T1R3: A human calcium taste receptor

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Many animals can detect the taste of calcium but it is unclear how or whether humans have this ability. We show here that calcium activates hTAS1R3-transfected HEK293 cells and that this response is attenuated by lactisole, an inhibitor of hT1R3. Moreover, trained volunteers report that lactisole reduces the calcium intensity of calcium lactate. Thus, humans can detect calcium by taste, T1R3 is a receptor responsible for this, and lactisole can reduce the taste perception of calcium by acting on T1R3.

Many animals can satisfy their physiological need for calcium by locating and consuming calcium salts\(^1,2\). This "calcium appetite" is controlled by taste: Concentrated calcium salts are rejected by nutritionally replete animals but avidly ingested by calcium-deprived ones\(^3,4\). The mechanism involves changes in oral sensitivity to calcium\(^3–6\) but how calcium is detected is unclear. There is evidence, albeit incomplete, for calcium taste receptors in amphibians and rodents\(^2,7–11\) but it is unknown whether humans can detect calcium per se rather than as a combination of, for example, bitter and sour taste components\(^12\).

One receptor implicated in the detection of calcium taste by mice is T1R3\(^13\). Polymorphisms in \(\text{Tas1r3}\), the gene encoding T1R3, are associated with the calcium preferences of 40 inbred mouse strains, and \(\text{Tas1r3}\) knockout mice are indifferent to concentrations of \(\text{CaCl}_2\) and calcium lactate that wild-type littermates avoid\(^13\). Moreover, the electrophysiological response of the chorda tympani nerve elicited by oral calcium is less pronounced in \(\text{Tas1r3}\) knockout mice than wild-type littermate controls\(^13\). But despite this evidence, T1R3 seems an unlikely candidate to be a calcium taste receptor because it has well-established roles as a detector of sweet and umami (monosodium glutamate) tastes\(^14,15\). Critically, the taste intensities of most sweeteners and monosodium glutamate are inhibited by lactisole \([\text{sodium 2-(4-methoxyphenoxy)propanoate}]\)\(^15–17\). This raises the possibility of using lactisole to test whether T1R3 influences calcium taste. To accomplish this, we first determined whether hT1R3 was sensitive to calcium and lactisole in vitro.

Results

HEK293 cells transiently transfected with hT1R3 and a chimeric G-protein, \(\text{G}\alpha_{16}\)-gust44, responded to calcium (but not magnesium) in a dose-dependent manner (EC\(_{50}\) = 53 mM; Fig. 1a). Cells transfected with \(\text{G}\alpha_{16}\)-gust44 but not T1R3 were completely unresponsive to either mineral (not shown). The response to calcium was inhibited by lactisole (EC\(_{50}\) = 3 mM; Fig. 1b). This was not simply a nonspecific inhibitory effect on cellular responses because the response to carbachol was unaffected by lactisole (Fig. 1c).

T1R3 is a shared subunit of both the sweet taste receptor (T1R2+T1R3) and umami taste receptor (T1R1+T1R3). Consistent with earlier characterizations of the T1R3 receptor\(^18–20\), HEK293 cells expressing hT1R3 alone did not respond to sweeteners or to monosodium glutamate (data not shown). However, hT1R3 could account entirely for the response of dual-transfected cells to calcium (Fig 1a,b and d): Transfection with hT1R3 alone or both hT1R3 and hT1R2 supported the response whereas transfection with hT1R2 alone did not (Fig. 1d).

Human subjects were trained to recognize calcium by taste and then were asked to rate various taste solutions. They rated calcium lactate solution as having a predominant calcium taste with minor bitter and sour intensities. Lactisole decreased the intensity of the calcium taste (Fig. 2). It did this despite simultaneously increasing calcium lactate’s sourness, bitterness, saltiness and overall intensity (Supplementary Table 1). As expected, lactisole significantly reduced the overall intensity and sweetness intensity of sucrose\(^14,15\) and decreased the sweetness intensity of urea. It also caused slight but significant increases in the bitter, umami and calcium intensities of sucrose. Consistent with earlier work, lactisole did not influence the taste perception of citric acid, NaCl, quinine hydrochloride or MSG+IMP\(^15\) (Supplementary Table 2).
Discussion

These results, together with evidence that T1R3 is a calcium taste receptor in mice\(^1\), suggest that humans can detect calcium by taste, that T1R3 is a receptor responsible for this, and that lactisole can reduce the taste perception of calcium by acting on T1R3. The results are consistent with what little is known about the molecular mechanisms of calcium detection. A calcium-binding pocket has been delineated in the Venus fly trap region of the calcium-sensing receptor, CaSR, and the same residues are conserved in T1R3\(^2\). Lactisole interacts with the transmembrane domain of T1R3, restricting the molecule to its ground state conformation, and thus inhibiting sweet and umami taste\(^1\). It appears reasonable that the same mechanism is responsible for lactisole’s effects on calcium taste.

T1R3 is unlikely to be the only receptor involved in the detection of calcium salts by humans. Our subjects were trained to recognize calcium specifically but, even so, they rated calcium lactate as having bitter and sour components. Although lactisole reduced the calcium intensity of calcium lactate it also increased its sourness, saltiness, bitterness, and overall intensity. This probably reflects disinhibition of the lactate anion from mixture suppression, allowing it to exert a greater effect on intensity than when accompanied by the calcium cation\(^2\). The concentration of lactisole required to effectively inhibit calcium (and also umami taste) was higher than that required to inhibit sweetness\(^1\). Perhaps this is because sweet taste detection depends entirely on T1R3 (dimerized with T1R2) whereas calcium and umami taste compounds recruit additional receptors that are insensitive to lactisole. The existence of additional receptors can also explain why lactisole’s effect on calcium taste intensity was largest with the highest concentration of calcium lactate we tested, and the effect was a reduction but not an elimination of calcium taste intensity. One possibility is that lactisole blocks the action of T1R3 but leaves active CaSR, the calcium-sensing receptor, which may function as a taste receptor\(^2\).

T1R3 is a detector of sweet and umami tastes so the present findings raise the question of why calcium is not perceived as being sweet or umami-like. There is no confusion between calcium taste and sweet or umami taste (Fig. 2) and no evidence for an interaction between calcium and the sweet or umami taste qualities (Supplementary Table 2). Perhaps there is a subpopulation of taste cells that...
are armed with T1R3 and wired specifically as calcium-sensitive. We have speculated that T1R3 in these cells dimerizes with CaSR or another receptor although the finding made here that T1R3 can detect calcium by itself (Fig. 1) suggests that a T1R3 homodimer is responsible. Human T1R3-transfected HEK293 cells did not respond to magnesium, which contrasts with results we have found previously with mice: Unlike wild-type controls, Tásir3 knockout mice do not avoid MgCl2. We have also found that (a) lactisole does not affect intensity ratings of magnesium chloride solutions made by humans (unpublished results), and (b) HEK293 cells transfected with mouse (as opposed to human) T1R3 respond readily to MgCl2 (see supplementary material, Figure 1). Thus, we suspect that there is a species difference in the response of T1R3 to magnesium. Of course, species differences in the response of T1R3 are not without precedent; there are well-known differences in the response of the human and rodent forms of T1R3 to lactisole and to artificial sweeteners (e.g.1,2).

Our focus here is on T1R3 as a taste receptor but T1R3 has also been identified in the gastrointestinal tract so it may signal the presence of high concentrations of calcium in the stomach or intestines. T1R3 is also found in human pancreas and liver but it is unlikely to act as a postabsorptive calcium sensor because, at least under our experimental conditions, the lowest calcium concentrations to activate T1R3-mediated responses were well above the 1–2 mM concentrations found in blood.

We are often asked whether calcium is a basic taste, akin to sweet, sour, salty, bitter and umami. Our demonstration of a receptor in the oral cavity fulfills a universally accepted criterion for a basic taste but there is little evidence to support whether or not others criteria might be met. For example, is a specific taste quality or a central representation of the palatability of calcium solution in rats. Physiol. Behav. 84, 335–342 (2005).

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Author contributions
MGT and PJ conceived and designed the experiments. SV and PJ performed the cell assay experiments. LA performed the human tests and preliminary data analyses. MGT conducted statistical analyses and wrote the paper.

Additional information
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