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The sweet taste of true synergy: positive allosteric modulation of the human sweet taste receptor

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A diet low in carbohydrates helps to reduce the amount of ingested calories and to maintain a healthy weight. With this in mind, food and beverage companies have reformulated a large number of their products, replacing sugar or high fructose corn syrup with several different types of zero-calorie sweeteners to decrease or even totally eliminate their caloric content. A challenge remains, however, with the level of acceptance of some of these products in the market-place. Many consumers believe that zero-calorie sweeteners simply do not taste like sugar. A recent breakthrough reveals that positive allosteric modulators of the human sweet taste receptor, small molecules that enhance the receptor activity and sweetness perception, could be more effective than other reported taste enhancers at reducing calories in consumer products without compromising on the true taste of sugar. A unique mechanism of action at the receptor level could explain the robust synergy achieved with these new modulators.

The search for non-caloric sweeteners

Consuming foods that are high in calories and fat while living a sedentary lifestyle causes an energy imbalance that is at the root of various health conditions such as obesity, type II diabetes, and cardiovascular diseases, which have reached epidemic proportions in some countries. In the USA alone, more than 34% of people are overweight [1], resulting in more than $147 billion in associated annual medical costs [2]. Obesity is also estimated to cause approximately 9% of all deaths in adults each year in the US [3]. As a result, more than ever before, food and beverage companies have been under pressure to cut calories and sugar to improve the overall nutritional value of their products. However, more than 100 years of research has failed to produce a zero-calorie sweetener that fully reproduces the taste of sugar [4]. Alternative sweeteners have to be used at high concentrations and can exhibit objectionable off-tastes (bitter, metallic, liquorish, cooling), inadequate temporal properties (slow onset and/or lingering of sweet taste), or even a limited sweetness intensity at these levels [4–6]. Because many zero-calorie sweeteners, on their own, exhibit objectionable off-tastes, there has been a large research effort over the past several decades aimed at identifying the ideal combinations or blends of zero-calorie sweeteners that would deliver a formulation with the closest taste to sugar [7–11]. As a result of this work, several zero-calorie sweeteners have been reported to exhibit synergistic properties when mixed with one another or with a carbohydrate sweetener [7,12,13]. These are conditions in which the sweetness intensity of the sweetener mixture is reportedly greater than the sum of the sweetness intensity of each of the individual components. Even so, only minimal sweet taste enhancement can be achieved with these blends, and apparent synergy in taste tests can only be observed at lower sweetener levels. At higher levels, mostly sweetness additivity or even sweetness suppression is perceived [13].

The discovery of genes coding for the sweet taste receptor [14–18] has been a game changer. It has enabled the use of high-throughput screening technologies and classical discovery approaches to identify entirely new flavor ingredients, such as positive allosteric modulators (PAMs), answering the shortcomings of zero-calorie sweeteners and addressing the needs of manufacturers. In contrast to receptor agonists, PAMs for the human sweet taste receptor are absolutely tasteless at the intended use level but significantly enhance the activity of orthosteric agonist(s) of the receptor. These properties, coupled to their specific mechanism of action on the sweet taste receptor, could explain the remarkable level of synergy achieved with sucralose and sucrose in taste tests. Here we briefly discuss the biology of the sweet taste receptor, the discovery of the first class of PAMs, their mechanism of action at the receptor level, and how these PAMs compare with other reported sweet taste enhancers at enhancing sweet taste in humans.

The human sweet taste receptor

A considerable sequencing effort using a subtracted cDNA library derived from lingual tissue and analysis of genome sequence data for corresponding human G protein-coupled receptors (GPCRs) linked to the *Sac* locus led to the identification of 3 separate genes, *TAS1R1*, *TAS1R2* and *TAS1R3*, expressed specifically in taste receptor cells of the tongue and the soft palate [14–16,19–21]. These genes code respectively for three family C GPCRs, T1R1, T1R2 and T1R3, that are characterized by a large extracellular venus

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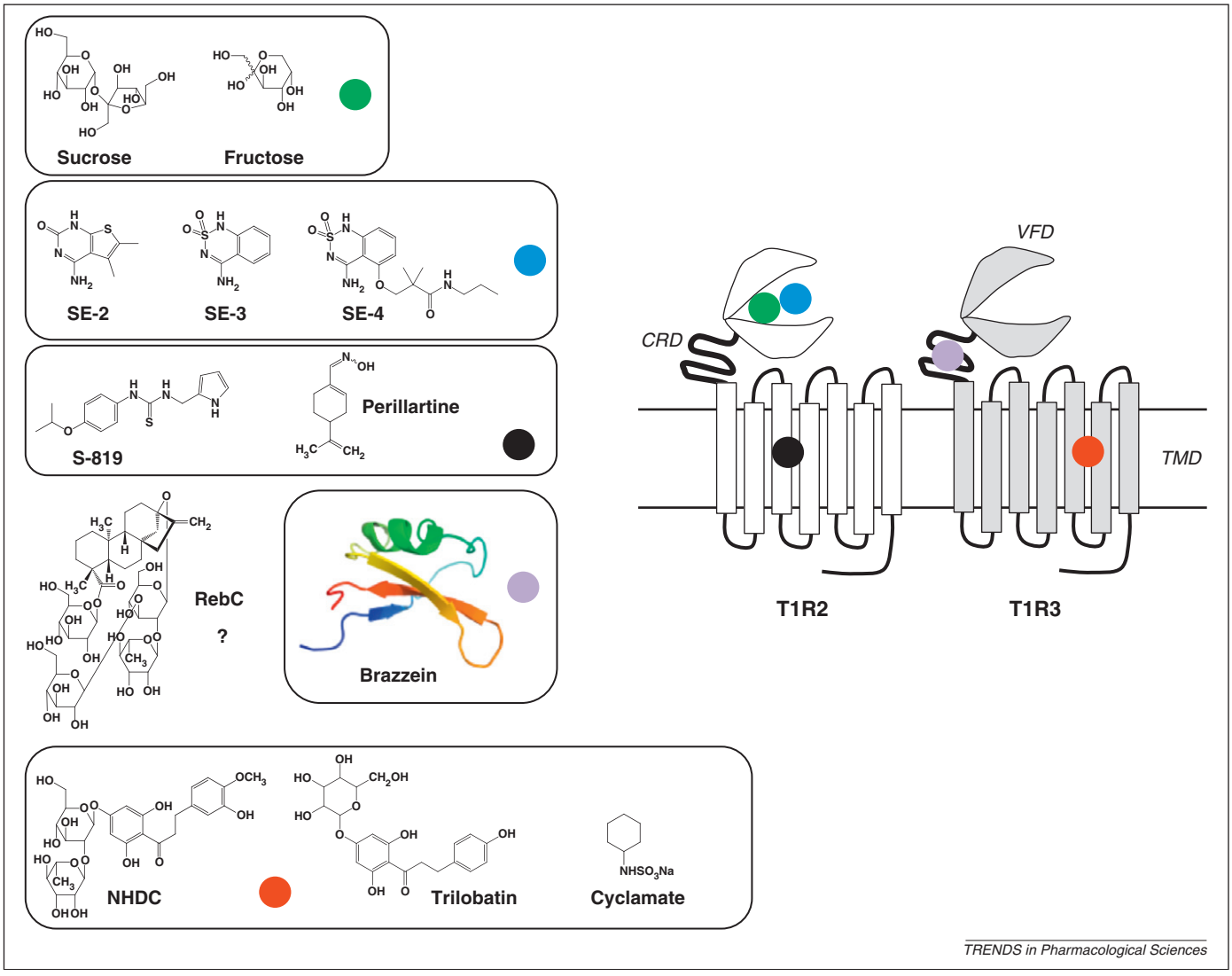

Figure 1. Structure of the sweet taste receptor and depiction of representative modulators. The sweet receptor is composed of two different subunits named T1R2 and T1R3 which in turn contain three distinct domains, the venus flytrap domain (VFD), the cysteine-rich domain (CRD) and the heptahelical transmembrane domain (TMD). Agonists and positive allosteric modulators interact on these different domains of the heterodimer, as depicted by the colored dots within the receptor subunits. The site of interaction for RebC has not yet been identified.

flytrap domain (VFD) linked to a canonical 7-transmembrane domain (TMD) via a short cysteine-rich domain (CRD) (Figure 1). Studies in cell-based assays and in knockout mice demonstrated that the sweet taste receptor functions as an heterodimeric receptor made of the T1R2 and T1R3 subunits, whereas the T1R1 and T1R3 subunits form a heterodimeric receptor for umami taste [the savory taste of monosodium glutamate (MSG)] [17,18,22,23]. Identification of genes encoding the sweet taste receptor has allowed the development of specific cell-based assays that have been used to identify the function of the receptors. Dozens of different synthetic and natural sweeteners have been characterized pharmacologically, and their rank order of potency in the assays correlates with their relative sweetness intensity in taste tests [24,25]. At least four different ligand-binding domains have been identified on the T1R2 and T1R3 subunits (Figure 1) [24,26,27]. This explains the propensity of the sweet receptor to be activated by a significant number of structurally distinct agonists that display several orders of magnitude differences in apparent affinity. Agonists can either activate the receptor by stabilizing the closed conformation of the VFD of hT1R2 [28–30] or by interacting with the TMD of T1R2 or T1R3 [26,28,31–33]. Additional residues located in the CRD of hT1R3 seem also important for receptor activation by sweet proteins [34]. The TMD of hT1R2 functionally interacts with the G protein [28]. It is not yet clear how the signal is transmitted to this domain from the VFD of hT1R2 and the TMD or the CRD of hT1R3.

Identification of PAMs for the human sweet taste receptor

Research over the past 15 years has revealed that family C GPCRs are very prone to allosteric modulation [35,36]. PAMs have been identified for several members of the metabotropic glutamate receptor family and the $GABA_B$ receptor. Every synthetic PAM identified for these receptors binds to the TMD to enhance the activity, and sometimes increase the affinity, of the natural ligand (glutamate, γ-aminobutyric acid, or calcium) interacting within the VFD [35–37]. Upon binding to the receptors, these small molecules exhibit no or little intrinsic agonist

activity on their own or are ago-potentiators (i.e. molecules acting both as agonists on their own and as enhancers for the endogenous agonists) [38,39]. Some of these PAMs, which have now entered clinical trials, are intended to treat conditions such as pain, anxiety, depression, and epilepsy [35,36]. Cinacalcet, a PAM of the calcium-sensing receptor, is the first of its kind to reach the market and is used as a calcimimetic to treat secondary

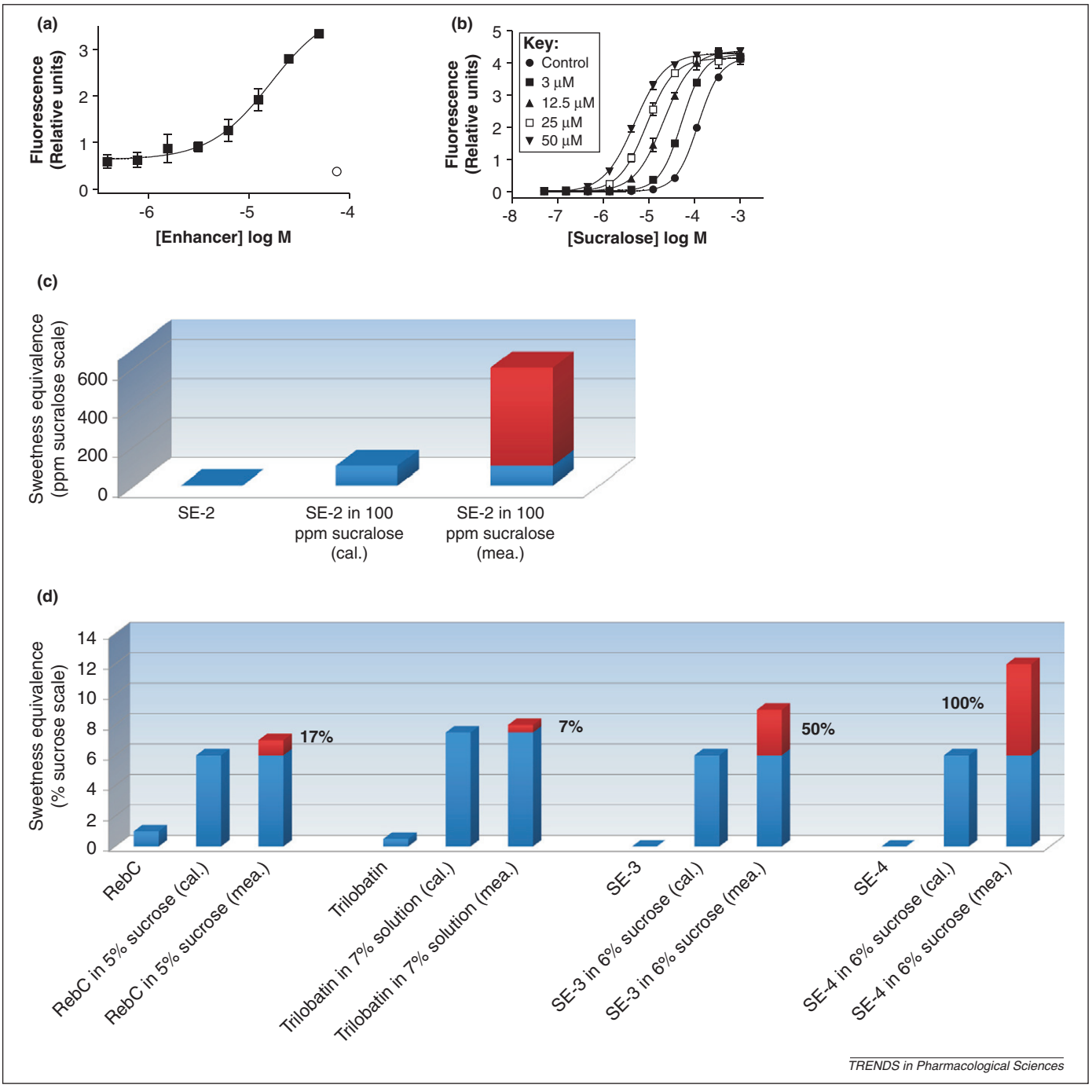

Figure 2. Effect of positive allosteric modulators and sweeteners in a cell-based assay for the sweet taste receptor and in taste tests. **(a)** SE-2 (Figure 1 for structure) dose-dependently activates the sweet taste receptor in the presence of a sub-optimal concentration of sucralose (closed circles). By contrast, SE-2 has no activity in the absence of sucralose (open circles), a typical behavior for a PAM. **(b)** SE-2 increases the potency of sucralose in the assay. SE-2 dose-dependently induces a leftward shift in the sucralose dose-response without affecting the bottom asymptote of the curve, another typical behavior for a PAM. **(c)** Evaluation of the effect of SE-2 on sucralose sweetness intensity in human panelists. 100 μM SE-2 brings the sweetness intensity of a 100 ppm sucralose solution (251 μM) to a level produced by a 600 ppm sucralose solution (1.506 mM). Note that 100 μM SE-2 does not elicit a sweet taste on its own. The net enhancement effect – the difference in sweetness intensity between the measured sweetness intensity obtained in taste tests (mea.) and the calculated sweetness intensity of the mixture (cal.) – is depicted in red. **(d)** Evaluation of the effects of two sweeteners, RebC and trilobatin and of two PAMs, SE-3 and SE-4, on the sweetness intensity of sucrose in human panelists. 285 ppm RebC (300 μM) brings the sweetness intensity of a 5% (w/v) sucrose solution (146 mM) to a level produced by a 7% (w/v) sucrose solution (204 mM). At this concentration, RebC elicits alone a sweetness intensity roughly equivalent to a 1% (w/v) sucrose solution (29 mM). 100 ppm trilobatin (229 μM) brings the sweetness intensity of a 7% (w/v) sucrose solution (204 mM) to a level produced by a 8% (w/v) sucrose solution (234 mM). At this concentration, trilobatin elicits alone a sweetness intensity roughly equivalent to a 0.5% (w/v) sucrose solution (15 mM). 100 μM SE-3 brings the sweetness intensity of a 6% (w/v) sucrose solution (175 mM) to a level produce by a 9% (w/v) sucrose solution (263 mM). At this concentration, SE-3 does not alone elicit a sweet taste. 25 μM SE-4 brings the sweetness intensity of a 6% (w/v) sucrose solution (175 mM) to a level produced by a 12% (w/v) sucrose solution (351 mM). At this concentration, SE-4 does not alone elicit a sweet taste. Data presented in (a–c) and taste test data with SE-3 in (d) were adapted from [40] with permission. Taste test data with RebC, trilobatin, and SE-4 in (d) were based on information from [49], [44] and [41], respectively.

hyperparathyroidism and hypercalcemia [37]. Utilizing a similar approach, involving a specific cell-based assay, high-throughput screening, and assay-guided chemical optimization, the first examples of PAMs for the sweet taste receptor were identified at Senomyx in 2007 [40].

One of these molecules, SE-2 (Figure 1 for structure), significantly enhances the activity (Figure 2a) and the potency (Figure 2b) of the zero-calorie sweetener sucralose in a cell-based assay [40]. In simple taste tests (in which the sweetener is dissolved in water), SE-2 allows for a reduction of four- to sixfold in the level of sucralose while preserving the sweetness intensity (Figure 2c) [40]. Notably, SE-2 is inactive in the cell-based assay in the absence of sucralose (Figure 2a) and is not sweet on its own at the intended use level (Figure 2c). Further optimization of the initial lead resulted in the identification of other potent and efficacious PAMs, such as SE-3 and SE-4 (Figure 1 for structures). These PAMs display an altered specificity, allowing a reduction of up to 50% in the amount of sucrose (table sugar) while maintaining the sweetness intensity (Figure 2d) [40,41]. Product prototypes SE-2 and SE-4 elicit unmatched levels of sweet taste enhancement [41,42]. Moreover, SE-2 can be used to reduce the off-tastes associated with high levels of sucralose in consumer products [42]. Importantly, in contrast to many non-caloric sweeteners, at use levels neither SE-2 nor SE-4 exhibits any bitterness or metallic off-taste or imparts undesirable temporal effects, and products containing these enhancers taste identically to the fully sweetened equivalent [41,42].

In marked contrast to PAMs for other family C GPCRs (which interact within the TMD) SE-2, SE-3 and SE-4 interact in close proximity to the sweetener agonist binding site located within the VFD of hT1R2 (Figure 1) [43]. Mutagenesis and modeling analysis suggest that, upon agonist binding near the hinge region and closure of the VFD, a binding pocket for the enhancers is created towards the outer rim, allowing hydrophobic interactions between crucial residues and the enhancer that further stabilize the agonist-bound closed conformation of the receptor (Figure 3) [43]. We believe that this mechanism of action explains, in part, the remarkable level of functional selectivity for these PAMs, which enhance either sucrose or sucralose, but no other sweeteners [40]. Direct or indirect interactions are thought to occur between the enhancer and the glucose moiety in the sucrose or sucralose molecule [43]. The absence of interaction with other sweeteners binding within the VFD or steric hindrance by more voluminous sweeteners could preclude PAMs from interacting with high affinity. Alternatively, it is also possible that less voluminous sweeteners, such as fructose, could stabilize a slightly different closed conformation which does not facilitate the binding of this class of enhancers.

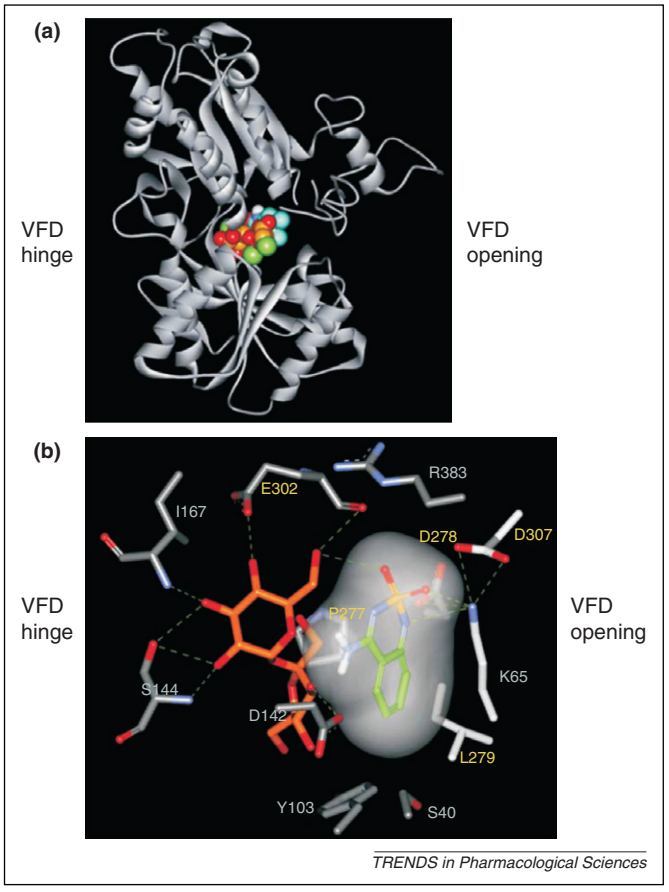

Figure 3. A molecular model of the T1R2 VFD domain. **(a)** The T1R2 VFD domain in a closed conformation with bound sucralose (carbon atoms in gold, oxygen in red, and chlorine in green) and SE-2 (carbon atoms in cyan). Sucralose and SE-2 are adjacent to each other between the two lobes of the flytrap. **(b)** A close view of the ligand-binding pocket seen from above the upper lobe with sucrose and SE-3 bound. Designations for lower lobe amino acid residues are labeled in yellow and upper lobe residues are in grey. Sucrose is in gold; SE-3 is in green and is encased in a grey surface. The three residues crucial for enhancer activities (K65, L279 and D307) are in white, and the seven residues crucial for sucrose/sucralose activities (S40, Y103, D142, D278, E302, P277, R383) are in grey. Figure adapted with permission from [43].

Enhancing sweet taste with a sweetener or a PAM?

Conclusions about the ability of sweeteners to enhance sweet taste can diverge based on the methods used to evaluate the taste test data [13]. Moreover, in all cases, the specific mechanism of action at the core of the stated effect has not been defined. Typically, the sweeteners are used at or near their own sweetness detection thresholds, therefore limiting their use (and potential effects) to concentrations where they do not exhibit significant inherent sweetness. The reported enhancement effects under these strict conditions are relatively small and are not always reproducible (see below). Some of the most popular sweeteners reported to exhibit enhancement properties include thaumatin, mogroside, neohesperidin dihydrochalcone (NHDC), and cyclamate (Figure 1 for structure depiction of some of these sweeteners) [7,13,44]. Often, such sweeteners will change the temporal properties rather than the magnitude of the sweetness intensity (i.e. prolonging the duration of the sweet taste when a lingering sweetener is used in combination with another sweetener having a shorter duration). Alternatively, some sweeteners will improve or enhance the overall flavor and sweet taste characteristic of another sweetener by masking its associated bitterness, metallic taste and other off notes [9,11,45,46]. Although these types of sweetener blends could have actual beneficial use in the food and beverage industry, they do not necessarily correspond to mixtures of compounds having *bona fide* robust synergistic effects. In fact, a thorough analysis of the effect of NHDC using full psychophysical taste function of sucrose with and without

NHDC at a sweetness detection threshold concentration suggests that it is not a taste enhancer in humans [47], a finding that was corroborated in another independent investigation [4].

Recently, two additional sweeteners have been reported to exhibit synergistic effects with sucrose in human volunteers. Trilobatin (Figure 1), a natural sweetener derived from the sweet plant *Lithocarpus polystachyus*, is also an analog of NHDC. When used at its approved use level of 100 ppm (229 μM), a level near its detection threshold and which provides a sweetness intensity equivalent to a 0.5% (w/v) sucrose solution, it increases the sweetness intensity of a 7% sucrose solution to that seen with an 8% sucrose solution (Figure 2d) [44]. When considering its own sweetness level at 100 ppm, trilobatin apparently enhances the sweetness intensity of a 7% sucrose solution by a 0.5% (w/v) sucrose equivalence, or by about 7% (Figure 2d), a modest rise when compared with a prototypical PAM that can essentially double the sweetness intensity of a sucrose solution (up to a 100% increase) (Figure 2d).

Rebaudioside C (RebC) (Figure 1) is another natural sweetener isolated from the plant *Stevia rebaudiana* and a close analog of the newly marketed sweetener RebA (e.g. Truvia™) [48]. According to Redpoint Bio, 285 ppm (300 μM) of RebC is considered a threshold concentration for sweetness, corresponding to a level greater than water and less than a 2.5% (w/v) sucrose solution [49]. When added to a 5% (w/v) sucrose solution, 285 ppm RebC boosts the sweetness intensity to the equivalent of a 7% (w/v) sucrose solution (Figure 2d) [49]. Here again, if 300 μM RebC produces a sweetness equivalent to at least a ~1% (w/v) sucrose solution (a value based on internal evaluations at Senomyx using paired comparison and in agreement with the range stated above) it then boosts the sweetness intensity of a 5% (w/v) sucrose solution by less than 17%.

Despite the relatively smaller enhancement effects provided by sweeteners in general, blends and new sweetener formulations remain very popular in the industry. They can improve the overall sweetness profile and mouth feel of food products, and sometimes even provide cost-savings to food and beverage companies. However, the beneficial effects of sweetener combinations are clearly not the result of robust synergy between each of the components (as shown above) but are instead due to their complementary flavor characteristics (different off-tastes and temporal properties) and physicochemical properties (different affinities, off-rates, solubility and different level of interactions with other components in the final product). If the goal is to enhance sweetness significantly using a low concentration of modulator and preserve the true taste of sugar, then PAMs undoubtedly offer a better outcome than any sweetener or combination of sweeteners reported to date. A unique aspect of these types of PAMs is that a separate enhancer could need to be developed for each sweetener, creating a family of sweetener-specific PAMs with high efficacy.

A priori, sweeteners would not be the ideal choice to use as enhancers because, *in vivo*, they probably do not saturate enough of the receptor sites at concentrations close to their sweetness detection threshold. Indeed, it is likely that both the enhancer and the sweetener need to occupy, at the same time, at least a significant fraction of the receptor sites to produce a clear functional interaction (i.e. synergy) in taste tests. One could therefore assume that careful assessment of the sweeteners reported to exhibit sweet-enhancement properties, at concentrations that allow a greater level of receptor occupancy, should reveal and even reinforce the notion of synergy between sweeteners. An internal investigation at Senomyx, using a sweet-receptor cell-based assay and several popular sweeteners interacting with the TMD of hT1R3 (trilobatin or NHDC) [44] and even RebC [49] has yet to expose a definite functional synergy with sucrose or other sweeteners *in vitro* (G. Servant *et al.*, unpublished observations). The inability of sweet modulators such as these agonists to promote an obvious and reproducible level of synergy with sweeteners acting on the VFD of hT1R2 is puzzling because, for several family C GPCR members, allosteric modulators acting within the TMD can exhibit a significant degree of synergy with orthosteric agonists acting within the VFD [35–37]. It is conceivable that further screening efforts could identify novel and efficacious positive allosteric modulators that exhibit less agonist activity than a typical sweetener and that interact within the TMD of the hT1R2 or hT1R3 subunits. Indeed, a negative allosteric modulator of the sweet taste receptor, lactisole [50], interacting within the TMD of hT1R3 [33,51] has already been identified. However, after screening more than half a million different compounds on several different sweeteners, the only sweet taste enhancers that have been identified and that provide robust effects in taste tests have been found to operate through the VFD of hT1R2. In addition, most novel modulators acting within the TMD domains of the sweet receptor that have been identified at Senomyx act as full agonists (G. Servant *et al.*, unpublished observations). It is possible that the high level of constitutive activity of the human sweet taste receptor [50] prevents the identification of PAMs acting through the TMD (i.e. molecules that would otherwise show no agonist activity become full agonists because of the constitutive activity) [52]. To this point, the far greater enhancement effect provided by the current PAMs over any other allosteric modulators suggests that targeting the VFD could be a better approach for the sweet taste receptor.

Concluding remarks

PAMs for the sweet taste receptor are unique and represent a significant breakthrough in the effort to control caloric intake. They could revolutionize the field of flavor development for sweetened consumer products. Relative to other sweet taste enhancers (i.e. the use of some non-caloric sweeteners) PAMs offer a superior approach to lowering the caloric content in food and beverages while preserving the desired taste. The success of this approach has now prompted a larger effort to identify additional PAMs from synthetic and natural sources and which are capable of enhancing other caloric sweeteners.

Conflict of interest statement

G.S., C.T. and D.S.K. are employees of Senomyx Inc. G.S., C.T., X.L. and D.S.K. have a personal financial interest in the form of stock ownership or options ownership of

Senomyx Inc. and are inventors on several patents and patent applications in the area of cell-based assays for taste receptors and taste receptor modulators.

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