Calcium-Deprived Rats Avoid Sweet Compounds

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ABSTRACT To characterize the link between calcium status and sweet solution intake, rats fed a diet containing 25 mmol Ca²⁺/kg (Ca-25, low calcium) or 150 mmol Ca²⁺/kg (Ca-150, control) were given 48-h two-bottle tests with a choice between water and various concentrations of a nutrient (sucrose, Polycose, ethanol and/or corn oil). Rats fed the Ca-25 diet had significantly lower sucrose intakes and preferences over the entire range tested (10–320 g/L) even though the same (Experiment 1), or identically treated (Experiment 2a) rats had normal Polycose and ethanol intakes and normal (Experiment 1, 2b) or significantly greater (Experiment 2a) corn oil intakes. In addition, rats fed the Ca-25 diet had significantly lower intakes relative to rats fed the Ca-150 diet of other sweeteners (30 mmol/L D-phenylalanine, 1 mmol/L saccharin and 0.3 mmol/L aspartame), significantly higher intakes of 0.5 mg/L capsaicin and 300 mmol/L monosodium glutamate, and normal intakes of 10 g/L or 80 g/L safflower oil and 10 g/L peanut oil. In a three-cup macronutrient selection experiment (Experiment 3), calcium-deprived rats ate significantly less of a high sucrose carbohydrate source and significantly more of a protein source than did controls. These results suggest that calcium deficiency reduces the rat's liking for sweetness, irrespective of the type or form of sweetener, and that this is not due to a general reduction in energy intake. J. Nutr. 128: 1232–1238, 1998.

KEY WORDS: • macronutrient intake • Polycose • ethanol • diet selection • rats

Fifty years ago, McCay and Eaton (1947) reported that calcium-deprived rats reduced sugar solution intake. Relative to rats fed a complete diet, those fed a low calcium diet drank less 100 g/L sucrose solution or cola beverage, but not water. Allowing the calcium-deprived rats to eat bone meal or calcium carbonate elevated sucrose and cola intakes to near normal. In another study reported in the same paper (McCay and Eaton 1947), rats fed a low-micronutrient diet had low 100 g/L sucrose intakes that were elevated by calcium supplementation. These findings are difficult to interpret because sucrose or cola was the only fluid available. However, they are complemented by recent work that used 24- or 48-h two-bottle tests with water as one of the choices and saccharin or sucrose as the other. Rats fed a low calcium diet drank significantly less 100 or 316 mmol/L sucrose and 1, 2.5, 3.16 or 10 mmol/L sodium saccharin than did controls (Coldwell and Tordoff 1996a, Tordoff et al. 1990, Tordoff 1992b). This did not appear to be due to a generalized reduction in intake of all compounds because the same rats greatly increased their intakes of NaCl and several other salts, and had normal intakes of representative sour and bitter compounds (e.g., 2.5 mmol/L citric acid and 0.368 mmol/L sucrose octaacetate). Of the 31 compounds tested in one study, only phosphates and the sweeteners were avoided by calcium-deprived rats (Coldwell and Tordoff 1996a).

The work described above shows that calcium-deprived rats selectively reduce intakes of sucrose, cola and saccharin. These compounds have a sweet taste in common but different chemical structures and postigestive actions, suggesting that calcium status influences oral sensitivity rather than postigestive mechanisms. However, an alternative hypothesis is that the reduction in sucrose intake is due to a general reduction in energy intake. This may include reduced intake of nonnutritive saccharin because the sweet taste of this compound is an innate indicator of energy consumption (Tordoff 1988).

To test this, and to examine the phenomenon in more detail, we compared the ingestive responses of calcium-deprived and replete control rats to several nutrients and taste compounds.

MATERIALS AND METHODS

Animals and diets. All experiments were approved by the Monell Chemical Senses Center Animal Care and Use Committee and complied with the NIH guidelines (NRC 1985). The experiments involved male Sprague-Dawley CD-VAF rats, aged 21–23 d when purchased from Charles River Laboratories (Stony Ridge, NY). Group sizes and body weights at the start of testing are presented in Table 1. Each rat was housed individually in a stainless steel cage with a mesh front wall and floor (19.5 × 17.5 × 24.5 cm). The temperature was maintained at 23°C and illumination was on a 12-h light:dark cycle with lights off at 1800 h. Deionized water was continuously available from a 300-mL inverted glass water bottle with a stainless steel spout and rubber stopper. When available, test solutions were provided in a similar bottle, with the tips of the solution and water bottle spouts positioned ~3 cm above the cage floor and 3–5 cm apart.

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Food was presented as a powder in a glass jar attached by a stainless steel spring to the front wall of the cage. The food formulation was based on a calcium-free version of the AIN-76A diet (AIN 1980). Briefly, the mineral mix involved substituting K2HPO4 for CaHPO4, MgSO4 for K2SO4 and K-citrate, and lowering MgO content (see Tordoff 1992a for diet formulation). These manipulations provided a diet that was virtually calcium free, with the same amounts of phosphorus and other essential nutrients and minerals as in the AIN-76A diet. Calcium carbonate was added to this basal diet to provide calcium at either 150 or 25 mmol Ca\(^{2+}\)/kg.

The diets were prepared commercially (Dyets, Bethlehem, PA) and their calcium content was verified before use. We consider the 150 mmol Ca\(^{2+}\)/kg diet (Ca-150) to be a control diet and the 25 mmol Ca\(^{2+}\)/kg diet (Ca-25) to be a low-calcium diet. In comparison, the AIN-76A diet formulation calls for 129 mmol Ca\(^{2+}\)/kg diet and Purina Laboratory Chow (no. 5001, St. Louis, MO) contains 250 mmol Ca\(^{2+}\)/kg diet. Maximal levels of growth can be obtained with diets containing 40 or 68 mmol Ca\(^{2+}\)/kg (Bernhardt et al. 1969, Chandler and Crangle 1962), and maximal bone mineralization requires 45 mmol Ca\(^{2+}\)/kg diet (Bernhardt et al. 1969). We used a low-calcium rather than a calcium-free diet to be comparable with most of our recent work [e.g., (Coldwell and Tordoff 1996a and 1996b, Tordoff et al. 1993, Tordoff and Okiyama 1996)]. Total calcium deprivation of young male rats is fatal in ~5wk, whereas rats fed a low-calcium diet have almost normal growth rates and survive indefinitely. However, they have reduced plasma concentrations of ionized and total calcium, elevated plasma concentrations of parathyroid hormone, 1,25-dihydroxyvitamin D and calcitonin, and reduced bone calcium stores (Tordoff et al. 1993, Tordoff 1996a and 1997, Tordoff and Okiyama 1996).

Experiment 1: comparison of nutrient solutions with equal energy density. Rats (n = 24) were fed either the Ca-150 or Ca-25 diet for 18d before tests began. Then, all of the rats received a series of four 48-h two-bottle tests with a choice between water and 180 g/L sucrose, 100 g/L ethanol, 180 g/L Polycose or 80 g/L corn oil. These drinks all provided ~3000 kJ/L. Polycose is a commercially prepared soluble mixture of maltooligosaccharides (glucose polymers; Ross Laboratories, Columbus, OH). It is a non-sweet carbohydrate, with taste properties that have been investigated extensively by Sclafani and colleagues (see Sclafani 1987). The sucrose, ethanol and Polycose were dissolved in deionized water. Because the corn oil is insoluble, it was suspended in water by homogenization with 3 g/L xanthan gum (Sigma Chemical, St. Louis, MO; for simplicity, the suspension is referred to here as a solution). To control for side preferences (i.e., the rat's proclivity to drink from the left or right bottle), the positions of the water and solution bottles were switched every 24h. Between each 48-h test was a day in which a single bottle of water was provided.

**Experiment 2a: ascending concentration series of sucrose, Polycose, ethanol and corn oil.** Experiment 1 showed that relative to controls fed the Ca-150 diet, rats fed the Ca-25 diet drank similar amounts of Polycose, ethanol and corn oil but less sucrose solution. However, both groups had much higher intakes of 180 g/L sucrose solution than equicaloric solutions of ethanol or corn oil, raising the possibility that a floor effect masked differences between the groups in intake of these solutions. To provide a more detailed examination of the effect of calcium deprivation on intake of nutrient solutions, groups of rats fed the Ca-150 or Ca-25 diet were given a range of concentrations of the four nutrients used in Experiment 1.

General procedures for this experiment were similar to those of Experiment 1. Eight groups of 12 rats were fed either the Ca-150 or Ca-25 diet for 16d. They then received a series of six 48-h two-bottle choice tests between water (or 3 g/L xanthan gum for the corn oil group) and sucrose, Polycose, ethanol or corn oil. The concentrations used were 10, 20, 40, 80, 160 and 320 g/L sucrose, Polycose and corn oil, and 2.5, 5, 10, 20, 40 and 80 g/L ethanol. Each rat received all six concentrations of one of the nutrients in ascending order. The positions of the water and solution bottles were switched every 24h to control for side preferences, and a 24-h period with only water available was interspersed between each 48-h test. So that the entire test series took 17d.

**Experiment 2b: intake of miscellaneous solutions by rats fed the low-calcium diet.** The rats used in Experiment 2a were fed their appropriate diets and were given 4d with only water to drink before the start of Experiment 2b. The rats were assigned to new groups of eight that were matched for previous experience, i.e., each group contained two rats from the sucrose, Polycose, ethanol and corn oil groups. These rats were then tested with the following compounds: 10 g/L corn oil, 10 g/L safflower oil, 3 mmol/L monosodium glutamate, 0.5 mg/L capsaicin, 3 mmol/L D-phenylalanine or 1 mmol/L saccharin. After a day with only water to drink, the rats were reassigned to new groups matched for experience on both previous tests, and were given one of the following (as well as water) to drink: 80 g/L peanut oil, 80 g/L safflower oil, 300 mmol/L monosodium glutamate, 0.25 mg/L capsaicin, 30 mmol/L D-phenylalanine or 0.3 mmol/L aspartame. Most of the compounds were dissolved in water. However, the oils were suspended in 3 g/L xanthan gum; to help the capsaicin dissolve, this was first prepared in 0.2 g/L ethanol, which was then diluted in water. The “water” bottles for the oil and capsaicin tests also contained these small amounts of xanthan gum or ethanol.

The test compounds were chosen for several reasons as follows: 1) Three potential sweeteners were tested. Saccharin was tested to allow comparison with earlier work with this compound (Tordoff et al. 1990, Tordoff 1992b). D-Phenylalanine is an amino acid–type sweetener that is liked by some mouse strains but to which others are indifferent (Ninomiya et al. 1984, Ninomiya and Funakoshi 1993). The two concentrations tested discriminate among these strains. Aspartame is an artificial sweetener that rats and mice mildly prefer or do not distinguish from water (Sclafani and Abrams 1986).

2) Three oils were tested. The 10 g/L corn oil was retested (from Experiment 2a) because the results of that experiment were equivocal. The other oils (10 and 80 g/L safflower oil, 80 g/L peanut oil) were tested to see whether the difference in corn oil intake between rats fed the Ca-25 diet and those fed the Ca-150 diet was generalized to

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**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration of calcium deprivation</th>
<th>Ca-150</th>
<th>Ca-25</th>
<th>Ca-150</th>
<th>Ca-25</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>150</td>
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<td>134 ± 5</td>
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<td><strong>2a</strong></td>
<td></td>
<td>18</td>
<td>48</td>
<td>48</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>138 ± 2</td>
<td>132 ± 2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2b</strong></td>
<td></td>
<td>39</td>
<td>48</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>331 ± 5</td>
<td>280 ± 5**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3</strong></td>
<td></td>
<td>20</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>157 ± 5</td>
<td>153 ± 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Body weights are means ± SEM. Relative to the group fed the Ca-150 diet, *P < 0.05, **P < 0.0001.
2. Ca-150, rats fed diet containing 150 mmol Ca\(^{2+}\)/kg; Ca-25, rats fed diet containing 25 mmol Ca\(^{2+}\)/kg. The significant difference in body weight between rats fed the Ca-150 diet and the Ca-25 diet in Experiment 2 but not Experiments 1 and 3 was probably due to the larger number of rats tested.
other oils. 3) Two concentrations of monosodium glutamate were tested because this compound produces the prototypical umami taste (Kawamura and Kake 1987). It has also been suggested to have a sweet taste to rodents (Yamamoto et al. 1991). 4) Two concentrations of capsaicin were tested because this compound is an irritant that stimulates the trigeminal system.

**Experiment 3: diet selection by calcium-deprived rats.** Experiments 1 and 2 used two-bottle preference tests to examine the acceptance of nutrients solutions by calcium-deprived rats. However, more traditional selection studies involve choosing among various diets or diet components. In the final experiment in this series, we examined the food selection of calcium-deprived rats given a choice among solid sources of protein, fat, and a sweet carbohydrate to determine whether the results found with fluid intake applied to solid nutrients.

Twenty-nine rats were tested. Initially, 15 were fed the Ca-25 diet and the other 14 were fed the Ca-150 diet. After 20 d, the effectiveness of the dietary manipulation was confirmed by measuring blood calcium concentrations. A 40-μL blood sample was taken from the tip of the tail of each rat and immediately analyzed using a CIBA-Corning 634 clinical calcium electrode (Medfield, MA). The rats were then weighed and transferred to cages designed to facilitate diet selection studies. Each cage was 42 cm wide × 36 cm deep × 17 cm high, with a mesh front wall and floor, and stainless steel sides, back and roof. Nutrient sources were available from 6 cm square × 3 cm high stainless steel cups with “spill-proof” lids, which gave access to the food through a hole 3 cm in diameter. The containers were held in place in each corner of the cage by 6.5-cm wide stainless steel channels. The macronutrient sources used were (per kg): protein, 1000 g vitamin-free casein; fat, 950 g Primoex (Purina and Gamble, Cincinnati, OH; similar to Crisco) and 50 g safflower oil; carbohydrate, 600 g cornstarch, 300 g dextrin and 100 g sucrose. These sources are similar to those used by other investigators, but no vitamins or minerals were mixed into them. Deionized water was available from an inverted 300-mL bottle with a rubber stopper and stainless steel drinking tube. This was mounted in the middle of the front wall of the cage。

After 24 h and again at 48 h, intake of each of the three nutrient sources was recorded. The position of the cups was not changed in this experiment.

**Data analyses and presentation.** Food and fluid intakes were measured daily by weighing the food cups and/or bottles (± 0.1 g). Fluid intakes were converted to milliliters, assuming that 1 g of water or solution had a volume of 1 mL. Diet, macronutrient source and solution intakes were converted to kilojoules, assuming that 1 g of nutrient was equivalent to 16.7, 16.7 or 37.7 kJ for protein, carbohydrate and fat, respectively. We derived total fluid intakes (water + solution intake), solution preference ratios (solution intake/total intake), and total energy intake (food intake + solution intake) and the percentage of total intake consumed as each macronutrient source (Experiment 3).

For Experiments 1 and 2a, each measure was analyzed by separate two-way mixed design ANOVA with factors of diet condition (Ca-150 or Ca-25 diet) and solution type (Experiment 1) or concentration (Experiment 2a). The significance of differences between individual pairs of means was determined using post-hoc Tukey’s tests. In Experiments 2 and 3, there was little benefit gained from direct comparison of intakes of nutrients or solutions’ thus separate data analyses were conducted for each. For Experiments 2b and 3, analyses were conducted using Student’s t tests.

On the basis of preliminary analyses, two steps were taken to simplify description of the results. First, the effects of solution concentration on solution intake in Experiment 2a are not reported. Our analyses provided no new insights into these well-characterized concentration-response functions (e.g., Castonguay et al. 1984, Richter 1977). Second, the results of analyses of food intakes and total energy intakes were complex and added little to interpretation. These too are not reported here.

All statistical tests were conducted using a criterion for significance of 0.05. Values presented in the text are means ± SEM.

**RESULTS**

**Experiment 1: comparison of nutrient solutions with equal energy density.** Relative to solution intakes of rats fed the Ca-150 diet, rats fed the Ca-25 diet drank significantly less sucrose, but consumption of the other solutions did not differ [Fig. 1; diet × solution interaction, F(3,66) = 14.1, P < 0.00001]. Rats fed the Ca-150 diet drank similar amounts of sucrose and Polycose, which were both consumed in significantly greater amounts than were ethanol or corn oil. Rats fed the Ca-25 diet drank significantly less sucrose than Polycose, but significantly more sucrose than ethanol or corn oil. Water intakes demonstrated a reciprocal pattern, i.e., when sucrose was available, rats fed the Ca-25 diet drank significantly more water than did rats fed the Ca-150 diet. When the other solutions were available, there was no effect of diet on water intake (Fig. 1).

**Experiment 2a: ascending concentration series of sucrose, Polycose, ethanol and corn oil.** Rats fed the Ca-25 diet had significantly lower sucrose intakes and preferences than did rats fed the Ca-150 diet at all six concentrations tested [Fig. 2; main effect of diet, solution intake, F(1,122) = 49.7, P < 0.00001; preference, F(1,22) = 51.9, P < 0.00001]. The differences between the groups were generally greater at high sucrose concentrations than at low ones [diet × concentration interaction, solution intake, F(3,66) = 8.09, P < 0.00001; preference, F(5,110) = 2.78, P < 0.05]. Rats fed the Ca-150 diet had strong preferences for all concentrations of sucrose, whereas those fed the Ca-25 diet showed milder preferences for low concentrations and were indifferent (i.e., their preferences did not differ reliably from 50%) to 160 and 320 g/L sucrose. The changes in preference were a result of differences in both solution intake and water intake. Rats fed the Ca-150 diet drank little or no water when they had access to sucrose solution; rats fed the Ca-25 diet drank significantly more water at all concentrations than did rats fed the Ca-150 diet [main effect, F(1,122) = 56.6, P < 0.00001], but particularly when given 160 or 320 g/L sucrose to drink [diet × concentration interaction, F(5,110) = 7.41, P < 0.00001].

There were no differences between rats fed the Ca-25 diet and the Ca-150 diet in Polycose intake or ethanol intake, or their associated water intakes or preferences (Fig. 2). There were also no significant differences between the two diet groups in preference for corn oil or water intakes when corn oil was available. However, intake of corn oil was significantly higher in rats fed the Ca-25 diet than in those fed the Ca-150 diet [main effect, F(1,122) = 4.34, P < 0.05]. Although the difference appeared largest at the lowest concentration (Fig. 2), the
FIGURE 2 Daily intake of a range of concentrations of sucrose, Polyose, ethanol or corn oil by rats fed diets containing 150 (Ca-150) or 25 (Ca-25) mmol Ca\(^{2+}\)/kg. Upper panels show intake of solutions, middle panels show intake of water, which was available when solutions were given, and lower panels show solution preference (solution intake/total fluid intake). Note that intakes of ethanol and corn oil are plotted against a different scale than are intakes of sucrose and Polyose. Values are means ± SEM, n = 12 in each of eight groups.

interaction between diet and corn oil concentration was not significant [F(5,110) = 2.13, P < 0.07].

Experiment 2b: intake of miscellaneous solutions by rats fed low calcium diet. Calcium deficiency significantly increased the preference for 300 mmol/L monosodium glutamate \([t(14) = 3.27, P < 0.01]\) and significantly decreased preferences for 30 mmol/L D-phenylalanine \([t(14) = 3.41, P < 0.005]\), 1 mmol/L saccharin \([t(14) = 2.20, P < 0.05]\), 0.3 mmol/L aspartame \([t(14) = 2.52, P < 0.05]\) and 10 g/L safflower oil \([t(14) = 2.26, P < 0.05]; Table 2\). It also significantly attenuated the avoidance of 0.5 mg/L capsaicin \([t(14) = 3.11, P < 0.01]\). With the exception of the change in preference for 10 g/L safflower oil, which was apparently due to high water intakes of rats fed the Ca-25 diet \([t(14) = 3.29, P < 0.01]\), all of the changes in preference were due to changes in solution intakes; relative to controls fed the Ca-150 diet, rats fed the the Ca-25 diet had significantly higher intakes of 300 mmol/L monosodium glutamate \([t(14) = 4.82, P < 0.001]\) and 0.5 mg/L capsaicin \([t(14) = 2.84, P < 0.05]\), and significantly lower intakes of 30 mmol/L D-phenylalanine \([t(14) = 3.71, P < 0.005]\), 1 mmol/L saccharin \([t(14) = 3.16, P < 0.01]\) and 0.3 mmol/L aspartame \([t(14) = 2.73, P < 0.05]\). There were no significant differences between the groups in intake of 10 g/L corn oil, 10 g/L or 80 g/L safflower oil, 80 g/L peanut oil, 3 mmol/L monosodium glutamate, 3 mmol/L D-phenylalanine or 0.25 mg/L capsaicin (Table 2).

Experiment 3: Diet selection by calcium-deprived rats. Immediately before the diet selection test, the 15 rats fed the Ca-25 diet had significantly lower blood ionized calcium concentrations than did the 14 rats fed the Ca-150 diet \([1.19 ± 0.03 \text{ vs. } 1.34 ± 0.01 \text{ mmol/L}, t(27) = 4.47, P < 0.001]\).

During the 48-h test with a choice between macronutrient sources, there was no difference between the two groups in total energy intake \([\text{Fig. } 3; t(27) = 1.45, \text{ not significant (NS)}]\). However, relative to controls, the calcium-deprived rats ate significantly more of the protein source [raw intakes, \(t(27) = 2.86, P < 0.01\); proportion of total, 33 ± 4 vs. 17 ± 5%, \(t(27) = 2.73, P < 0.05\)] and significantly less of the carbohydrate source [raw intakes, \(t(27) = 2.64, P < 0.05\); proportion of total, 46 ± 4 vs. 62 ± 4%, \(t(27) = 2.87, P < 0.01\)]. There was no difference between the groups in fat intake [raw intakes, \(t(27) = 0.72, \text{ NS}\); proportion of total, 21 ± 2 vs. 21 ± 2%, \(t(27) = 0.16, \text{ NS}\)].

**DISCUSSION**

Relative to the calcium-replete controls, rats fed the low-calcium diet had reduced preferences for a range of sucrose solutions and other sweet compounds (saccharin, D-phenylalanine, aspartame), and ate less of a sweet carbohydrate source in a macronutrient selection experiment. Intakes of non-sweet nutrients by the same or identically treated rats either did not differ from controls (Polyose, ethanol, various oils), or were slightly elevated (corn oil in Experiment 2a, protein source in

**TABLE 2**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Solution intake Ca-150</th>
<th>Solution intake Ca-25</th>
<th>Water intake Ca-150</th>
<th>Water intake Ca-25</th>
<th>Solution preference Ca-150</th>
<th>Solution preference Ca-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mmol/L D-Phenylalanine</td>
<td>21 ± 6</td>
<td>22 ± 2</td>
<td>17 ± 3</td>
<td>18 ± 4</td>
<td>53 ± 7</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>30 mmol/L D-Phenylalanine</td>
<td>40 ± 8</td>
<td>17 ± 3**</td>
<td>15 ± 5</td>
<td>21 ± 2</td>
<td>75 ± 7</td>
<td>42 ± 7*</td>
</tr>
<tr>
<td>1 mmol/L Saccharin</td>
<td>63 ± 8</td>
<td>38 ± 6*</td>
<td>8 ± 2</td>
<td>12 ± 3</td>
<td>89 ± 2</td>
<td>72 ± 4*</td>
</tr>
<tr>
<td>0.3 mmol/L Aspartame</td>
<td>29 ± 6</td>
<td>15 ± 3*</td>
<td>22 ± 7</td>
<td>20 ± 4</td>
<td>58 ± 5</td>
<td>42 ± 4*</td>
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<tr>
<td>10 g/L Corn oil</td>
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<td>16 ± 4</td>
<td>11 ± 1</td>
<td>20 ± 4</td>
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<td>10 g/L Safflower oil</td>
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<td>11 ± 3</td>
<td>13 ± 2</td>
<td>27 ± 4*</td>
<td>47 ± 8</td>
<td>28 ± 4*</td>
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<td>80 g/L Safflower oil</td>
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<td>32 ± 5</td>
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<tr>
<td>80 g/L Peanut oil</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>13 ± 1</td>
<td>19 ± 2*</td>
<td>45 ± 7</td>
<td>38 ± 5</td>
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<td>3 mmol/L MSG2</td>
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<td>26 ± 6</td>
<td>17 ± 3</td>
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<td>300 mmol/L MSG</td>
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<td>54 ± 4**</td>
<td>22 ± 6</td>
<td>20 ± 5</td>
<td>62 ± 4</td>
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<td>0.25 mg/L Capsaicin</td>
<td>8 ± 4</td>
<td>9 ± 2</td>
<td>26 ± 4</td>
<td>35 ± 6</td>
<td>21 ± 5</td>
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</tr>
<tr>
<td>0.5 mg/L Capsaicin</td>
<td>5 ± 1</td>
<td>9 ± 1**</td>
<td>27 ± 1</td>
<td>22 ± 5</td>
<td>15 ± 2</td>
<td>32 ± 5*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8 per group. *P < 0.05, **P < 0.01 relative to rats fed Ca-150 diet.

2 MSG, monosodium glutamate.
carbohydrate source (cornstarch, dextrin and sucrose); FAT, fat source.

electrophysiologic and genetic evidence implicating impaired peripheral gustatory mechanisms (Ninomiya et al. 1984, Ninomiya*, Ninomiya1236 TORDOFF AND RABUSA

sweetness by calcium-deprived rats is related to some adverse Opiate-mediated antinociception is modulated by calcium, 32 ñ P < 0.05 relative to group fed Ca-150 diet. Values are means ± SEM, n = 15 (Ca-25), n = 14 (Ca-150). PRO, protein source (casein); CHO, carbohydrate source (cornstarch, dextrin and sucrose); FAT, fat source (vegetable shortening and safflower oil).

Experiment 3). Taken together, these results indicate that calcium deprivation reduces the rat’s preference for sweetness.

Several less interesting explanations for the difference in sweet intake between calcium-deprived and control rats can be dismissed. In this respect, a particularly important result was that calcium-deprived animals drank more Polycose than equicaloric sucrose (Experiment 1). This shows that the low sucrose intakes of the calcium-deprived rats were not due to constraints on the capacity to ingest large amounts of energy or fluid, or to a general reduction in carbohydrate intake. Sucrose and Polycose solutions differ in osmolarity and postigestive actions, but these were apparently unimportant for the results because calcium-deprived rats showed reliable reductions in intake of both hyper- and hypotonic sucrose solutions, as well as artificial sweeteners, which have virtually no osmotic effects and widely differing metabolic effects (if any).

It is likely that rats fed the low-calcium diet found their maintenance diet more palatable than did controls because calcium is unpalatable to humans and generally avoided by rats (Tordoff 1994, Tordoff 1996b). This raises the possibility that the reason for the reluctance of the calcium-deprived rats to drink sucrose was that they were less motivated to ingest a second source of energy. However, we think this is unlikely for the following reasons: 1) deprived animals are generally more inclined to sample novel foods (Rezin 1976); 2) the difference in diet palatability did not affect intake of non-sweet nutrients; and 3) the reduction in sweet intake was obtained even in the macronutrient self-selection experiment in which rats did not have access to the maintenance diet.

The findings that calcium-deprived rats avoid phosphates (Coldwell and Tordoff 1996a), which exacerbate calcium deficiency, and ingest NaCl, which temporarily ameliorates it (Tordoff 1997), raise the possibility that the avoidance of sweetness by calcium-deprived rats is related to some adverse metabolic consequence of drinking sucrose. We have found that rats fed a low-calcium diet have lower plasma concentrations of glucose and insulin than do controls (Tordoff 1997). They also show an attenuated feeding response to challenges with 2-deoxyglucose or insulin (unpublished data). Thus, there may be a subtle derangement of glucose metabolism induced by calcium deprivation. However, we observed no obvious changes in the disposal of ingested glucose (assessed from plasma glucose concentrations) and no effects of drinking glucose on plasma concentrations of calcium or calcitropic hormones (Tordoff 1997). In any case, the possibility of an adverse metabolic effect of glucose on intake is difficult to reconcile with the reduced intake of nonnutritive sweeteners and normal intake of Polycose by calcium-deprived rats.

Our work with various concentrations of sucrose, 1 mmol/L saccharin, 30 mmol/L D-phenylalanine and 0.3 mmol/L aspartame, extends earlier studies showing that calcium deprivation reduces intake of sucrose (100, 292 or 316 mmol/L); cola, and other concentrations of saccharin [1, 3.16 and 10 mmol/L]; (Coldwell and Tordoff 1996a, Tordoff et al. 1990, Tordoff 1992b)]. We did not see a diet-induced difference in 3 mmol/L D-phenylalanine intake or preference, but this may be because the rats could not detect this low concentration. However, the results with 30 mmol/L D-phenylalanine were particularly interesting. For this sweet compound, rats fed the Ca-150 diet showed a moderately strong preference (75%), whereas those fed the Ca-25 diet were indifferent (42%). A similar failure to show any interest in D-phenylalanine has been seen in some strains of mice, and for these animals there is electrophysiologic and genetic evidence implicating impaired peripheral gustatory mechanisms (Ninomiya et al. 1984, Ninomiya and Funakoshi 1993). The possibility that calcium deficiency induces changes in the detection of sweetness is also supported by findings that calcium-deprived rats have reduced intakes of sweet solutions in short-term (20 or 30 min) acceptance tests (Coldwell and Tordoff 1996b, Tordoff 1997).

The possibility that dietary calcium deficiency impairs the action of sweet amino acid-type receptors is appealing, but speculative because there is no obvious mechanism by which calcium deficiency might influence sweetness perception. Sweet transduction is believed to be receptor mediated (e.g., Lindemann 1996); thus it is difficult to see how extracellular concentrations of calcium could be involved directly in the transduction event. Perhaps calcium facilitates the sweet molecule’s interaction with the receptor or is required for optimal receptor configuration. Another possibility is that extracellular concentrations of calcium might influence intracellular calcium concentrations, and thus second messenger pathways, synaptic transmission or action potential propagation. It is also possible that changes in hormones or other factors induced by calcium deficiency, rather than low levels of calcium per se, could be responsible for the changes in preference.

The possibility that a more general mechanism is involved is underscored by findings that deficiencies of sodium and zinc also decrease sweet intake and/or sweet preference (Beuchamp et al. 1990, Catalanotto and Lacy 1977, Catalanotto 1978, Rains and Shay 1995, Richter 1941). This is not a function of general malnutrition because no changes in saccharin intake were observed in rats deprived of iron, magnesium or phosphorus (Tordoff 1992b). One possibility is that deficiencies in calcium and other minerals interfere with brain opioid levels, which govern intake of sweet compounds. In support of this, calcium, sodium and zinc antagonize the binding of morphine and/or enkephalins to central nervous system receptor sites (Lujan et al. 1978, Stengaard-Pedersen 1982). Opiate-mediated antinociception is modulated by calcium,
calcium and vitamin D deficiency (Bazzani et al. 1984, Ben-Sreti et al. 1983, Contreras et al. 1988, Stengaard-Pedersen 1982). Intake of saccharin and other sweet solutions is increased by morphine and centrally administered opioid peptides (e.g., Gospell and Majchrzak 1989), and decreased by opioid receptor antagonists (Beczowska et al. 1993, Cooper 1983, Gospell and Majchrzak 1989). Although the issue is not resolved, some selectivity of the opioid system for sweet intake is apparent. For example, under some conditions, the nonspecific opioid antagonist, naloxone, inhibits intake of a sucrose-containing diet but not a Polycose- or cornstarch-containing diet (Weldon et al. 1996). Moreover, specific (K3) opioid receptor antagonists reduce intake of saccharin but not maltose dextrin solutions (Beczowska et al. 1993), akin to the results with Polycose found here. It remains to be determined whether the mild dietary calcium deficiency used here is sufficient to affect central opioid regulation and, assuming that this occurs, whether restoration of opioid function can restore intake of sweet solutions to normal.

There were several examples of calcium-deprived rats in- gesting more of a substance than did controls. In one experiment (Experiment 2a), rats fed the low calcium diet drank significantly more of a range of corn oil concentrations than did replete controls. However, this effect was fairly small, did not affect corn oil preferences, did not replicate in two other experiments (80 g/L corn oil in Experiment 1; 10 g/L corn oil in Experiment 2b) and was not seen with other oils (Experiment 2b) or a solid fat source (Experiment 3). We are inclined to dismiss it as spurious. More clearly, calcium-deprived rats had greater intakes of 0.5 mg/L capsaicin and 300 mmol/L monosodium glutamate than did controls. Because capsaicin is an oral irritant, the increased intake of this compound suggests that calcium deprivation might interfere with the trigeminal system. However, some caution in interpretation is warranted because a difference in capsaicin intake was seen at only one of the two concentrations tested, and no differences were found in alcohol intake, which is also an oral irritant. The results with monosodium glutamate argue for a deficit in umami taste in calcium deficiency and against the hypothesis that monosodium glutamate is sweet to rats (Yamamoto et al. 1991). However, this result is consistent with findings that calcium-deprived rats drink large volumes of sodium salts (Tordoff 1992a,1992b and 1996a, Tordoff et al. 1993, Tordoff and Coldwell 1996b) and, more generally, that sodium intake in compensation for reduced intake of the carbohydrate source.

On the basis of their work with rats, McCay and Eaton concluded that “the volume of sugar solution consumed may serve as a criterion of calcium deficiency...” (McCay and Eaton 1947, p. 359). It is intriguing to speculate whether this might have implications for humans. We are aware of only two epidemiologic studies in which the population was segregated on the basis of calcium intakes; neither study presented sugar intakes (Barger-Lux et al. 1992, Holbrook and Barrett-Connor 1991). More pertinently, a small study found that diet calcium intake was inversely related to intake of soft drinks, which might be considered as markers of sweet intake (Guenther 1986). However, this could be due to soft drinks displacing milk consumption. Thus, it remains to be seen whether the reduction in sweet intake and preference produced by eating a low calcium diet applies to humans as well as rats.

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**LITERATURE CITED**


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