

# Female Rats show a Bimodal Preference Response to the Artificial Sweetener Sucralose

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## Abstract

The preference of female Sprague–Dawley rats for sucralose, a non-nutritive sweetener derived from sucrose, was evaluated in 23 h two-bottle tests with water or saccharin. Overall, the rats displayed weak or no preferences for sucralose (0.25–4 g/l) over water but strong preferences for saccharin (0.5–8 g/l) over water and saccharin (1 g/l) over sucralose (0.5 g/l). The rats also preferred a saccharin + sucrose mixture to sucrose, but sucrose to a sucralose + sucrose mixture. There were marked individual differences in sucralose preferences: about half the rats preferred sucralose to water at some concentrations while most remaining rats avoided sucralose. Both subgroups preferred saccharin to sucralose. Sucralose appears to have an aversive off-taste that reduces its palatability to rats.

**Key words:** artificial sweeteners, individual differences, sex differences, species difference, taste preferences

## Introduction

Since 1933 (Hausmann, 1933), the artificial sweetener saccharin has been extensively used in rat studies investigating taste, nutrition, learning, drug addiction and many other topics. Saccharin solutions have a sweet, i.e. sucrose-like taste to rats and are preferred to water over a range of concentrations. However, a significant limitation of saccharin as a motivational stimulus is that it is not all that attractive to rats. That is, based on a variety of behavioral measures which minimize postingestive factors, the most preferred saccharin solutions (2–4 g/l) are isohedonic to only dilute sucrose solutions (20–40 g/l; Smith and Sclafani, 2002). The weak incentive value of saccharin, relative to sucrose, is illustrated by the results of sham-feeding tests in which ingested solutions drain out of a gastric fistula as the animal drinks. Whereas the sham-feeding intake of sucrose increases with concentration and peaks at ~70 ml/30 min, the sham-feeding intake of saccharin increases and then decreases with concentration and peaks at only 14 ml/30 min (Smith and Sclafani, 2002). The bitter aftertaste of saccharin may be one reason for its limited palatability to rats (Dess, 1993). In addition, recent research on sweet taste receptors in mice indicate that while saccharin and sucrose both simulate the T1R2/T1R3 heteromeric receptor complex, only natural sugars stimulate the low-affinity T1R3 homomeric receptor (Zhao *et al.*, 2003).

Saccharin has been replaced in many food products by newer artificial sweeteners that have a more sucrose-like taste quality and less bitter aftertaste (Warshaw, 1990; Schiffman *et al.*, 1995). One such sweetener is aspartame (NutraSweet®). Rats and mice, however, show little or no preference for aspartame solutions (Sclafani and Abrams, 1986; Bachmanov *et al.*, 2001) which is attributed to the failure of aspartame to stimulate the rodent T1R2/T1R3 taste receptor (Li *et al.*, 2002; Zhao *et al.*, 2003). Sucralose (1',6'-dichloro-1',6'-dideoxy-β-D-fructofuranosyl-4'-chloro-4'-deoxy-α-D-galactopyranoside) is a newer non-nutritive sweetener derived from sucrose which is also being used in many food products (Splenda®) (Knight, 1994). It has a sweet taste quality very similar to sucrose but is ~600 times sweeter than sucrose on a weight basis (Warshaw, 1990; Knight, 1994). Behavioral reports indicate that mice prefer sucralose solutions to water over a range of concentrations (0.1–10 g/l) (Bachmanov *et al.*, 2001). *In vitro* studies indicate that sucralose stimulates the rat sweet taste receptor, T1R2/T1R3 (Li *et al.*, 2002). However, the behavioral response of rats to sucralose has not been documented. The present study, therefore, determined the rats' preference for sucralose in 23 h/day sweetener versus water choice tests. Sucralose and saccharin preferences were also compared to determine if sucralose is a more potent sweetener than saccharin to rats. In addition, the preferences for mixtures of

sucralose + sucrose and saccharin + sucrose were compared. This was of interest because of prior findings showing that rats avidly consume dilute sugar solutions (glucose or sucrose) containing saccharin (Sclafani *et al.*, 1987; Smith *et al.*, 1982). Female rats were studied because they typically display stronger preferences for saccharin and sugar solutions than do male rats (Valenstein *et al.*, 1967).

## Materials and methods

### Subjects

Experiment 1 used naive, female Sprague–Dawley rats ( $n = 12$ ) born in our laboratory from stock obtained from Charles River Laboratories (Wilmington, MA). The animals were 72–85 days of age and weighed 233–289 g (mean 261 g) at the start of the experiment. The findings obtained with these animals were unexpected and therefore additional naive, female Sprague–Dawley rats ( $n = 12$ ) obtained from Charles River Laboratories were studied in a second experiment. They were 72 days of age and weighed 220–236 g (mean 224 g) as the start of testing. The animals were individually housed in standard wire-mesh cages in a vivarium maintained at 21°C under a 12:12 h light:dark cycle. Powdered chow (No. 5001; PMI Nutrition International, Brentwood, MO) was available *ad libitum*.

### Taste solutions

Sucralose (McNeil Specialty, New Brunswick, NJ) solutions were prepared using tap water at concentrations of 0.25 g/l (0.025%, 0.6 mM), 0.5 g/l (0.05%, 1.5 mM), 1 g/l (0.1%, 2.5 mM), 2 g/l (0.2%, 5 mM) and 4 g/l (0.4%, 10.1 mM). Saccharin (sodium saccharin; Sigma Chemical Co., St Louis, MO) solutions were prepared at concentrations of 0.5 g/l (0.05%, 2.4 mM), 1 g/l (0.1%, 4.9 mM), 2 g/l (0.2%, 9.7 mM), 4 g/l (0.4%, 19.5 mM) and 8 g/l (0.8%, 39.0 mM). Sucrose (Domino Foods, Inc., Yonkers, NY) solutions were prepared at 20 g/l (2%, 58.4 mM) with or without added sweetener (0.5 g/l sucralose or 1 g/l saccharin). Sweetener concentrations were selected based on prior rodent studies and human reports that saccharin and sucralose are 300× and 600×, respectively, sweeter than sucrose on a weight basis (Warshaw, 1990; Bachmanov *et al.*, 2001; Smith and Sclafani, 2002).

### Procedure

#### Experiment 1

The animals were divided into two subgroups ( $n = 6$ ) which were given a series of 23 h/day two-bottle tests with sweetener versus water. One subgroup was first tested with sucralose at concentrations of 0.25–4 g/l; the concentrations were presented for 4 days each in an ascending order. The rats were then given water only for 2 days followed by saccharin versus water tests (4 days each) at saccharin concentrations of 0.5 to 8 g/l. The rats in the second subgroup were similarly tested but with saccharin first and sucralose second. All rats

were then given a two-bottle test (2 days) with 0.5 g/l sucralose versus 1 g/l saccharin.

The rats were next given water only for 3 days followed by consecutive one-bottle tests with 0.5 g/l sucralose and 1 g/l saccharin for 2 days each, with the order of sweetener presentation counterbalanced.

In a final series of two-bottle tests (2 days each), the rats were given the choice between 0.5 g/l sucralose + 20 g/l sucrose versus 20 g/l sucrose only and 1 g/l saccharin + 20 g/l sucrose versus 20 g/l sucrose only; the order of testing was counterbalanced.

#### Experiment 2

The rats were given a series of three two-bottle choice tests (4 days each). They were tested with 0.5 g/l sucralose versus water, 1 g/l sucralose versus water and 2 g/l saccharin versus water, in that order.

In the two-bottle tests, the left-right positions of the test fluids were alternated daily to control for side preferences. Fluid intakes were recorded to the nearest 0.1 g using an electronic balance interfaced to microcomputer.

### Analysis

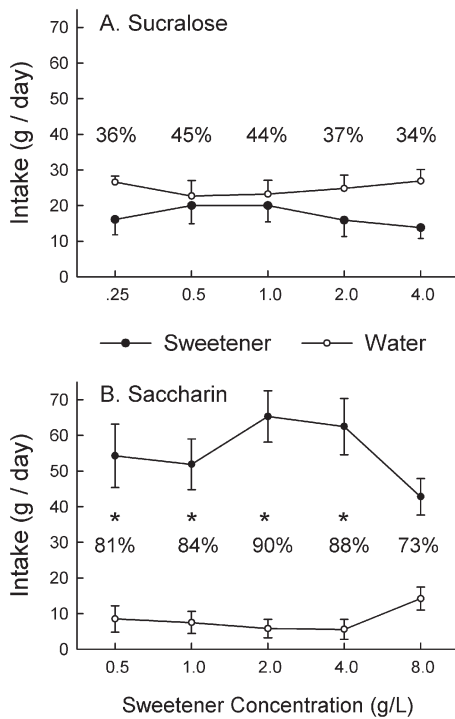
Sweetener and water intakes were averaged over the 2 or 4 days of testing and were evaluated using repeated measures analysis of variance (fluid and concentration) procedures followed by simple main effects tests, where appropriate. Additional analysis were performed using a mixed design including a between group and within group (fluid and concentration) as variables (see Results). A significant difference between the two-bottle intake of sweetener and water was taken as the primary evidence for a sweetener preference. Sweetener intakes were also expressed as percent scores (sweetener intake/total intake × 100) and analyzed following an inverse sine transformation (Kirk, 1995).

## Results

### Experiment 1

Preliminary analysis indicated that the order of testing (sucralose or saccharin first) did not affect solution intakes or preferences and the data for subgroups were therefore combined.

As illustrated in Figure 1A, in the sucralose versus water choice tests, the rats failed to drink more sweetener than plain water. Intakes of sucralose and water did not significantly differ, nor did intakes change as a function of sweetener concentration. In contrast, the rats consumed significantly more saccharin than water [ $F(1,11) = 26.80$ ,  $P < 0.001$ ; Figure 1B]. Intakes varied as a function of concentration [ $F(1,1) = 7.87$ ,  $P < 0.05$ ] and there was a Fluid × Concentration interaction [ $F(4,44) = 8.15$ ,  $P < 0.001$ ]. Overall, the rats consumed much more saccharin than sucralose [55.3 versus 17.1,  $F(1,11) = 26.24$ ,  $P < 0.001$ ]. In

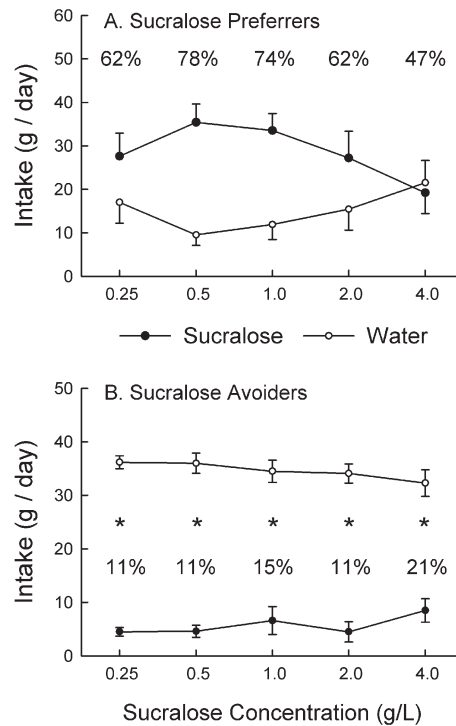


**Figure 1** (A) Mean ( $\pm$ SEM) intake of sucralose and water during two-bottle preference tests. (B) Mean ( $\pm$ SEM) intake of saccharin and water during two-bottle preference tests. The numbers in the graphs represent the mean of the individual rats' percentage sweetener intakes. Asterisks indicate significant ( $P < 0.05$ ) difference between sweetener and water.

addition, the percent saccharin intakes were greater than percent sucralose intakes [ $F(1,11) = 29.34, P < 0.001$ ].

Inspection of the sucralose data revealed that six rats preferred sucralose to water at least at one concentration (0.5 g/l), while the remaining six rats avoided sucralose. The rats were therefore divided into sucralose preferring (SP) and sucralose avoiding (SA) subgroups and their sweetener intakes were compared at all concentrations. As illustrated in Figure 2A, SP rats drank more sucralose than water at all concentrations except the highest [Fluid  $\times$  Concentration interaction [ $F(4,20) = 6.71, P < 0.01$ ] although none of the individual comparisons reached significance according to the simple main effects tests. In contrast, the SA rats strongly avoided sucralose and drank more water than sweetener at all concentrations [ $F(1,5) = 164.63, P < 0.001$ ]. Overall, the absolute and percent saccharin intakes of the SP and SA groups did not differ significantly. However, one of the SA rats avoided saccharin at all concentrations; the remaining SA and SP rats preferred saccharin to water at three or more of the concentrations tested (data not shown).

When given the choice between the 0.5 g/l sucralose and 1 g/l saccharin, the rats drank significantly more saccharin than sucralose (Table 1,  $F(1,10) = 26.20, P < 0.01$ ). This was true for both the SP and SA subgroups (Table 1). The results of the one-bottle tests revealed that the rats consumed significantly more saccharin than sucralose or water, and the



**Figure 2** (A) Mean ( $\pm$ SEM) intake of sucralose and water during two-bottle preference tests for rats classified as sucralose preferring ( $n = 6$ ). (B) Mean ( $\pm$ SEM) intake of sucralose and water during two-bottle preference tests for rats classified as sucralose non-preferrers ( $n = 6$ ). The numbers in the graphs represent the mean of the individual rats' percentage sweetener intakes. Asterisks indicate significant ( $P < 0.05$ ) difference between sweetener and water.

intakes of the sucralose and water did not differ [Table 1,  $F(2,10) = 10.10, P < 0.001$ ]. The SA rats tended to drink less sucralose than did the SP rats, but this difference was not significant. The SA rats also drank slightly, but not significantly less sucralose than plain water. One bottle intakes were not influenced by the order of sweetener presentation.

In the choice test with sucralose + sucrose versus sucrose, overall the rats consumed more sucrose than the mixture [Table 1,  $F(1,10) = 13.48, P < 0.01$ ]. There was a Subgroup  $\times$  Sweetener interaction [ $F(1,10) = 11.11, P < 0.01$ ]; the SA rats, but not the SP rats consumed substantially more sucrose than mixture. When given the choice between saccharin + sucrose versus sucrose, the rats consumed substantially more of the mixture than of plain sucrose [Table 1,  $F(1,10) = 36.01, P < 0.001$ ]. Eleven of the 12 rats strongly preferred the mixture to plain sucrose; the remaining rat, which was the SA rat that was also a saccharin avoider, drank similar amounts of the saccharin + sucrose and sucrose solutions.

### Experiment 2

When given the choice of 0.5 g/l sucralose and water, seven rats preferred sucralose (77–90%) while five rats avoided or were indifferent to sucralose (17–49%). The rats were there-

**Table 1** Mean ( $\pm$ SEM) intake of sucralose, saccharin, and water in one- and two-bottle tests of experiment 1

Two-bottle sucralose versus saccharin test	Sucralose, 0.5 g/l	Saccharin, 1 g/l	Percentage sucralose
All rats	7.2 $\pm$ 2.7	46.7 $\pm$ 7.1 <sup>a</sup>	16.1 $\pm$ 6.9
Sucralose preferrers, ( <i>n</i> = 6)	11.6 $\pm$ 5.5	42.0 $\pm$ 9.2 <sup>a</sup>	25.5 $\pm$ 12.9
Sucralose avoiders ( <i>n</i> = 6)	3.9 $\pm$ 1.0	59.2 $\pm$ 8.3 <sup>a</sup>	6.8 $\pm$ 2.1
One-bottle sucralose, saccharin and water tests	Sucralose, 0.5 g/l	Saccharin, 1 g/l	Water
All rats	37.6 $\pm$ 2.2	63.7 $\pm$ 7.8 <sup>b</sup>	39.1 $\pm$ 1.7
Sucralose preferrers ( <i>n</i> = 6)	41.5 $\pm$ 2.5	57.4 $\pm$ 9.1	38.2 $\pm$ 3.0
Sucralose avoiders ( <i>n</i> = 6)	33.6 $\pm$ 3.0	70.0 $\pm$ 13.0 <sup>b</sup>	40.0 $\pm$ 1.8
Two-bottle sucralose (0.5 g/l) + sucrose (2 g/l) versus sucrose (2 g/l) test	Sucralose + sucrose	Sucrose	Percentage S + S
All rats	21.0 $\pm$ 5.2	56.6 $\pm$ 9.2 <sup>c</sup>	29.9 $\pm$ 8.2
Sucralose preferrers ( <i>n</i> = 6)	34.9 $\pm$ 6.3	38.1 $\pm$ 10.3	51.0 $\pm$ 10.8
Sucralose avoiders ( <i>n</i> = 6)	7.2 $\pm$ 1.6	74.9 $\pm$ 11.7 <sup>c</sup>	8.8 $\pm$ 1.4
Two-bottle saccharin (1 g/l) + sucrose (2 g/l) versus sucrose (2 g/l) test	Saccharin + sucrose	Sucrose	Percentage S + S
All rats	90.5 $\pm$ 11.8	7.3 $\pm$ 2.9 <sup>d</sup>	90.9 $\pm$ 4.1

<sup>a</sup>Two-bottle test: saccharin > sucralose,  $P < 0.05$  or less.

<sup>b</sup>One-bottle test: saccharin > sucralose and water,  $P < 0.05$  or less.

<sup>c</sup>Two-bottle test: sucrose > sucralose + saccharin,  $P < 0.05$  or less.

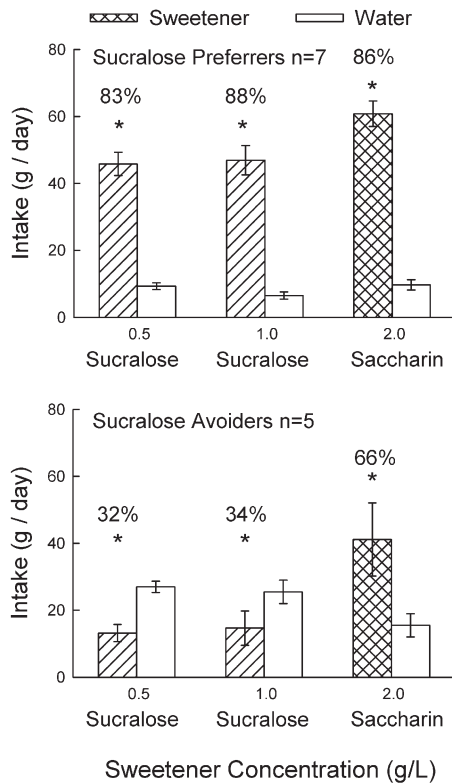
<sup>d</sup>Two-bottle test: saccharin + sucrose > sucrose,  $P < 0.001$ .

fore divided into sucralose preferring (SP) and sucralose avoiding (SA) subgroups (Figure 3). Analysis of the sucralose versus water tests revealed that intakes did not vary as a function of concentration. Overall, the rats consumed more sucralose than water [32.8 versus 15.5 g/day,  $F(1,10) = 15.16$ ,  $P < 0.001$ ]; their percent sucralose intake was 64%. However, the Subgroup  $\times$  Solution interaction was significant [ $F(1,10) = 57.30$ ,  $P < 0.001$ ]. Whereas the SP rats consumed more ( $P < 0.05$ ) sucralose than water, the SA rats consumed less ( $P < 0.05$ ) sucralose than water. In addition, the SP rats consumed more ( $P < 0.05$ ) sucralose and less ( $P < 0.05$ ) water than did the SA rats. When tested with saccharin, the rats consumed more saccharin than water [56.6 versus 12.1 g/day,  $F(1,10) = 39.13$ ,  $P < 0.001$ ] and the intakes of the SP and SA subgroups did not differ. Eleven of the 12 rats preferred saccharin to water (78–90%). The remaining rat, which was a sucralose avoider (14% sucralose preference), also avoided saccharin (16% saccharin preference). A comparison of the 1 g/l sucralose and 2 g/l saccharin tests revealed that the rats overconsumed more saccharin than sucralose, relative to water [52.6 versus 12.1 and 33.5 versus 14.4 g/day,  $F(1,10) = 23.06$ ,  $P < 0.01$ ]. The SP rats did not differ in their 1 g/l sucralose and 2 g/l saccharin percentage intakes (88 versus 86%), although they consumed

more fluid in the saccharin preference test than in the sucralose test [ $F(1,6) = 29.47$ ,  $P < 0.01$ ]. The SA rats, on the other hand, drank less sucralose, but more saccharin than water [Subgroup  $\times$  Sweetener interaction,  $F(1,4) = 14.58$ ,  $P < 0.05$ ] and differed in their percent sucralose and saccharin intakes [34 versus 66%,  $t(4) = 3.21$ ,  $P < 0.05$ ].

## Discussion

The present results revealed a bimodal response of female Sprague–Dawley rats to the artificial sweetener sucralose: about half the rats preferred sucralose solutions to water at one or more concentrations tested, while most of the remaining rats avoided sucralose. This contrasts with their more uniform response to saccharin: 22 out of 24 rats in experiments 1 and 2 preferred saccharin to water at one or more of concentrations. Furthermore, in a direct choice test the rats preferred 1 g/l saccharin to 0.5 g/l sucralose by 84%. Although saccharin versus sucralose preferences were not tested at other concentrations, the sweetener versus water preference profiles observed in experiment 1 suggest that saccharin would be preferred to sucralose over a wide range of concentrations. The rats in the first experiment also consumed less sucralose than saccharin in one-bottle tests and only saccharin increased fluid intake relative to plain



**Figure 3** (A) Mean ( $\pm$ SEM) intake of sucralose, saccharin and water during two-bottle sweetener versus water tests for rats classified as sucralose preferrers ( $n = 7$ ). (B) Mean ( $\pm$ SEM) intake of sucralose, saccharin and water during two-bottle sweetener versus water tests of rats classified as sucralose avoiders ( $n = 5$ ). The numbers in the graphs represent the mean of the individual rats' percentage sweetener intakes. Asterisks indicate significant ( $P < 0.05$ ) difference between sweetener and water.

water. They also preferred a plain sucrose solution to a sucralose + sucrose mixture, but strongly preferred a saccharin + sucrose mixture to plain sucrose.

Even among the subset of rats that preferred sucralose, their avidity for sucralose was less than that for saccharin as indicated by the results of the sweetener versus water, sucralose versus saccharin, and one-bottle tests. The relatively weak and bimodal preference response to sucralose was unexpected based on *in vitro* findings showing that the rat's sweet taste receptor (T1R2/T1R3) is at least as responsive to sucralose as to saccharin (Li *et al.*, 2002). Also, Bachmanov *et al.* (2001) reported that sweet 'taster' (C57BL/6ByJ) and 'nontaster' (129P3/J) mouse strains displayed preferences to sucralose as strong as their preferences to saccharin in 24 h/day sweetener versus water tests, although preference thresholds were higher in the nontaster strain. We confirmed this finding and observed sucralose preferences of 89–95% at suprathreshold concentrations for each strain (C57BL/6ByJ, 0.5–4 g/l; 129P3/J, 1–4 g/l; unpublished findings). Male mice were used in the Bachmanov *et al.* (2001) study and our experiment whereas female rats were used in the present study which raises the possibility that sex differences may

account for the different sucralose preferences observed in mice and rats.

In a preliminary test to determine if male rats show a stronger sucralose preference than do female rats, we gave a large group of naive male rats (Sprague–Dawley; Charles River Laboratories) a two-bottle test with 0.5 g/l sucralose versus water (2 days). Only 7 of the 42 rats tested preferred sucralose to water (72–94%); 34 rats avoided sucralose (5–39%), and one rat was indifferent (56%). Overall, the male rats consumed significantly less sucralose than water [18.9 versus 34.9 g/day,  $t(41) = 3.32$ ,  $P < 0.001$ ] and their sweetener preference was only 27%. Thus, rather than having a stronger preference for sucralose than female rats, these preliminary data indicate that male rats show a stronger sucralose avoidance. Bello *et al.* (2004) also investigated sucralose preference in male Sprague–Dawley rats. They observed that male rats preferred plain water as well as saccharin solutions to sucralose over a range of sucralose concentration (0.1–10 g/l). It appears, therefore, that rats and mice differ in their preferences for sucralose, which is surprising given the general similarity in their taste preference profiles for sugars and non-nutritive sweeteners.

The marked individual differences in the rats' preference for sucralose may occur because of allelic variations in their gene coding for the T1R3 sweet taste receptor as is seen in different inbred mouse strains (Reed *et al.*, 2004). However, the finding that most rats that failed to prefer sucralose were adverse rather than indifferent to sucralose indicates that sucralose had an aversive 'off-taste' to them. The source of this off-taste and why some rats but not others avoided sucralose is uncertain. Two of 24 female rats also avoided saccharin and it is not uncommon for some rats to avoid saccharin (Dess, 1993). Saccharin is assumed to have a bitter taste component to rats (Dess, 1993) and perhaps sucralose also has a bitter taste quality to some rats. However, humans report that sucralose has a less intense bitter taste than does saccharin (Schiffman *et al.*, 1995). The synergistic effect on sweetener intake of mixing saccharin with sugar is thought to result, in part, from the sugar blocking the bitter off-taste of saccharin (Smith *et al.*, 1982). The absence of a synergistic effect of mixing sucralose and sucrose suggests that the off-taste that limits sucralose differs in quality from that of saccharin. More sophisticated behavioral tests (e.g. taste aversion generalization tests) are required to identify the aversive nature of sucralose.

In an earlier study of aspartame preference we reported that half of the female rats tested displayed a mild preference for aspartame over water while the remaining rats were indifferent or avoided aspartame (Sclafani and Abrams, 1986). However, the aspartame preference was obtained only at high concentrations (50–100 g/l) and it did not appear to represent a sweet taste response. Consistent with this view, aspartame preference was not correlated with sugar or saccharin preferences in rats selectively bred for saccharin intake (De Francisco and Dess, 1998). More

recent studies of sweet taste receptors in rats indicate that aspartame, unlike sucralose, does not stimulate the T1R2/T1R3 receptor complex (Li *et al.*, 2002). Thus, it would appear that the sucralose and aspartame preferences displayed by some rats are mediated by different taste receptors. Nevertheless, it would be of interest to compare sucralose and aspartame preferences in the same group of rats.

In conclusion, female and male Sprague–Dawley rats show a relatively weak and variable preference for sucralose and many rats actually avoid the sweetener. Whether more positive and uniform responses to sucralose would be obtained using concentrations lower or higher than those studied here or using different strains of rats remain to be determined. Further studies are also needed to characterize the aversive taste component of sucralose. Based on the present findings, sucralose is not a candidate to replace saccharin in rat studies requiring an attractive, non-nutritive sweetener.

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