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PERSPECTIVE

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Constitutive activity of the sweet taste receptor: Heavy water sweetness and beyond

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Abstract

The present work proposes an explanation for a recent observation that has conclusively proven that heavy water, that is, water containing the non-radioactive isotope of hydrogen deuterium, is mildly sweet, at variance with the tasteless common water. No firm explanation was proposed for this unexpected behavior. Yet, the subject is far from being an irrelevant curiosity, as the explanation of yet unidentified properties of the sweet receptor can help us to understand the molecular bases of food appreciation that have direct repercussions on pathologies such as diabetes and obesity. Here, a simple but convincing structural explanation of the taste of heavy water is proposed that is based on the influence of heavy water on the conformation of the active form of the receptor. The explanation requires the concept of "*constitutive receptor activity*", that is, a notion well accepted in many areas of pharmacology but clearly neglected in reference to taste receptors. We discuss how constitutive activity also explains other properties such as the recognition of sweet proteins that are several thousand times sweeter than small carbohydrates.

KEYWORDS

constitutive activity, isotopes, sweet proteins, sweet taste

1 | INTRODUCTION

Taste receptors are specialized proteins located on specialized taste cells within taste buds that trigger the taste sensation upon detection of specific chemical compounds in food and beverages (Bachmanov et al., 2014). Taste receptors are primarily present on the tongue but are also found in other areas of the oral cavity and in extraoral locations like the gastrointestinal tract. These receptors allow us to distinguish among the five basic tastes: sweet, salty, sour, bitter, and umami. While several different proteins account for the reception of the bitter taste, the sour, salty, and umami tastes are related to two receptors and, remarkably, only one receptor triggers the sweet taste. This peculiar difference is usually ascribed to the necessity to have many different sensors to detect, and therefore find protection from, the several poisoning compounds, usually bitter, that surround us in the environment while the sweet sensation suffices with only one specialized reception.

In the present work, we discuss the physico-chemical significance of a recent observation that is directly relevant for understanding the functioning of the sweet receptor. It was noticed that heavy water has a mild sweet taste (Ben Abu et al., 2021; Hansen & Rustung, 1935), implying that it interacts with our unique sweet receptor, at variance with the tasteless properties of regular water. Heavy water (D_2O) is chemically similar to regular water (H_2O), but instead of ordinary hydrogen, it contains deuterium, a non-radioactive isotope of hydrogen with one proton and one neutron in its nucleus. To find a rational explanation for the sweetness of heavy water is by far not a mere curiosity or a

frivolous topic for gourmets: it sheds light on the functioning of the sweet taste receptor, whose structure without ligands is still not available (Juen et al., 2025), and provides further information about our understanding of taste, needed both for increasing our basic knowledge and to design better sweeteners, essential for people with disabilities like diabetes, obesity, or cardiac problems.

2 | THE SWEET TASTE RECEPTOR: STRUCTURE AND ACTIVATION STATES

The sweet receptor was functionally characterized in 2002 as a heterodimer comprising T1R2 and T1R3, both belonging to the Class C G-protein coupled receptors (GPCRs) (Li et al., 2002). These are a subfamily of GPCRs uniquely characterized by large extracellular domains and by constitutive dimerization, which often involves either or both homo- and hetero-dimers. These receptors, unlike other GPCRs, have their ligand binding sites primarily located in the extracellular domains, rather than within the transmembrane domain (Li et al., 2002). As with other class C GPCRs, T1R2 and T1R3 include a large extracellular Venus Fly Trap domain, which undergoes conformational changes associated with receptor activation. T1R3 had previously been associated with the saccharin preference gene (Sac) (Kitagawa et al., 2001; Max et al., 2001), but its co-assembly with T1R2 was demonstrated to be essential for a full response to a broad spectrum of sweeteners. Although the full experimental structure of the T1R2–T1R3 complex is not yet available, extensive sequence homology with mGluR1, whose structure was determined shortly after the identification of the sweet receptor (Kunishima et al., 2000), has enabled the construction of detailed three-dimensional models.

3 | HEAVY WATER: AN UNEXPECTEDLY SWEET SIP

It was suggested long ago that deuterated water tastes sweet (Hansen & Rustung, 1935). This observation was, however, contradicted shortly after by the eminent Nobel laureate Prof. Harold Urey, the very discoverer of deuterium, who believed that heavy water had the same taste as normal water (Urey & Failla, 1935). Deuterium, as opposed to its close isotope hydrogen, which differs only by an additional neutron in the nucleus, is a non-radioactive element that is non-toxic in small amounts, but is harmful to living organisms in large doses. At the time, nobody, of course, dared to contradict Prof. Urey, although, not being a "professional taster" and probably expecting a clear sweetness rather than a modest increase, he might not have been in the best position for a definite judgment. Notwithstanding, the possibility that heavy water had a different taste from normal water remained as a halo in literature.

Recently, a detailed paper on this subject suggested, based on detailed and rigorous experimental bases, that indeed deuterated water has an intrinsic, albeit mild sweet taste (Ben Abu et al., 2021). The authors found that, according to a human sensory panel of taste experts, the perceived sweetness of D_2O increased in a D_2O-dose dependent manner, reaching an average of 3.3 sweetness, typical of what is described, using a 9-point scale, as slightly sweet.

It is puzzling that the same authors found that mice could not taste heavy water as different from normal water, but the difference between tests on humans and mice might be related to the difficulty of detecting a very mild difference between D_2O and H_2O using behavioral assays.

The compelling question is therefore why? Can we explain this observation based on what we currently know at the atomic level about the structure and the functioning of the sweet receptor?

This effect is unlikely to result from conventional ligand-receptor interactions. Ben Abu et al. (2021) could not reach a firm hypothesis on this important point. Detailed modeling of the sweet receptor's binding sites failed to identify specific sites that would directly accommodate water molecules, let alone distinguish between H_2O and D_2O (Ben Abu et al., 2021). Thus a different explanation is owed to be found.

4 | RECEPTORS WITH A SECRET LIFE

Before discussing a reasonable answer, it is essential to revisit a crucial concept on receptors widely recognized by pharmacologists but not always well known by the largest audience. Receptors are not just passive on/off switches. As pharmacologists now widely accept, many receptors—including G protein-coupled receptors (GPCRs)—exist in a dynamic equilibrium between active and inactive conformations, even when no ligand is present. This behavior is known as "*constitutive receptor activity*" (Berg & Clarke, 2018; Milligan, 2003). Despite this fundamental principle of receptor pharmacology, early studies of the sweet taste receptor largely omitted explicit mention of constitutive activity. The first indirect acknowledgments appeared in structural modeling studies of sweet-tasting proteins (Morini et al., 2005; Temussi, 2002), where homology models of the sweet receptor were constructed using glutamate receptor templates that included ligand-free active conformations (Kunishima et al., 2000).

Interestingly, these authors of the glutamate receptor structure had also shown that the extracellular domain of mGluR1 exists in three different forms: one

complexed with two molecules of glutamate and two forms free of any ligand. Both the receptor complexed with glutamate (1ewk.pdb) and the uncomplexed free form II (1ewv.pdb) are classified as open-closed conformations and correspond to the active state of the receptor, whereas the other ligand-free form (I) (1ewt.pdb) is in an open-open conformation and corresponds to the inactive state of the receptor. Combining two sequences (T1R2 and T1R3) with two conformations amounts to four possible heterodimers. The first heterodimeric T1R2–T1R3 model corresponded to only one of the two possible active models (Temussi, 2002). Shortly after, Morini et al. (2005) built all models of the human sequences and used them to identify all possible sites of interaction. The four T1R2–T1R3 heterodimers are two inactive, ligand-free, open-open forms, and two active closed-open forms. In all cases, the active forms (whether with ligands or ligand free) are more compact than the resting/inactive forms.

A more direct recognition came from Galindo-Cuspinera et al. (2006), who provided experimental evidence for constitutive activity in their study of the so-called "water taste" phenomenon. In this case, rinsing the mouth with water after exposure to an inverse agonist restored a baseline sweet perception, which the authors correctly attributed to reactivation of the receptor's constitutive state.

5 | A LIKELY ANSWER TO THE QUESTION OF WHY HEAVY WATER IS MILDLY SWEET

Based on the mentioned knowledge, it is thus reasonable to suggest a likely interpretation for the sweet taste of heavy water using the concept of *constitutive receptor activity*. The explanation requires that the sweet taste receptor (T1R2–T1R3), like the majority of receptors, exists in the absence of any ligand, as an equilibrium between an inactive and an active form. These ligand-free active forms are central to understanding the receptor's constitutive activity. Notably, such activity provides a plausible framework for interpreting how certain stimuli, including sweet proteins and heavy water, modulate sweet perception without conventional orthosteric binding. New findings suggest that proteins are slightly more compact and rigid in D_2O than in H_2O (Tempra et al., 2023). This difference could subtly shift the receptor's equilibrium toward its compact, active state—leading to a faint sweet taste without any ligand. Heavy water may tip the balance toward activation—not by binding, but simply by changing the environment around the receptor (Figure 1). It is thus not about a direct docking site but about the physical *context* influencing the receptor's conformation and the ability of heavy water to stabilize proteins.

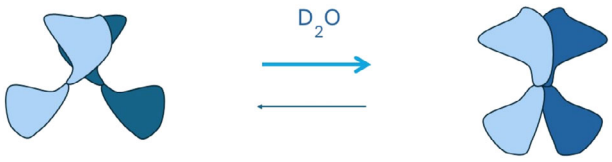

FIGURE 1 Cartoon illustrating the shift of the equilibrium between two ligand-free forms of the sweet receptor induced by immersion of the receptor in heavy water.

It is in order to recall that heavy water, while behaving like ordinary water in the majority of aspects, can influence protein stability in specific ways because of subtle but well distinct physico-chemical properties. Key differences between D_2O and H_2O are (1) the hydrogen/deuterium bond strength: D_2O forms stronger, more stable hydrogen bonds; (2) the density and viscosity: D_2O is ~10% denser and more viscous, possibly affecting protein dynamics; and (3) the dielectric constant and solvent effects that could also influence receptor conformational stability. Among these differences, the increased strength of hydrogen bonding will directly favor compact conformations, like the active form of the ligand-free receptor. At the same time, an increase in solution viscosity will slow down protein dynamics, thus favoring protein rigidity.

6 | SWEET PROTEINS: LARGE MOLECULES, POTENT EFFECTS

The concept of constitutive activity is further reinforced and supported also by another well-known fact: the extraordinarily high potency of sweet proteins. Some of the most potent sweet compounds are not small organic molecules but proteins—such as brazzein, thaumatin, and monellin—derived from tropical plants (Morris, 1976). These macromolecules exhibit sweetness intensities up to five orders of magnitude greater than sucrose (Morini et al., 2005), despite having structures that seem incompatible with the receptor's known small-molecule binding sites. Initial hypotheses proposed the existence of "sweet fingers"—localized structural motifs on the protein surface resembling small sweeteners—which could potentially interact with orthosteric sites (Tancredi et al., 2004). However, this approach failed to produce peptides that retained sweetness, suggesting an alternative mechanism. A compelling explanation was proposed by Temussi (2002): sweet proteins may not interact directly with the orthosteric site but rather bind to an external site on the receptor, stabilizing the ligand-free active conformation. This model, later referred to as the *wedge model*, was supported by in silico docking studies demonstrating that these proteins fit into a large extracellular cavity formed by the active receptor conformation (Morini

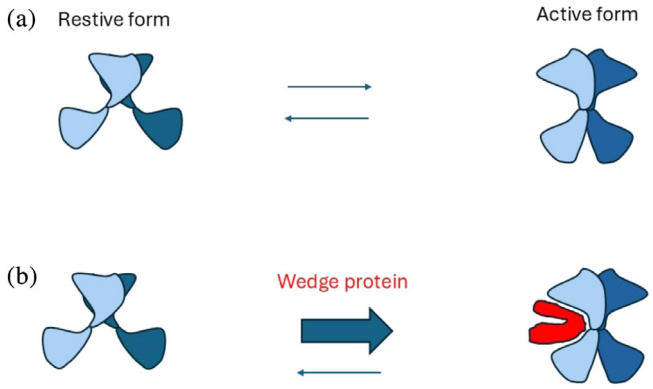

FIGURE 2 Cartoon illustrating the shift of the equilibrium between two ligand free forms of the sweet receptor induced by a wedge shaped protein. (a) The two free form conformations of the VFT domain. (b) The shift induced by a wedge shaped protein.

et al., 2005). In this model, sweet proteins act as conformational stabilizers, shifting the equilibrium between inactive and active states in favor of the latter (Figure 2). This elegant explanation matched experimental data but was, unfortunately, often misrepresented or ignored. Some later studies tried different angles (Jiang et al., 2004; Kim et al., 2022), but the new models could not easily accommodate large proteins like thaumatin. The wedge model still stands as the only one flexible enough to explain how all sweet proteins might work. Despite initial resistance or misinterpretation in the literature, this mechanism remains one of the few that can coherently explain the potent sweetness of structurally diverse sweet proteins, particularly given their size and limited structural similarity.

7 | CONCLUSIONS

From mind-blowingly sweet tropical proteins to the quiet sweetness of heavy water, these unusual taste phenomena make one thing clear: the sweet receptor is not just a passive player waiting for ligands to knock. Instead, it exists as a dynamic equilibrium of states, capable of subtle activation even in the absence of traditional ligands—a behavior explained only through the lens of *constitutive receptor activity*. Ignoring this intrinsic activity limits our understanding. Embracing it unlocks the full richness of taste biology—and may explain some of its most delicious sweet mysteries.

AUTHOR CONTRIBUTIONS

Annalisa Pastore: Conceptualization; validation; supervision. **Piero Andrea Temussi:** Conceptualization; writing – original draft; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in pdb at https://uk.search.yahoo.com/search?fr=mcafee&type=E210GB1406G0&p=pdb. These data were derived from the following resources available in the public domain: pdb, https://uk.search.yahoo.com/search?fr=mcafee&type=E210GB1406G0&p=pdb.

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REFERENCES

Bachmanov AA, Bosak NP, Lin C, Matsumoto I, Ohmoto M, Reed DR, et al. Genetics of taste receptors. Curr Pharm Des. 2014;20(16):2669–83. https://doi.org/10.2174/13816128113199990566

Ben Abu N, Mason PE, Klein H, Dubovski N, Ben Shoshan-Galeczki Y, Malach E, et al. Sweet taste of heavy water. Commun Biol. 2021;4(1):440. https://doi.org/10.1038/s42003-021-01964-y

Berg KA, Clarke WP. Making sense of pharmacology: inverse Agonism and functional selectivity. Int J Neuropsychopharmacol. 2018;21(10):962–77. https://doi.org/10.1093/ijnp/pyy071

Galindo-Cuspinera V, Winnig M, Bufe B, Meyerhof W, Breslin PA. A TAS1R receptor-based explanation of sweet 'water-taste'. Nature. 2006;18(7091):354–7. https://doi.org/10.1038/nature04765

Hansen K, Rustung E. Untersuchen under die Biologische Wirkungen von "Schwerem Wasser" bei Warmblutingen Tieren. Klin Wochenschr. 1935;14:104–8.

Jiang P, Ji Q, Liu Z, Snyder LA, Benard LM, Margolskee RF, et al. The cysteine-rich region of T1R3 determines responses to intensely sweet proteins. J Biol Chem. 2004;279(43):45068–75. https://doi.org/10.1074/jbc.M406779200

Juen Z, Lu Z, Yu R, Chang AN, Wang B, Fitzpatrick AWP, et al. The structure of human sweetness. Cell. 2025;188:4141–4153.e18. https://doi.org/10.1016/j.cell.2025.04.021

Kim TY, Woo EJ, Yoon TS. Binding mode of brazzein to the taste receptor based on crystal structure and docking simulation. Biochem Biophys Res Commun. 2022;592:119–24. https://doi.org/10.1016/j.bbrc.2022.01.004

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochem Biophys Res Commun. 2001;283:236–42.

Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, et al. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature. 2000;407:971–7.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. Proc Natl Acad Sci USA. 2002;99:4692–6.

Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, et al. Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus sac. Nat Genet. 2001;28:58–63.

Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. Mol Pharmacol. 2003;64(6):1271–6. https://doi.org/10.1124/mol.64.6.1271

Morini G, Bassoli A, Temussi PA. From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. J Med Chem. 2005;48(17):5520–9. https://doi.org/10.1021/jm0503345

Morris JA. Sweetening agents from natural sources. Lloydia. 1976; 39(1):25–38.

Tancredi T, Pastore A, Salvadori S, Esposito V, Temussi PA. Interaction of sweet proteins with their receptor. A conformational study of peptides corresponding to loops of brazzein, monellin and thaumatin. Eur J Biochem. 2004;271:2231–40.

Tempra C, Chamorro VC, Jungwirth P. Effects of water deuteration on thermodynamic and structural properties of proteins and biomembranes. J Phys Chem B. 2023;127(5):1138–43. https://doi.org/10.1021/acs.jpcb.2c08270

Temussi PA. Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2-T1R3 receptor. FEBS Lett. 2002;526(1–3):1–4. https://doi.org/10.1016/s0014-5793(02)03155-1

Urey HC, Failla G. Concerning the taste of heavy water. Science. 1935;81(2098):273. https://doi.org/10.1126/science.81.2098.273-a

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