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Taste after-images: the science of "water-tastes"

V. Galindo-Cuspinera and P. A. S. Breslin

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, Pennsylvania 19104 (USA),
Fax: 215 898 2084, e-mail: breslin@monell.org

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We tend to think of water as a tasteless liquid. But those of us who have tasted pure, distilled, de-ionized water have noticed a mild bitter taste from it. How could the absence of a chemical ligand have a taste? We understand the bitterness from pure water as the gustatory after-image resulting from rinsing away the constituents of saliva, such as salts, bicarbonate, proteins, etc. Just as color after-images appear clearly when gazing at a clean white wall or white sheet of paper, so too do water-taste after-images appear when tasting pure water after exposure to certain substances (Fig. 1) [1–3]. There are even instances where the "water-tastes" that follow a taste stimulus are surprisingly intense, and can present various taste qualities, even sweet [2]. One example of strong sweet 'water-taste' came to our attention while studying the taste properties of the common sweetener sodium saccharin (Na-saccharin). Although Na-saccharin is used commercially as a sweetener, high concentrations of saccharin are not sweet and instead taste predominantly bitter (Fig. 2a). Curiously, rinsing away strong saccharin solutions with sips of water produced a pronounced sweet "water-taste" (Fig. 2b). This serendipitous observation led to a full investigation of the phenomenon [4].

We hypothesized that saccharin was not sweet at high concentrations because it inhibits the sweetener receptor at these intensities, a form of auto-inhibition. We tested the idea that sweet water taste and sweetness inhibition were functionally connected. Several experiments confirmed that all the compounds we examined that elicited sweet water-tastes were also able to inhibit a wide variety of sweeteners. Compounds that accounted for this effect included lactisole [Na-(±)-2-(P-me-

Figure 1. Taste after-images. The phenomenon of sweet 'water-taste.'

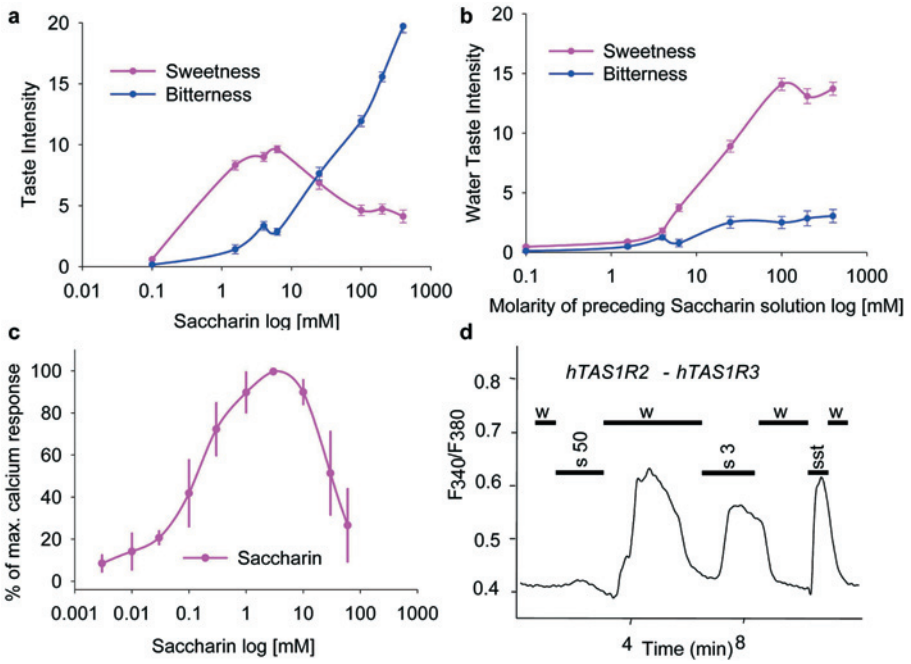

Figure 2. *In vivo* (human) and *in vitro* (HEK cells) responses to Na-saccharin. (*a*) Concentration–intensity relationship for Na-saccharin, measured during the second tasting of a given concentration (n = 14 subjects). Intensity was rated on the gLMS scale. (*b*) 'Water-taste' concentration-intensity curve for Na-saccharin. The taste intensity of deionized water was measured after two consecutive exposures to the same Na-saccharin solution (10-s delay between exposures, n = 14 subjects). (*c*) Functional properties of hTAS1R2-hTAS1R3. FLIPR calcium responses averaged over three independent experiments (n =3) to Na-saccharin of cells expressing hTAS1R2–hTAS1R3. d, Typical single-cell imaging traces of cells expressing hTAS1R2–hTAS1R3, to examine effects of rinsing off Na-saccharin (40 responders out of 108 cells, n = 3). Treatment conditions were wash (w), 50 mM Na-saccharin (s-50), 3 mM Na-saccharin (s-3), and 0.1 mM somatostatin14 (sst). Horizontal bars indicate the duration of the treatment. Adapted by permission from Macmillan Publishers Ltd: Nature [4], copyright 2006.

thoxy-phenoxy)-propionic acid], $MgSO_4$ (neither of which taste sweet when sampled directly), and high concentrations of two sweeteners: Na-saccharin and acesulfame-K. Thus, we confirmed our hypothesis that the sweetener Na-saccharin is counterintuitively also a sweetness inhibitor at high concentrations.

We also demonstrated that two alternative hypotheses of sweetness suppression, cognitive mixture suppression by the bitterness of high concentrations of Na-saccharin and sweetness adaptation effects by high saccharin, can not explain the observed phenomena. According to the former theory, it is possible that the strong bitter taste of high Na-saccharin concentrations could mask the sweet taste [5]. The data, however, do not support this notion, since there was no correlation between perceived bitter taste intensity and sweet taste intensity among individuals. For the second theory, it is possible that sweetness of concentrated Na-saccharin adapts rapidly and this accounts for its own lack of sweetness and its ability to block sweetness of other compounds. This hypothesis was not supported by an experiment in which multiple presentations of a concentrated sucrose and Na-saccharin mixture that was only mildly sweet did not interfere with the sucrose eliciting strong sweetness when presented alone at the end of the series of presentations. Furthermore, sweeteners that do not elicit a sweet water-taste, such as Na-cyclamate or aspartame, did not inhibit the sweetness of other sweet substances. This demonstrates that there is a link between the kinetics of sweetener inhibition and sweet "water-taste" elicitation [6, 7].

In collaboration with our co-authors Marcel Winnig, Bernd Bufe, and Wolfgang Meyerhof, we used calcium imaging to monitor the activation of immortalized human kidney cells (HEK293/Ga16gust44) transfected with the heteromeric human sweet taste receptor hTAS1R2-hTAS1R3 [4]. We found that responses to increasing concentrations of Na-saccharin or acesulfame-K peaked and declined, following a bell-shaped function, in a manner that closely paralleled the loss of sweetness perceived by human tasters (Fig. 2 c, c.f. 2a). Similarly, calcium imaging revealed that high concentrations of Na-saccharin inhibited the response of other sweeteners and that both Na-saccharin and lactisole produced a calcium flux 'off-response' when rinsed from single cells (there was a rise in the activity when the ligand was removed, analogous to the perceptual sweet water-taste) (Fig. 2 d response to s 50, c.f. 2b). To explain this phenomenon, we proposed

a two-state allosteric model for the sweetness receptor, where substances such as Na-saccharin or acesulfame-K bind to a high-affinity activating site at low concentrations, while at high concentrations, the same compounds bind to a lower-affinity site that shifts receptor equilibrium toward an inactive conformation. In this model, water rinses result in the removal of these compounds from the low-affinity inhibitory site causing rebound activation and the consequent perception of sweetness.

This rebound is driven by the constitutive activity of the TAS1R2-TAS1R3 receptor, which causes the coordinated shift from inhibition over to the basal activation state. We demonstrated that the sweet receptor shows constitutive activity by measuring a drop in resting calcium levels when the hTAS1R2-hTAS1R3 inverse agonist, lactisole, is applied to HEK293 cells that express this receptor. The rinsing away of lactisole, which is not sweet when first tasted, elicits a pronounced sweet water-taste and activates hTAS1R2-hTAS1R3-expressing cells when rinsed away. By studying the interaction of sweet 'water-taste' compounds with the TAS1R2-TAS1R3 sweetener receptor, we have shown that the sweet 'water-taste' phenomenon is directly related to sweet taste inhibition and that the perceived sweetness from water is the result of removing an inverse agonist from the receptors and their coordinated return to constitutive activity. Based on these findings, it should be possible to identify novel sweet taste inhibitors simply by determining if they stimulate sweet water-taste.

At this moment, it is unclear whether similar mechanisms will work with other taste systems such as umami or bitter taste. First, there are no demonstrations that TAS1R1-TAS1R3 umami receptors or TAS2R bitter receptors show similar constitutive activity, although there is reason to believe they will, since many G-protein-coupled receptors (GPCRs), such as the family-C GPCRs to which the TAS1Rs belong, have intrinsic activity [8]. Second, lactisole, a very effective general sweetness inhibitor, has also been shown to inhibit umami taste in humans but with considerably less potency [9, 10]. There are no reports that rinsing away lactisole stimulates umami water-taste, although the effect might be masked perceptually by the strong sweet water-taste of lactisole. There are, however, instances where bitter water-tastes are stimulated, as mentioned above [1, 3], cases in which the taste is not very strong. Interestingly, common salt is a general purpose bitter taste inhibitor [5], and water after tasting weak NaCl can have a mild bitter taste [1]. It is unclear how sodium salts inhibit bitter taste, but one hypothesis is that the cations allosterically modulate the GPCRs via interactions with acidic side chains on residues [11]. Therefore, it is possible that sodium ions are reducing intrinsic activity in TAS2Rs and their rinse is stimulating a mild bitter water-taste, parallel to the sweet water-taste described. A similar result would be expected when an allosteric inhibitor of the TAS1R1-TAS1R3 umami receptor has been identified. More research is needed to test these hypotheses.

Another question that still remains is the binding location for inhibition by high concentrations of Na-saccharin on the receptor. We have demonstrated by the use of human-rat chimeric receptors that Na-saccharin inhibits TAS1R3 in the trans-membrane portion of the protein, similar to lactisole's binding region. But beyond this regional statement we cannot assert precisely where or how. Zubare-Samuelov et al. [12] have shown that saccharin can be internalized into the cell, which opens the possibility that the binding site responsible for inhibition could be on intracellular portions of the protein. The fact that inhibition by Na-saccharin is slightly delayed relative to the sweet taste of the agonist might be due to the transit time it takes saccharin to be internalized. Zubare-Samuelov et al. [12] proposed that the internalization of Na-saccharin might explain its prolonged taste, but this may be an unrelated phenomenon. Thus, future research should identify the allosteric inhibitory site(s) responsible for sweetness inhibition and sweet water-taste by Na-saccharin and compare its relationship to the known lactisole-binding site on TAS1R3 [13].

Although not all water-taste phenomena have been explained, our findings have opened new possibilities for means to approach complex taste sensations and have illustrated the potential of combining perceptual-psychophysical studies with functional molecular assays to unravel the mechanisms for taste modulation and perception, in this specific case, the science behind sweet 'water-taste'.

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