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Some Basic Psychophysics of Calcium Salt Solutions

Michael G. Tordoff

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104-3308, USA

Correspondence to be sent to: Michael G. Tordoff, Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104-3308, USA

Abstract

Detection thresholds and the taste qualities of suprathreshold concentrations of calcium salt solutions were assessed. Average taste detection thresholds for calcium chloride ($CaCl_2$), lactate (CaLa), hydroxide, phosphate and gluconate ranged between 8 and 50 mM, with no reliable differences among the various salts. Between-subject variability ranged over four orders of magnitude and reliability coefficients for repeated detection threshold tests of $CaCl_2$ averaged $r = 0.52$. In an odor detection test, subjects could reliably discriminate 100 but not 1 mM $CaCl_2$ and CaLa from water. The taste of suprathreshold concentrations (1–100 mM) of $CaCl_2$ and CaLa was considered unpleasant. At 1 mM, $CaCl_2$ solution was rated as 35% bitter, 32% sour, 29% sweet and 4% salty. At higher concentrations the sweet component diminished and the salty component increased, so that 100 mM $CaCl_2$ was rated as 44% bitter, 20% sour, 1% sweet and 35% salty. CaLa solutions were considered to be significantly less bitter and marginally more sour than equimolar $CaCl_2$ solutions. Thus, the taste of calcium varied with both the form and concentration of salt tested, but included both sour and bitter components. Saltiness was identified only in high (≥32 mM) concentrations of $CaCl_2$, and thus was not necessarily a component of calcium taste. **Chem. Senses 21: 417–424, 1996.**

Introduction

There is clear evidence that animals can detect and recognize calcium (Hellwald, 1931; Richter and Eckert, 1937; Widmark, 1944; Scott *et al.*, 1950; Junge and Brodwick, 1970; Hughes and Wood-Gush, 1971; Frank, 1974; Kitada, 1978; Hyman and Frank, 1980a, b; Kurihara *et al.*, 1980; Boudreau *et al.*, 1983; Jacobs *et al.*, 1988; Tordoff *et al.*, 1990; Bigiani and Roper, 1991; Nakamura and Kurihara, 1991; Coldwell and Tordoff, 1993). However, there has been little attempt to examine whether the same is true of humans. Based on four early studies (Höber and Kiesow, 1898; von Skramlik, 1926; Fabian and Blum, 1943; Frings, 1948), Pfaffmann (1959) listed the median threshold for detection of $CaCl_2$ as 10 mM (range 2–30 mM). At suprathreshold levels, calcium salts have classically been considered bitter and/or salty. Kahlenberg (1901) wrote that 'the taste of $Ca(NO_3)_2$ is a trifle sharp in *n*/12½ solutions and distinctly bitter in *n*/6¼'. Moncrieff (1967, p. 266) mentioned (without attribution) that calcium salts are bitter and Frings (1946), apparently based on personal experience, stated that $CaCl_2$ is 'salty-bitter'. There have also been several attempts to match the intensity or taste quality of $CaCl_2$ with a reference solution, usually NaCl. Thus, von Skramlik (1926) found that $CaCl_2$ was 81% as salty as the same concentration of NaCl (reported in Pfaffmann, 1959) and Schiffman and Erickson (1971) found that 25 or 50 mM $CaCl_2$ was considered to be the same intensity as 200 mM NaCl. These authors also asked subjects to rate the similarity of $CaCl_2$ to 18 other taste solutions and to

describe them using 40 semantic differential scales. Multidimensional scaling suggested that the taste of $CaCl_2$ was approximately equally close to salty and bitter taste solutions and relatively distant from sweet taste solutions. The semantic differential scales suggested that $CaCl_2$ is 'generally a simple, minerally taste which is moderately bitter, smooth, warming and soft. Only one subject [of 4] considered it to have a salty component...Two subjects found it quite soapy, but not obnoxious. Two others found it obnoxious, but not soapy. For two subjects, $CaCl_2$ was rated slow developing, flat and nauseous. For the other two, it was rated fast developing and neither flat nor nauseous. It was uniformly considered nonfoodlike, but was rated poisonous by only one subject' (Schiffman and Erickson, 1971, p. 625).

The studies cited above used a calcium salt as part of a battery of tests of many solutions and thus were not focused on the taste of calcium *per se*. To provide a more detailed description of the chemosensory properties of calcium, we established detection thresholds for five calcium salts (chloride, lactate, gluconate, hydroxide, phosphate) and evaluated test–retest reliability of detection thresholds using $CaCl_2$. We also assessed the possibility that $CaCl_2$ and calcium lactate (CaLa) could be detected by olfaction. Finally, using suprathreshold concentrations of these compounds, we collected ratings of intensity and liking, as well as an assessment of the contribution of the four major taste qualities to the solution taste.

Materials and methods

Subjects

The four experiments were approved by the University of Pennsylvania Committee on Studies Using Human Beings. All subjects were 20- to 34-year-old women, who were either technicians at the Monell Center or students at the University of Pennsylvania. All subjects signed their informed consent. The purpose of the experiments was not explained until after the subjects were tested; they did not know they were tasting calcium salts. Each subject was tested at the same time of day, with at least 24 h and not more than 1 week between each test. She was requested not to eat or drink during the 2 h before each test.

Stimuli

The solutions used in tests were prepared from the purest salts available from Fisher Scientific (Pittsburgh, PA) or Sigma Chemical Corp. (St Louis, MO). They were prepared the day prior to each test by dissolving the appropriate quantity of salt in deionized water. The resulting solutions were kept in stoppered glass bottles at 4°C overnight, but allowed to warm to room temperature (~22°C) before use.

Detection thresholds

Eighteen subjects were used to compare the detection thresholds of five calcium salts: phosphate, hydroxide, chloride, lactate and gluconate. A detection threshold for each salt was determined during one test so that each subject was tested five times. The order of the tests was counterbalanced.

To find the approximate range of the threshold, the subject was given increasingly stronger samples of the calcium salt being tested, starting at 0.1 mM and increasing in one-third log steps, until they reported detecting a taste. As with all the taste tests, the sample was 10 ml in volume, presented in a disposable medicine cup, and the subject rinsed her mouth with deionized water between each test. A more accurate detection threshold was then determined using a two-cup, forced-choice staircase method. The subject was first presented with two cups, one containing deionized water and the other containing a sample of the salt concentration just detected. The task was to indicate which sample had a taste. If the calcium-containing cup was chosen, the next pair of samples presented was deionized water and a salt concentration one-third of a log step lower than the one just tested. If the calcium-containing cup was not chosen, the next pair of samples was deionized water and a salt concentration one-third of a log step higher than the one just tested. This procedure was repeated until five reversal points had occurred (i.e. a string of correct or incorrect responses ended). The threshold was considered to be the logarithmic mean of the last four reversal points.

To examine the reliability of the procedure, 15 subjects (two of whom had also been subjects in the previous experiment) were tested in a similar fashion except that on each of their three tests, the salt was always $CaCl_2$. In addition, a fourth test was conducted at the end of the experiment in which the 6-*n*-propylthiouracil (PROP) taste threshold was determined using the same methods, except the test solutions contained PROP in one-quarter log steps from 1 to 3162 mM.

During the experiment on taste quality (see below), some subjects mentioned that they could smell high concentrations of CaLa solution. To examine the extent to which

olfaction was playing a role in the detection of the calcium salts, 12 of the subjects who had been tested in the reliability experiment were given odor detection tests with 1 and 100 mM $CaCl_2$ and CaLa. Each subject was tested on two occasions, once with the low concentrations and once with the high concentrations. Each test consisted of two series of 12 counterbalanced trials, the second series being conducted in the opposite order to the first. On each trial, the subject was presented with three opaque 'sniff bottles' (plastic, 5 cm diameter, 15 cm high), two of which contained 20 ml water and one 20 ml calcium solution, or vice versa. The subjects were required to choose the bottle they believed to differ from the other two (i.e. pick the odd one). Thus, this test involved detection of oddity as well as incorporating a more traditional odor detection test, in which a single odor sample is always presented with two sample blanks.

Taste qualities

To characterize the taste of calcium as a function of the four major taste qualities, 15 subjects were given 24 × 10 ml samples to rate. Each subject was presented twice in counterbalanced order with a single cup of each of five concentrations of $CaCl_2$ (1, 3.16, 10, 31.6, 100 mM), the same five concentrations of CaLa and two water samples. The subject was asked to rate the solution's intensity and her 'liking' of it on 200 mm analog scales, anchored with the terms 'not intense' to 'very intense' and 'dislike extremely' to 'like extremely' respectively. She then was asked to score the degree of sweetness, sourness, bitterness and saltiness such that their total score on all four qualities was 100.

Results

Detection thresholds

Detection thresholds of individual subjects for the five calcium salts ranged over four orders of magnitude, although logarithmic mean detection thresholds were very similar (phosphate = 20 μM, hydroxide = 11 μM, chloride = 8 μM, lactate = 50 μM and gluconate = 9 μM; Figure 1). These values probably overestimate the mean detection thresholds because two subjects 'detected' four of the five salts at the lowest concentration of calcium offered (0.1 μM), three others 'detected' 0.1 μM calcium hydroxide and one other 'detected' 0.1 μM calcium gluconate. Some caution is required in interpreting these findings because the criterion used for detection was two successive correct trials,

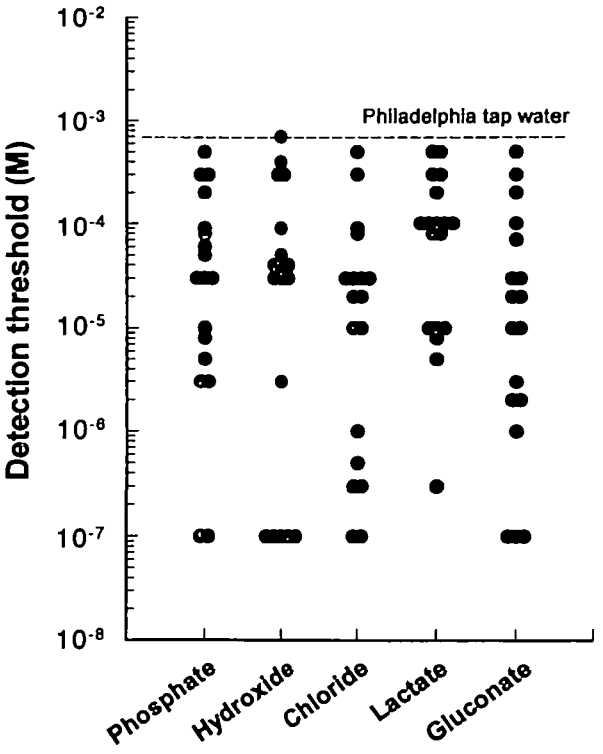

Figure 1 Detection thresholds of 18 young women for five calcium salts and their relationship to typical calcium levels in the local water supply (horizontal line).

which would be expected to be accomplished by chance on one out of four occasions. On the other hand, the possibility that two subjects could 'detect' the lowest of four of the five salts by chance is extremely small and we thus suspect these subjects could have detected lower concentrations of calcium if these had been available.

According to an analysis of variance conducted on the logarithm-transformed detection threshold values, there were no significant differences in detection threshold among the five calcium salts [$F(4,68) = 2.34$, $P = 0.063$] although there was a tendency for the threshold for lactate to be higher than those for the other salts. Correlation coefficients between individual tests ranged widely (Table 1).

The 15 subjects tested three times with $CaCl_2$ had similar mean detection thresholds to those tested with the five calcium salts (logarithmic mean of three tests = 16 mM; range 1–1000 mM; Figure 2). The average correlation between the three tests was $r = 0.52$ (test 1 versus 2, $r = 0.54$; test 1 versus 3, $r = 0.55$; test 2 versus 3, $r = 0.45$). The average detection threshold for PROP was 36 μM (range 1–270 μM) and the correlation between the mean calcium detection threshold and the detection threshold for PROP was r =

Table 1 Correlation matrix of logarithmic-transformed detection thresholds of 18 subjects for five calcium salts

	Phosphate	Hydroxide	Chloride	Lactate
Hydroxide	0.53*			
Chloride	0.48*	0.10		
Lactate	0.28	0.09	0.54*	
Gluconate	0.79*	0.41	0.56*	0.14

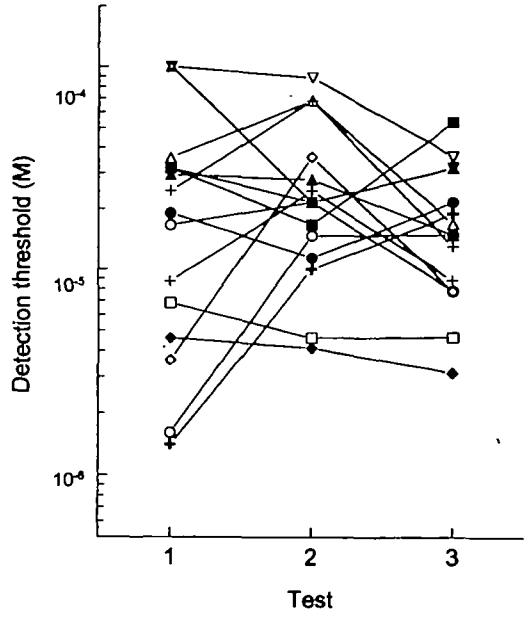

Figure 2 Detection thresholds of 15 women tested three times with $CaCl_2$.

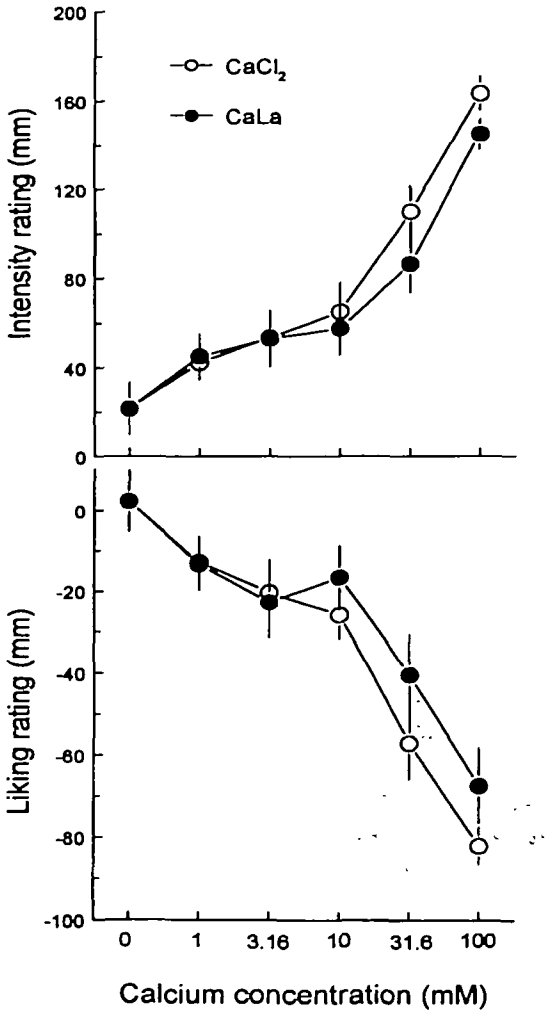

Figure 3 Rated intensity and liking of suprathreshold concentrations of calcium chloride ($CaCl_2$) and lactate (CaLa).

–0.40. This correlation was nonsignificant ($P = 0.14$), but significantly different from the intercorrelations of the three $CaCl_2$ tests ($P < 0.05$).

In the odor detection tests, subjects were given three bottles and asked to pick the sample that differed from the other two. In both low- and high concentration tests, there was no difference in the number of correct responses made on tests involving two odor samples and one blank sample compared with the more traditional one odor sample and two blank samples. The 12 subjects asked to detect the odor (or lack of odor) of 1 mM calcium solutions performed at chance levels (percentage of correct answers: $CaCl_2$ = 29 ± 6%; CaLa = 38 ± 8%; chance = 33%). They performed significantly better than chance with both 100 mM $CaCl_2$ and 100 mM CaLa [$CaCl_2$, correct answers = 47 ± 5%; $t(11) = 2.60$ $P < 0.025$; CaLa, correct answers = 92 ± 4%; $t(11) = 14.2$, $P < 0.0001$]. The subjects were significantly better at discriminating 100 mM CaLa from water than 100 mM $CaCl_2$ from water, $t(11) = 7.30$, $P < 0.0001$. According to binomial tests, all 12 subjects did significantly better than chance at discriminating 100 mM CaLa from water by smell, with only one subject getting fewer than 21 of 24 choices incorrect. On the other hand, only 5 of 12 subjects could discriminate 100 mM $CaCl_2$ from water by smell at better than chance levels and the best any subject scored was 18 of 24 trials correct.

Taste qualities

Data from each of the six dependent variables (ratings of intensity, liking and the four taste qualities) were analyzed by separate ANOVAs with factors of calcium source (chloride or lactate) and concentration. Because of the large

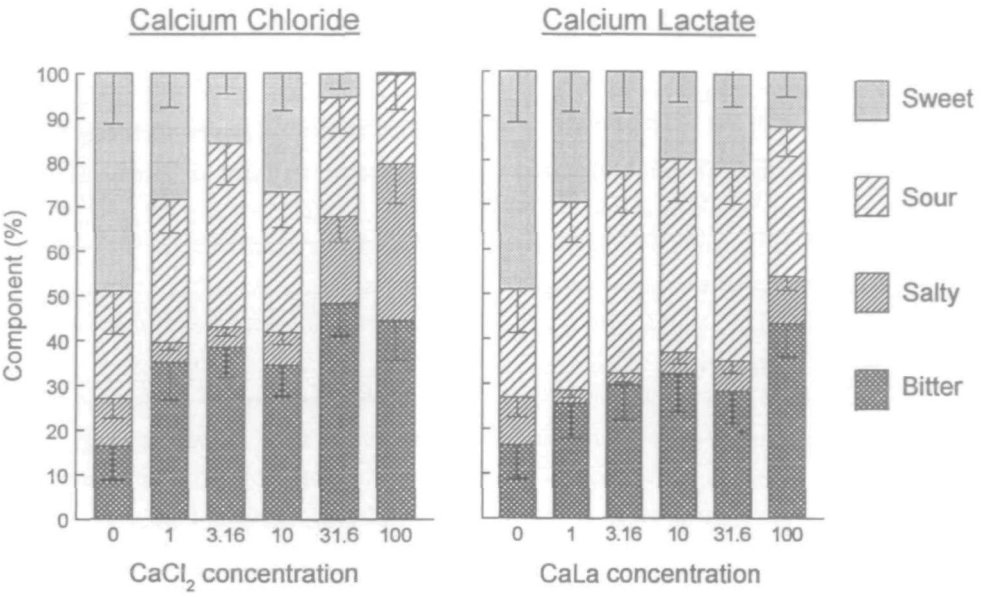

Figure 4 The contribution of sweet, sour, salty and bitter components to the taste of calcium chloride ($CaCl_2$) and lactate (CaLa) solutions. Data for water (0) are reported in both panels to aid comparison.

number of statistical tests conducted, the chance of making a type I error using the normal criterion for significance ($P < 0.05$) is high. On the other hand, a Bonferroni correction based on all comparisons appears inordinately strict [0.05/(2 solutions × 6 concentrations × 6 measures) = $P < 0.0007$]. Given the exploratory nature of the experiment, the significance level was set to allow some additional protection, at $P < 0.005$, with probability values between $P < 0.05$ and $P < 0.005$ considered to be equivocal. If $P < 0.00001$ then exact probabilities are given in the text so that cautious readers can apply other criteria.

Solution concentration significantly influenced ratings of intensity, liking and saltiness [respectively $F(4,56) = 43.2$, $P < 0.00001$; $F(4,56) = 22.9$, $P < 0.00001$; and $F(4,56) = 13.7$, $P < 0.00001$]. The effect of concentration on ratings of sweetness was equivocal [$F(4,56) = 3.54$, $P = 0.012$] and on the other taste qualities was nonsignificant. Ratings of intensity and saltiness increased with concentration whereas ratings of liking and sweetness decreased (see Figures 2 and 3). The form of calcium had significant effects on ratings of sourness [$F(1,14) = 12.6$, $P = 0.003$] and equivocal effects on ratings of bitterness and saltiness [respectively $F(1,14) = 4.86$, $P = 0.045$ and $F(1,14) = 5.39$, $P = 0.036$]. The $CaCl_2$ solutions were rated as more bitter, more salty and less sour than were the CaLa solutions. All other main effects were not significant and the only significant interaction was between the form and concentration of calcium on ratings of saltiness [$F(4,56) = 5.00$, $P = 0.0016$]. *Post hoc* Tukey's tests revealed this was due to the highest concentration of $CaCl_2$ being rated as significantly more salty than was the same concentration of CaLa ($P = 0.00019$). Only the 100mM $CaCl_2$ solution was considered to have a significantly greater salty component than water ($P = 0.0014$).

Discussion

On average, women could distinguish between water and calcium solutions in the order of 20 mM, a concentration ~100-fold lower than the detection thresholds found in earlier studies (see Introduction). There are several differences in methodology that could account for this. First, the method used here, which involved choosing between a pair of samples, one with and one without the calcium, is probably more sensitive than the identification of a single solution as containing or not containing calcium (Höber and Kiesow, 1898; von Skramlik, 1926; Fabian and Blum, 1943; Frings, 1948). Second, the deionized water used here for preparing calcium solutions and for rinsing between tests was probably purer than water available to earlier investigators. Thus, our subjects may have had less difficulty discriminating calcium in the test solution from 'endogenous' calcium and other contaminants in water. Third, our subjects were all women, whereas earlier studies tended to use men. It may be that women are more sensitive

to calcium, perhaps due to their higher calcium requirements (for reproduction) or to a greater need due to inadequate dietary calcium intake (Block *et al.*, 1985; Morgan *et al.*, 1985).

Mean detection thresholds for the five calcium salts tested were similar, suggesting that the anion had little influence on calcium detection. On the other hand, correlations between detection thresholds of different calcium salts ranged widely, from $r = -0.09$ to $r = 0.78$. This could potentially reflect differences in the basis of perception of different anions but it may also be due to random variation in the subjects' sensitivity to calcium from test-to-test. In support of the latter possibility, there was considerable variability in detection thresholds even when the same calcium salt ($CaCl_2$) was tested three times. The average test–retest reliability for $CaCl_2$ ($r = 0.52$) appears low. However, we have been unable to find in the literature comparable test–retest reliability measures for NaCl or other salts, and the value found here was not out of line with that seen with sucrose solutions ($r = 0.57$–0.86; Mattes, 1988). Thus, we suspect that the inherent limitations in the test procedure probably account for much of the variation in detection thresholds. Other sources of variability may come from innate differences in calcium detection (although this was unrelated to the ability to detect PROP), differences in nutritional status influencing calcium detection, or the use of different strategies to sample calcium (e.g. the thoroughness of rinsing between each test, the duration the solutions were held in the mouth).

The finding that 1 mM $CaCl_2$ and CaLa could not be discriminated from water by smell suggests that calcium detection was guided primarily by orosensory factors, presumably gustation. However, it remains possible that olfaction could contribute to the perception of low concentrations of calcium in the mouth if these stimulate smell via the retro-nasal route. The finding that a higher (100 mM) concentration of CaLa could be discriminated from water by smell was not surprising. Presumably the subjects could detect small quantities of volatile lactic acid produced by the interaction of the lactate with water. However, the ability of some subjects to discriminate 100 mM $CaCl_2$ from water by smell is more difficult to explain. One possibility is that some calcium may react with water to produce calcium hydroxide, which has a distinct odor. Another is that because the $CaCl_2$ used was only 99% pure, impurities were detected. It is also possible that the smell of $CaCl_2$ is 'real' and related to the smell of NaCl (Henkin and Bartter, 1966; Miller and Erickson, 1966; Bell *et al.*, 1979).

Our results of suprathreshold tests with $CaCl_2$ and CaLa suggest that solutions of these compounds are equally intense and equally disliked. There is one report that humans prefer low concentrations of calcium to water (Zoeteman *et al.*, 1978) and rats prefer low concentrations of calcium salts to water (Tordoff, 1994). However, these effects are subtle and may only be expressed when a choice is available. At all concentrations, both calcium salts tested had a substantial bitter component, with $CaCl_2$ being more bitter than CaLa. These findings are in broad agreement with early work suggesting calcium salts have a bitter taste (Kahlenberg, 1901; Frings, 1946; Moncrieff, 1967). However, unlike these early studies, here we found the two calcium salts tested also had a considerable sour component, with CaLa being more sour than $CaCl_2$. Saltiness was apparent at the highest (100 mM) concentration of $CaCl_2$ only. It would appear that the chloride ion imparts saltiness and either increases bitterness or reduces sourness. This may be due to its own action or to modification of the action of calcium on salt-, sour- and bitter-sensitive receptive elements.

Although these studies provide basic information about the chemosensory properties of calcium salt solutions, it is difficult to apply this information to the problem of low calcium intake in humans (cf. McCarron *et al.*, 1990; NIH Consensus Statement, 1994; Resnick, 1991). The biological significance of calcium detection in the micromolar range is unclear because sources of calcium in this range would be very rare. For example, the calcium content of Philadelphia's water supply during 1989–1994 averaged at ~0.75 mM and ranged between 0.35 and1.40 mM calcium, depending on the river from which the water was extracted and the season [Philadelphia Water Department (1994); calcium levels are lowest during periods of high rainfall]. Thus, calcium always contributes to the taste of tap water, although this may be masked by the taste of other compounds. Because calcium concentrations vary depending on the source of water and the season, it is likely that calcium contributes variably to the taste of tap water.

An obvious reason for low human calcium intakes is that high concentrations of calcium salts are strongly disliked. Although this was true of $CaCl_2$ and CaLa solutions, calcium occurs only in mixtures with other tasteful foods in the diet. Skimmed milk, which is probably the closest food in concentration and consistency to the pure solutions used,

here contains <32 mM calcium (Nutritionist-3 database), but ~92% of this is bound to casein or phosphate (for review see Robertson and Marshall, 1981, p. 113) and so may not be tasted. Pure sources of calcium are few and far between. Most people have tasted the calcium carbonate found in blackboard chalk or masonry lime. This is generally described as 'chalky', which is difficult to reduce to sweet, sour, salty and bitter components, and very unlike the tastes described by our subjects given calcium solutions. If mammals have specific calcium-sensitive receptors on the tongue, like those of the frog and mudpuppy (Junge and Brodwick, 1970; Kitada, 1978; Kurihara *et al.*, 1980; Bigiani and Roper, 1991), it may be inappropriate to reduce the taste of calcium to components based on the four taste modalities, or at least to infer any mechanistic principles from this.

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