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Advantame Sweetener Preference in C57BL/6J Mice and Sprague-Dawley Rats

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Abstract

Advantame is a new ultrahigh-intensity noncaloric sweetener derived from aspartame and approved for human use. Rats and mice are not attracted to the taste of aspartame and this study determined their preference for advantame. In 24-h choice tests with water, C57BL/6J mice and Sprague-Dawley rats were indifferent to advantame at concentrations of 0.01, 0.03, and 0.1 mM but significantly preferred 0.3 and 1 mM advantame to water. Both species also preferred 1 mM advantame to 1 mM saccharin in direct choice tests, but preferred 10 mM saccharin to 1 mM advantame, which is near the solubility limit for this sweetener. Mice also preferred 1 mM advantame to 1 mM sucralose or acesulfame K, but preferred both sweeteners at 10 mM to 1 mM advantame. In addition, mice preferred 1 mM advantame to 1 and 10 mM aspartame. Thus, advantame is a potent sweetener for rodents but, because of limited solubility, is not an effective alternative to saccharin, sucralose, or acesulfame K at higher concentrations.

Key words: acesulfame K, aspartame, saccharin, sucralose

Introduction

Advantame is a new ultrahigh-intensity noncaloric sweetener recently approved for human use by the U.S. Food and Drug Administration (2014). It is described as being approximately 20 000 times as sweet as sucrose which means that a very low concentration of advantame matches the sweetness potency of a dilute (3%) sucrose (Bishay and Bursey 2012). In contrast, the maximum sweetness intensity of advantame is estimated to match that of only 15.8% sucrose (Bishay and Bursey 2012). Advantame, which is derived from vanillin and the sweetener aspartame, is listed as 200 times more sweet than aspartame (Bishay and Bursey 2012). Toxicology studies have been performed with laboratory rodents and other species but data on the sweetener potency of advantame in rats and mice are not available (Otabe et al. 2011). The present study therefore investigated advantame preferences in C57BL/6J (B6) mice and Sprague-Dawley (SD) rats, 2 commonly used rodent strains in behavioral and physiological research on sweeteners. Overall, B6 mice and SD rats display little or no preference for aspartame in 24-h sweetener versus water tests and therefore it seemed unlikely that they would prefer advantame solutions (Sclafani and Abrams 1986; De Francisco and Dess 1998; Bachmanov et al. 2001). However, we observed significant advantame preferences in both species. We therefore performed additional tests which directly compared the preference for advantame to other noncaloric sweeteners including saccharin, sucralose, acesulfame K, and aspartame. Advantame was tested at a maximum concentration of 1 mM because of its limited solubility in water (Bishay and Bursey 2012). The other sweeteners were tested at the 1 mM concentration to compare their potency to advantame and also at 10 mM, a commonly used concentration with these sweeteners.

Experiment 1: advantame and other sweetener preferences in B6 mice

Materials and methods

Animals

Ten B6 mice (5 male, 5 female), born in our laboratory from stock purchased from the Jackson Laboratories, were studied. The mice were 8 weeks old at the start of testing and were singly housed in plastic tub cages with *ad libitum* access to chow (LabDiet 5001; PMI Nutrition International) and deionized water in a room maintained at 22 °C with a 12:12

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light:dark cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Test solutions

Advantame (Ajinomoto North America; MW 476.52) solutions were prepared at concentrations of 0.01, 0.03, 0.1, 0.3, and 1.0 mM using deionized water. The 1.0 mM concentration was the highest used because of the limited solubility of advantame in water (~1.8 mM or 0.099% at 25 °C). Saccharin (Sigma-Aldrich), aspartame (NuSci, HerbStore), sucralose (Tate & Lyle), and acesulfame K (Nutrinova) were prepared at 1 and 10 mM concentrations. Fluid was available through sipper spouts attached to 50-mL plastic bottles that were placed on top of the cage. The sipper spouts were inserted through holes positioned 3.7 cm apart in a stainless steel plate, and the drinking bottles were fixed in place with clips. Fluid intakes were measured to the nearest 0.1 g by weighing the drinking bottles on an electronic balance interfaced to a laptop computer. Daily fluid spillage was estimated by recording the change in weight of 2 bottles that were placed on an empty cage.

Procedure

The mice were adapted to drink water from 2 bottles for 1 week. They were then given a series of 2-day 2-bottle tests with advantame versus water at ascending concentrations of 0.01, 0.03, 0.1, 0.3, and 1.0 mM concentrations. They were then given 4 additional series of 2-day tests with saccharin, aspartame, sucralose, and acesulfame K versus advantame. In the first of these series, the mice were given 1 mM saccharin versus water for 2 days, 1 day of water only, and then 2 days of 1 mM saccharin versus 1 mM advantame followed by 2 days of 10 mM saccharin versus 1 mM advantame. Following 2 days of water only, the series was repeated using 1 and 10 mM concentrations of aspartame, sucralose, and acesulfame K, in that order, versus 1 mM advantame. The left–right position of the sweetener and water was alternated daily in these tests to control for side preferences.

Data an alysis

Daily solution and water intakes were averaged over the 2 days at each solution concentration. Sweetener intakes were also expressed as percent intakes (sweetener intake/total intakes × 100). Sweetener and water intakes were evaluated using ANOVA with solution (sweetener versus water or sweetener versus sweetener) and concentration as repeated measures. The significance of the 2-bottle sweetener preference at each concentration was evaluated by comparing sweetener versus water (or sweetener versus sweetener) intakes using paired *t*-tests corrected for multiple comparisons using the Bonferroni procedure. An initial analysis indicated that there were no main or interactive effects of sex so only the combined male and female data are presented.

Results

In the advantame versus water test series (Figure 1), the mice consumed comparable amounts of 0.01–0.1 mM advantame and water but significantly more 0.3 and 1 mM advantame than water (solution × concentration interaction, $F(4,44) = 28.1$, $P < 0.01$). Total solution intake (sweetener + water) at the 1 mM concentration exceeded that at the lower concentrations and also of mean water intakes recorded 2 days before and after the test series (9.4 vs. 6.3 g/day, $P < 0.01$). The percent preference for advantame increased ($P < 0.05$) at the 0.3 mM concentration and increased further at the 1.0 mM concentration ($F(4,36) = 41.7$, $P < 0.001$). Nine mice preferred 1.0 mM advantame to water by 80–96%, whereas the remaining mouse had a preference of 65% (see Figure 3).

In the saccharin series (Figure 2A), the mice consumed significantly more 1 mM saccharin than water ($T(9) = 7.4$, $P < 0.001$). They then consumed more 1 mM advantame than 1 mM saccharin but more 10 mM saccharin than

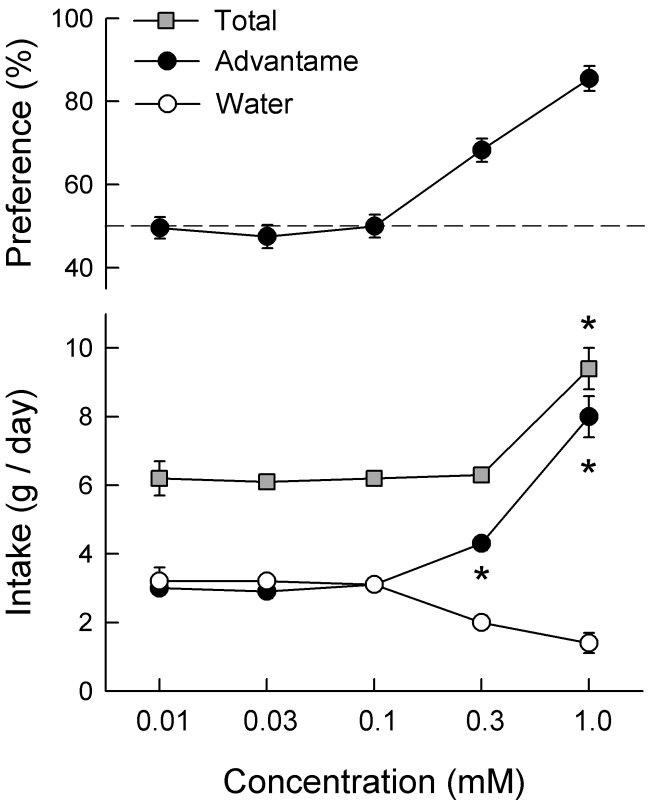

Figure 1 Experiment 1. Mean (± standard error of the mean) percent advantame preference (top) and intakes (bottom) of advantame and water in B6 mice during 2-day tests with ascending concentrations of 0.01–1 mM advantame. Significant ($P < 0.05$) differences between advantame and water are indicated by an asterisk (*).

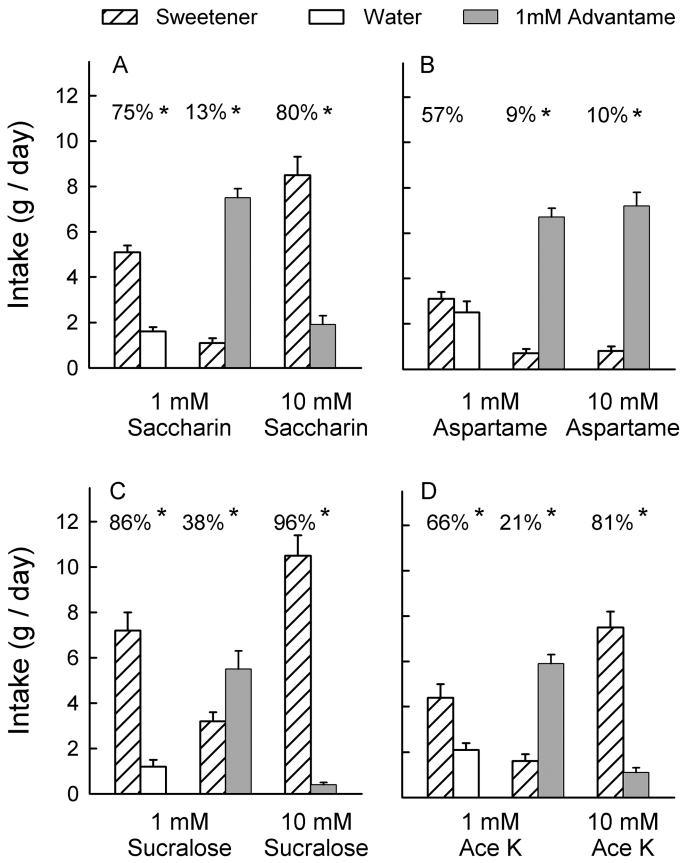

Figure 2 Experiment 1. Mean (+ standard error of the mean) intake of advantame and alternate sweeteners (**A**) saccharin, (**B**) aspartame, (**C**) sucralose, and (**D**) acesulfame K (Ace K) in B6 mice during 2-day sweetener versus water and sweetener versus advantame tests. Advantame was tested at a 1 mM concentration, whereas the alternate sweeteners were tested at 1 and 10 mM concentrations. Numbers atop bars represent mean percent preference for the alternate sweetener. Significant ($P < 0.05$) differences within each test are indicated by an asterisk (*).

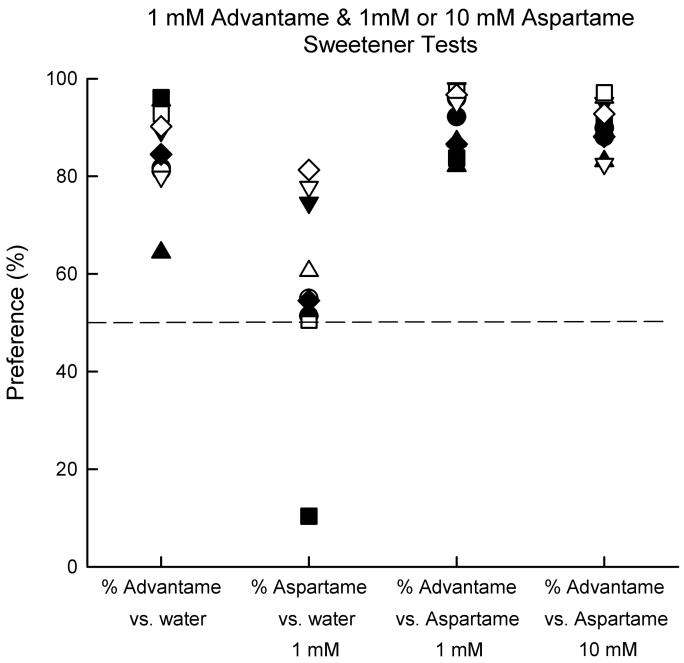

Figure 3 Experiment 1. Individual percent preferences of B6 mice during 1 mM advantame versus water, 1 mM aspartame versus water, 1 mM advantame versus 1 mM aspartame, and 1 mM advantame versus 10 mM aspartame 2-day choice tests.

1 mM advantame (sweetener × concentration interaction, $F(1,9) = 126.8$, $P < 0.001$). Total intakes increased from the 1–10 mM sweetener tests (8.5–10.5 g/day; $F(1,9) = 9.5$, $P < 0.05$). When offered aspartame (Figure 2B), in contrast, the mice, as a group, did not consume significantly more 1 mM aspartame than water. Four mice, however, preferred aspartame to water (by 61–81%), whereas 5 mice were indifferent and 1 mouse avoided aspartame (see Figure 3). In the 2 subsequent tests the mice consumed substantially more 1 mM advantame than 1 and 10 mM aspartame ($F(1,9) = 225.2$, $P < 0.001$). The advantame preference (~90%) was as strong in the aspartame "preferrers" as in the "nonpreferrers" (Figure 3).

In the next test (Figure 2C) the mice consumed significantly more 1 mM sucralose than water ($T(9) = 7.1$, $P < 0.001$). The 1 mM advantame was preferred to 1 mM sucralose but 10 mM sucralose was strongly preferred to 1 mM advantame ($F(1,9) = 77.7$, $P < 0.001$). Total sweetener intakes increased from the 1–10 mM test (8.6 vs. 10.9 g/day; $F(1,9) = 72.7$, $P < 0.001$). In the last test (Figure 2D), the mice consumed more 1 mM acesulfame K than water ($T(9) = 3.4$, $P < 0.01$). They then drank more 1 mM advantame than 1 mM acesulfame K, but more 10 mM acesulfame K than 1 mM advantame ($F(1,9) = 8.6$, $P < 0.05$). Total intakes did not significantly differ in the 1 and 10 mM sweetener tests (7.5 vs. 8.5 g/day). The preference for 10 mM sweeteners over 1 mM advantame was greater for sucralose than for saccharin or acesulfame K (96% vs. 80% vs. 81%, $F(2,18) = 9.8$, $P < 0.001$).

Experiment 2: advantame and saccharin preferences in SD rats

Materials and methods

Animals

Twelve SD rats (6 male, 6 female) were purchased from Charles River Laboratories; they were 11 weeks old at the start of testing. The rats were singly housed in wire mesh cages with *ad libitum* access to chow and deionized water in a room maintained at 22 °C with a 12:12 light:dark cycle.

Procedure

The rats were adapted for 1 week to drink water from two 120-mL drinking tubes. They were then given a series of 2-day, 2-bottle tests with advantame versus water at ascending concentrations of 0.01, 0.03, 0.1, 0.3, and 1.0 mM

concentrations. The rats were next given a series of 2-day tests with 1 and 10 mM saccharin as described in Experiment 1. Tests with additional sweeteners were not conducted because of the limited availability of advantame.

Fluid intakes were analyzed as in Experiment 1. An initial analysis indicated male rats consumed more total fluid during the advantame versus water test series (45.4 vs. 29.1 g/day; $F(1,10) = 30.8$, $P < 0.001$) but there were no sex differences in advantame preference or saccharin intake or preference. Consequently, only the combined male and female data are presented.

Results

In the advantame versus water test series, the rats did not differ in their sweetener and water intake at 0.01–0.1 mM concentrations, but consumed significantly more advantame than water at the 0.3 and 1 mM concentrations (solution × concentration interaction, $F(4,44) = 28.1$, $P < 0.001$) (Figure 4). The percent preference for advantame increased from the 0.1–1.0 mM concentrations ($F(4,44) = 32.8$, $P < 0.001$). With the exception of one low preferrer (58%) the rats preferred 1.0 mM advantame to water by 78–99%.

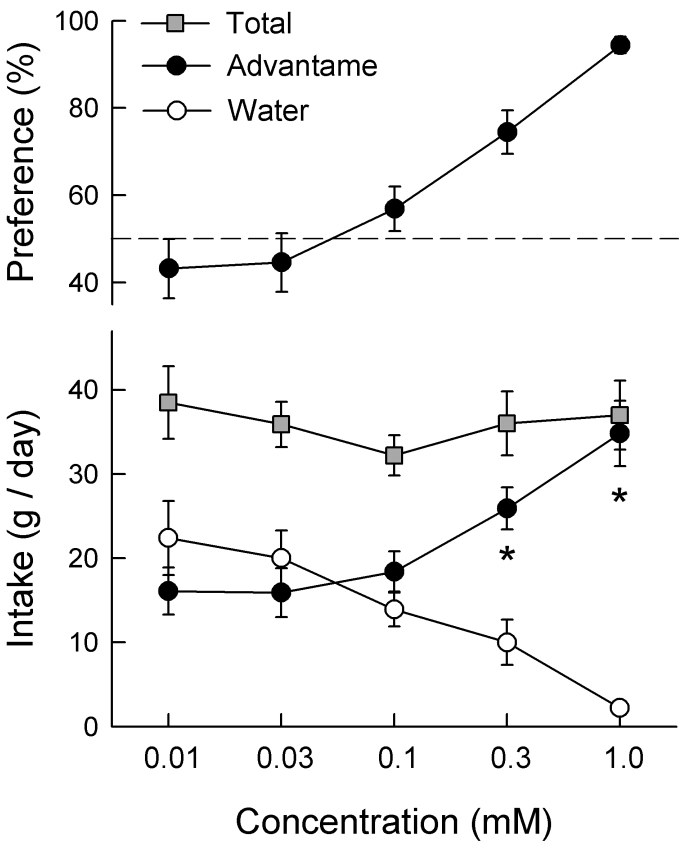

Figure 4 Experiment 2. Mean (± standard error of the mean) percent advantame preference (top) and intakes (bottom) of advantame and water in SD rats during 2-day tests with ascending concentrations of 0.01–1 mM advantame. Significant ($P < 0.05$) differences between advantame and water are indicated by an asterisk (*).

Total fluid intake did not vary during the advantame test and did not differ from the water intakes recorded before and after the test series (37.3 vs. 35.5 g/day).

In the saccharin test (Figure 5), the rats consumed significantly more 0.1 mM saccharin than water ($t(11) = 7.0$, $P < 0.01$). They then consumed more 1 mM advantame than 1 mM saccharin but more 10 mM saccharin than 1 mM advantame (sweetener × concentration interaction, $F(1,9) = 67.1$, $P < 0.001$). Total intakes increased from the 1–10 mM test (41.1 to 60.2 g/day; $F(1,11) = 27.5$, $P < 0.001$). In addition, the rats consumed twice as much fluid during the 10 mM saccharin test than during the water-only days before and after the test (60.2 vs. 30.7 g/day, $F(11) = 7.5$, $P < 0.001$).

Discussion

This study revealed that B6 mice and SD rats significantly preferred advantame to water at 0.3 and 1.0 mM concentrations. The animals were indifferent to the lower concentrations tested (0.01–0.1 mM), which are sweet to humans (Bishay and Bursey 2012). In particular, 0.01 mM advantame is reported to be equivalent to 10% sucrose in sweetness (Bishay and Bursey 2012). While showing identical percent preference profiles, the mice and rats differed in their avidity for advantame as discussed below.

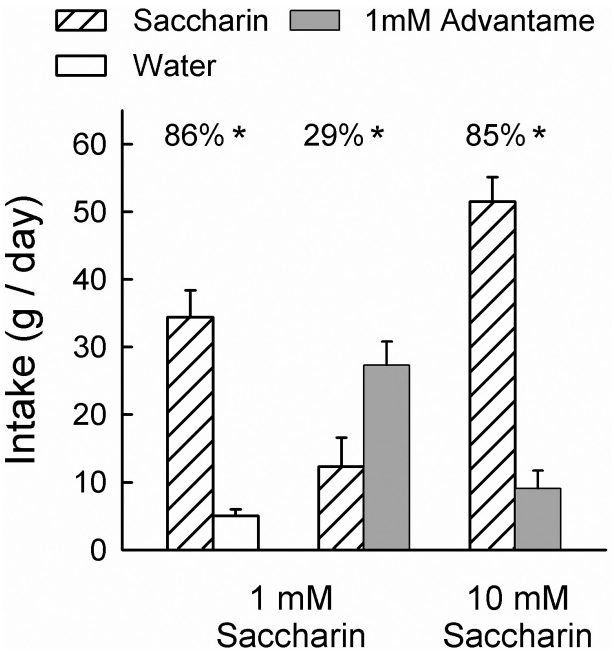

Figure 5 Experiment 2. Mean (+ standard error of the mean) intake of saccharin and advantame in SD rats during 2-day saccharin versus water and saccharin versus advantame tests. Advantame was tested at a 1 mM concentration, whereas saccharin was tested at 1 and 10 mM concentrations. Numbers atop bars represent mean percent preference for the saccharin solution. Significant ($P < 0.05$) differences within each test are indicated by an asterisk (*).

Compared with the other preferred noncaloric sweeteners, advantame was the most potent at the 1 mM concentration. That is, B6 mice significantly preferred 1 mM advantame to 1 mM saccharin, sucralose, and acesulfame K. Similarly, SD rats preferred 1 mM advantame to 1 mM saccharin. Thus, while less potent to rodents than humans, advantame can be considered a high potency sweetener to rats and mice relative to the other preferred sweeteners tested. However, because of its limited solubility, the sweetness efficacy of advantame is less than that of the other preferred sweeteners. That is, the rats and mice significantly preferred 10 mM saccharin, a commonly used saccharin concentration, to 1 mM advantame. The mice also significantly preferred 10 mM sucralose and 10 mM acesulfame K to 1 mM advantame.

Although advantame is synthesized from aspartame, its preference profile to rodents is quite different from that of its precursor. Consistent with prior findings (Bachmanov et al. 2001), overall the B6 mice did not prefer aspartame to water although there was considerable variability in their preference (Figure 3). Yet, in the 2-sweetener tests, the mice were consistent in preferring 1 mM advantame to 1 and 10 mM aspartame. As suggested for rats, which show similar variability in their aspartame preference (Sclafani and Abrams 1986; De Francisco and Dess 1998), it may be that the aspartame preference displayed by some B6 mice is due to a taste quality other than sweetness (Sclafani and Abrams 1986). Further behavioral and physiological studies of advantame and aspartame may reveal the taste receptor sites activated by these 2 sweeteners.

The advantame preferences displayed by the B6 mice and SD rats expand the range of commercial noncaloric sweeteners that are acceptable to rodents. These include saccharin, acesulfame K, and stevia (Bachmanov et al. 2001; Sclafani et al. 2010). Mice also show a strong preference for sucralose, whereas most rats avoid this sweetener and only a few rats prefer sucralose to water (Bachmanov et al. 2001; Sclafani and Clare 2004; Bello and Hajnal 2005; Dess et al. 2009; Loney et al. 2011; Sclafani et al. 2014). Mice and rats do not prefer cyclamate, dulcin, or neohesperidin dihydrochalcone, which are perceived as sweet by humans (Fisher et al. 1965; Wagner 1971; Nowlis et al. 1980; Naim et al. 1982). Saccharin is by far the most extensively used noncaloric sweetener in rodent research. Although saccharin is highly preferred by rats, it is a relatively poor substitute for sucrose; the most preferred saccharin concentrations (0.2–0.4%) are comparable to only 2–4% sucrose solutions (Smith and Sclafani 2002). The relative preference for saccharin versus sucrose has not been investigated in mice, but we recently reported that 0.8% sucralose or a mixture of 0.1% sucralose and 0.1% saccharin are isopreferred to 8% sucrose and significantly preferred to 8% glucose and fructose in brief access tests (Sclafani et al. 2014). Given that 1 mM advantame (0.047%) is less preferred to 10 mM saccharin (~0.2%), sucralose (~0.4%), and acesulfame K (~0.2%), advantame is not likely to be a good substitute for sucrose and other sugars in rodent research.

Although the B6 mice and SD rats had identical preference thresholds (0.3 mM) for advantame, they differed in their avidity for the sweetener. That is, the mice increased their total fluid intake above water baseline when tested with 1 mM advantame whereas the rats showed no change in total intakes. It may be that the "avidity threshold" concentration, that is, the concentration at which the sweetener increases fluid intake above water baseline, is higher in SD rats than in B6 mice and is unattainable in SD rats because of the limited solubility of advantame. B6 mice and SD rats also have similar preference thresholds but different avidity thresholds for other noncaloric sweeteners. In a prior study we reported identical preference threshold concentrations in B6 mice and SD rats for saccharin (0.01%) and stevia (0.01%) as measured in 24-h sweetener versus water choice tests (Sclafani et al. 2010). Further analysis of the 2-bottle data revealed that the sweetener concentrations that increased total fluid intake above water baseline were higher in SD rats (0.1% for stevia and saccharin) than in B6 mice (0.01% for stevia, 0.03% for saccharin). The similar preference thresholds displayed by B6 mice and SD rats for advantame, saccharin, and stevia suggests that the rodents do not differ in their peripheral sweet taste sensitivity. Supporting this interpretation, genetic analysis revealed that the T1r3 receptor that determines sweet taste sensitivity is similar in SD rats and B6 mice (Lu et al. 2005). Thus, differences in central sensory and/or reward processing, rather than in peripheral taste sensitivity, appear to be responsible for the different sweetener avidities displayed by B6 mice and SD rats.

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