructose corn syrup; see Ventura et al., 2011), is less than half maximal.

The pronounced differences in the characteristics of the sucrose sweetness intensity concentration-response functions generated by different laboratories over the decades are perplexing and difficult to reconcile with the behavior of agonist-occupied receptors. Many studies of concentration dependence of bitter taste intensity also have been conducted, but as discussed above, the demonstrated nonselectivity of most representative bitter tastant agonists is the chief obstacle in associating their results with the functions of specific receptors. However, an important illustration of the conundrum of a departure from expected receptor-limited taste behavior in humans is evident in gLMS of the aforementioned study of Bufe et al. (2005); whereas absolute thresholds from the 2-AFC tests (determined from the inflection point of the sigmoidal psychometric functions) corresponded to the EC_{50} values obtained in the cell-based assays, bitterness intensity ratings occurred in concentration ranges that were substantially right-shifted relative to those exhibited in both the cell-based assays and the 2-AFC discrimination and continued linearly well beyond the range in which saturation occurred in the cell-based assays. Suitable explanations for why taste intensity ratings tend to be right-shifted from other measures of tastant receptor activity obtained both in vitro and in vivo have yet to be addressed, but at this point it is not unreasonable to assume that data from taste intensity ratings are revealing additional processes.

XI. Founding Concepts of Psychophysics in the Study of Taste

Although more recent focus has been drawn to peripheral mechanisms, the study of taste has been predominated by the conceptual framework of psychophysics. Evidence of its influence even can be seen in the ways in which taste cells and the receptors that mediate taste signals are classified and studied.

Gustav Fechner is attributed as the founder of psychophysics, with his 1860 publication of *Elemente der Psychophysik*. Fechner's experimental approach was driven by his philosophical grappling with metaphysics and particularly the mind-body problem, which is centered on the question of how to explain the seeming immaterialism of the mind in the face of a materialist view of a universe defined by physical laws (Heidelberger, 2003). For Fechner, there were two separate worlds, one of which is physical and the other mental. The only one we can be certain of is the mental, but without a fundamental belief in the physical it would be impossible to function. Evidence for the existence of the physical world was obtained through sensory input. It was reasonable to conclude the reality of the external, physical world since

causality could be observed by events depicted through the senses and because there are other sentient beings with similar minds that behaved in similar ways in response to the witnessed physical events (Heidelberger 2003). After thoroughly developing the philosophical foundations for a new science sufficient to the challenges of systematically addressing the connection between physical and mental world, Fechner began to define the dependent and independent variables that would be the basis of the methodology for quantifying sensory experience.

The earlier work of Ernst Weber on the minimal discriminable differences between two stimuli served as a guide for Fechner (Ross, 1995). Weber determined that to be able to reliably distinguish the difference between two weights, for example, they must differ by a constant fraction. The "just noticeable difference," or JND, process could be observed in all sensory modalities, but the actual JND fraction observed was modality specific (Norwich, 1987). For Fechner, the JND represented a constant unit that could be empirically determined as the fundamental building block of the mind (Romand, 2012).

Fechner identified this fraction as the Weber constant, defined as

$$k = \frac{\Delta I}{I}$$

where I = the intensity output of the stimulus in question and ΔI is the smallest increment in intensity that can be perceived by the subject (Lawless, 2013). Further experimentation on human perception of light and sound suggested to Fechner that the Weber fraction by itself was not sufficient to describe mathematically the scale of sensory experience across large ranges of intensity output, particularly when comparing results from different sensory modalities. Fechner improved upon Weber's model by noting that the sensory experience of a stimulus intensity output increased according to a logarithmic function, incorporating the Weber fraction such that

$$p = k \ln \frac{I}{I_o}$$

where p = perceived intensity, and I_0 is the lowest stimulus intensity that can be perceived (the threshold of stimulus perception). Thus, it was critically important to determine the threshold stimulus intensity and also the JND. In Fechner's theory, JNDs were units that would add up, and ratios of summated JNDs then would be experienced as the magnitude of the change in intensity. For example, 100 JNDs should be perceived as twice the intensity magnitude of 50 JNDs (Lawless, 2013).

As experimentation on human perception continued on into the 20th century, the notion that the JND was a fundamental, additive unit was increasingly questioned.

Although Fechner's theory of perception laid a solid structure for conducting experiments, empirical results often did not meet the expectations set by the logarithmic model. Stevens (1961) pointed out that debate over the validity of Fenchner's principles had been ongoing since the publication of *Elemente der Psychophysik* with no clear consensus after 100 years of scientific inquiry, and a new psychophysical theory was needed for resolution. Stevens proposed a "law" that would replace Fechner, which states "simply that equal stimulus ratios produce equal subjective ratios" (Stevens, 1957). For Stevens, the philosophical problem underlying Fechner's "mistake" was the failure to recognize that sensory perception is related to physical stimulus intensity along two different continua, not just one, and because of this a logarithmic model would not always result from sensory measurement. Processing of information from sensory input essentially categorized sensory input as composed of quantitative (how much) and qualitative (what kind) information. These two types of information progressed along, "prothetic" and "metathetic" continua, respectively. The process of discriminating among prothetic properties of stimuli was additive, "adding excitation to excitation" as in sound amplitude or loudness. In contrast, the metathetic discrimination process was one of substitution, one quality for another, or as stated by Stevens, "changing the locus of excitation" (Stevens, 1957). In the context of taste, prothetic and metathetic continua would be experienced as changes in concentration and taste quality, respectively. Prothetic continua in particular were argued to produce perceptual functions that were best fit to an exponential function:

$$\Psi = k\,S^n$$

where Ψ is the sensation experienced, the variable k is a proportionality constant to accommodate the different units used for the independent variables for different stimuli, and the exponent n indicates the rate at which the subjective experience of intensity magnitude increases with changes in physical stimulus.

But it is clear from the work of Stevens (1969) and others (Meiselman et al., 1972) that the exponent so critical to the theory varies considerably across sensory modalities and even within different qualities of the same sensory modality. The value of n is empirically determined and does not seem to have any theoretical basis for representing a particular constant of biologic or physiologic significance.

XII. Concluding Comments

For most of the history of the study of taste, the methodologies used to interrogate taste-related phenomena and the paradigm through which experimental results have been interpreted were derived from the field of psychology. Psychology is more than an empirical science—it also comprises a broad collection of philosophies that attempt to probe some of the most fundamental questions of human existence, such as the sources and meaning of consciousness and awareness. The study of sensory perceptions always has been central to addressing these types of essential questions. In the early years of psychophysics, the mental world was postulated to be composed of elemental units that were only indirectly accessed through sensory discrimination methods. Steven's introduction of the theory and methodology for scaling sensory "intensity" was considered revolutionary because they were taken as direct, quantifiable translations of previously inaccessible private events of sensation (Bartoshuk, 2000). Receptors were always assumed to exist as the initial point of ingress for taste stimuli, but it is fair to say that an appreciation of the properties of receptor function contributed little, if at all, to the development of these ideas. The methods and theory behind both discrimination testing and scaling from these early sources remain in practice today, with relatively few modifications as the predominant paradigm for in vivo assays of taste. Even in animal models where taste responses are operationally defined, measurements of overt behavior often are interpreted as a reflection of internal sensory experiences. Thus, changes in tastant concentration are said to be detectable as changes in taste "intensity" (Gautam et al., 2012), and "changes in an animal's perceptual experience of the taste of saline" are thought to occur from depletion of dietary sodium (St John, 2017). Such instances of the language used to describe taste-related behavior suggest a top-down view of sensory processing (Rauss and Pourtois, 2013).

A bottom-up perspective on the study of taste began in the mid-20th century when physiologists such as Beidler recognized that the characteristics of neural responses to varying concentrations of tastants were best explained by molecules acting on receptors according to mass action law. Beidler's reasoning was equivalent to receptor theory, which at the time was already well-developed in the field of pharmacology, a framework for interpreting the lawfulness observed in tissue responses to exogenously applied chemicals. Currently, TAS1R and TAS2R cell-based assays have promoted the characterization of tastants as receptor agonists and shed light on the potential contributions by individual receptors to taste responses that could result from the actions of tastant agonists on multiple receptor types. By doing so, the resulting concentration-response functions obtained have defined limits on taste-related phenomena, including what can be expected of behavior and perceptual experiences. Recombinant cell-based assays are the best means currently available for determining how well receptor occupancy and coupling explain what is observed of taste responses in vivo. The development of cell-based assays also has provided a highly efficient means of identifying new ligands for probing receptor activity, but far more work in this

regard is needed, particularly with respect to discovery of potent, highly selective antagonists that could unambiguously clarify the contribution of specific TAS2Rs to the bitter taste imparted by non-selective agonists.

Analysis of concentration-response functions obtained from in vivo taste experiments can in turn inform on the activity of the underlying receptors if the data are in accord with a pharmacologic model. The consistency evident in the concentration-response functions across most animal models of taste, human taste discrimination, and cell-based assays implies that this is a valid assumption. A central outstanding question awaiting further investigation pertains to the quantitative relationship between taste discrimination data and receptor occupancy implied by cell-based assays. What is the relationship between the fraction of agonist-occupied receptors to the probability of taste recognition? Under an assumption that intrinsic efficacy does not complicate a linear relationship between receptor occupancy and the concentration-response function of a cell-based assay, the work of Bufe et al. (2005) suggests that thresholds for recognition of some bitter tastants roughly equate to a fractional occupancy of 0.5. However, the threshold values obtained for sucrose taste compared with the EC_{50} values obtained from TAS1R2/R3 cell-based assays could indicate that a smaller fractional occupancy is sufficient. How much greater must the fractional occupancy be before errors in taste discrimination approach zero?

Results that depart from the expected behavior of agonist-receptor functions present opportunities for further exploration by way of reasoning or experimentation.

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