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Sodium saccharin can be more acceptable to rats than pure saccharin

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ABSTRACT

The artificial sweetener saccharin is available in several forms, including pure saccharin (S) and saccharin sodium salt hydrate (SSSH). Acceptance and preference relative to 2% sucrose for these two forms was assessed using both older female and young male rats. At the higher of two concentrations, ~0.4%, SSSH was more acceptable and more greatly preferred over 2% sucrose than was a similar concentration of S, whereas little difference between the two forms was detected at the lower concentration, ~0.1%. These results indicate the importance for researchers of care in choosing and reporting the form of saccharin they use.

1. Introduction

Even though no longer extensively used as a low-calorie sweetener in beverages, saccharin is still used in a wide range of commercial products. It is also used extensively in research involving rodents, including studies concerned with flavour preference learning (e.g. Harris et al., 2000), nutritional studies (e.g. Swithers and Davidson, 2008) and rat models of drug and alcohol dependence (e.g. Huynh et al., 2017). Its use in such research has continued because rats find it sweet, whereas they seem not to taste several other substances used as low-calorie sweeteners for humans, such as aspartame (Sclafani and Abrams, 1986). Furthermore, sucralose appears to be acceptable only for a proportion of rats (cf. Sclafani and Clare, 2004; Antenucci and Hayes, 2015) and stevia has weaker effects than saccharin (Sclafani et al., 2010). High quality saccharin is available in a variety of forms, including the two of interest here: pure (free-acid) saccharin (S; Aldrich 240931) and sodium saccharin salt hydrate (SSSH; Sigma S-1002). It is also available as calcium saccharin, a form that is also acceptable to rats (Cullen et al., 1970), although subsequent to 1970 this form of saccharin has been rarely used in behavioural research. Finally, it is worth noting that the widely-used commercial sweetener, *Sweet 'N Low ®*, has also been used in some rodent studies (e.g. Cichelli and Lewis, 2002); however, this contains both saccharin and glucose, a combination that is far more acceptable to rats than any form of saccharin alone (e.g. Smith and Foster, 1980; Valenstein et al., 1967).

Two studies have suggested that the form of saccharin used may be important in rodent studies. When mice were given 24-h 2-bottle choice tests between pure saccharin and water and between SSSH and water, they showed a high preference for both forms at low concentrations, but declining preference for pure saccharin when concentrations were increased (Warren and Warren, 1966). A second study used rats and an acceptance measure, namely, 24-h intake when a saccharin solution was the only fluid available; results again indicated that SSSH was more acceptable than pure saccharin (Valenstein, 1966).

Our decision to revisit this issue and produce more systematic data was prompted by an incidental finding in an experiment on bingeing by rats given intermittent access to highly palatable solutions (Rehn and Boakes, 2018). Running out of SSSH forced an unplanned switch from ~0.4% SSSH to ~0.4% S that led to an abrupt decrease in saccharin intakes.

The present study differs from the two described above (Warren and Warren, 1966; Valenstein, 1966) in that (a) it used rats of different ages and sex to test the generality of the results and (b) it included higher concentrations (~0.4%) than those used previously, as well as ~0.1%. Inspection of recent reports of experiments using saccharin (see General Discussion below) suggested that the concentrations most commonly used in research are in the range 0.1% to 0.25%. We chose to use the higher concentration of ~0.4%, since unpublished experiments in our laboratory have found that this can maximise saccharin intakes by rats; also larger saccharin-based flavour preferences can be supported by 0.4% than 0.2% (Harris et al., 2000) and intakes and preferences for saccharin solutions can be greater for 0.3% than for 0.1% saccharin solutions (e.g. Sclafani et al., 2010).

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2. Method

2.1. Materials

2.1.1. Subjects

Sixteen female Sprague-Dawley rats aged 17 weeks with an average weight of 253.3 g at the start of the experiment had previously served in an experiment involving visual and auditory stimuli that signalled food pellet delivery. Sixteen male Wistar rats aged 8 weeks with an average weight of 235.6 g at the start of the experiment had previously served in a cat odour avoidance experiment. Both rat squads were maintained on chow and water in their previous experiments and were naïve to the taste of sucrose and of any form of saccharin. Within their respective squads, rats were group-housed (n = 4/cage) in a temperature- and humidity-controlled room on a reverse 12:12 h light cycle (lights off at 0900). Prior to the start of the experiment, access to water was progressively decreased across each day from 4 h to 2 h to 1 h to 30 min. Within each squad, rats were divided into two groups matched for body weight. During the experiment, rats were given 30-min access to water following each experimental session except in Stage 1, as described below. Animals were maintained on *ad libitum* access to laboratory chow (Specialty Feeds ®, Glen Forrest, WA) throughout the experiment. Procedures were approved by the University of Sydney Animal Ethics Committee.

2.1.2. Solutions

As is common practice, nominally 0.1% and 0.4% w/v solutions of saccharin sodium salt hydrate were prepared by dissolving dry weights (1 g and 4 g respectively) of SSSH (Sigma S-1002) in 1 L of tap water (Sydney Water). With the intention of preparing equimolar solutions of pure saccharin, 0.893 g and 3.572 g of pure saccharin (S: Aldrich 240931) were dissolved in 1 L of tap water. Unfortunately, our calculations neglected to account for the level of hydrate in the SSSH powder and, according to the manufacturer, this could account for 4–15% of the total weight. As a result, what we describe as '0.1%' and '0.4%' elsewhere in this report refer to SSSH solutions that contained either 0.085-0.096% or 0.340-0.384% saccharin and to S solutions that contained either 0.09% or 0.36% saccharin. Thus, in terms of actual saccharin content all solutions were slightly weaker than intended.

We chose to dilute the saccharin solutions in tap water partly because, as the rats had always been maintained on tap water, using distilled water for the saccharin solutions would have introduced an additional change in taste. However, we acknowledge that the free-acid form of saccharin could interact with possible minerals, including sodium, in the tap water so what the rats tasted was actually a mixture of hydrated mineral salts of saccharin. Any such process would tend to decrease the difference between S and SSSH. Given that major differences were found, it seems that the use of tap water was not a major problem.

A 58 mM (2% w/v) sucrose solution in tap water was prepared using commercially-available cane sugar.

2.1.3. General procedures and apparatus

For drinking sessions rats were transferred to individual acrylic cages (36 × 20 x 18 cm; "drinking cages"), fitted with metal lids and with paper pellets as bedding. Solutions were provided in 100-mL plastic bottles with stainless steel spouts, containing ball-bearings. No water or food was available in the drinking chambers. The drinking bottles were weighed to the nearest 0.1 g before and after each drinking session to measure consumption. As described below, during testing all rats were first exposed to the two 0.1% saccharin solutions before being given the 0.4% solutions; this was to reduce neophobic reactions to the higher concentrations.

2.1.3.1. Stage 1 (days 1–3): pretraining. During Stage 1 rats were given 30-min access to water in the drinking chambers each day. Water was provided in a single bottle on Day 1 and in two bottles inserted on either side of the chambers on Days 2 and 3. On these days bottle positions were exchanged after 15 min into the drinking session to acclimatise rats to the two-bottle choice test procedure. After the Day 1 session each of the two matched groups of each squad received 2-h home cage access to either 0.1% S (S-first) or 0.1% SSSH (SSSH-first) solution. After the Day 2 session the S-first group received 2-h home cage access to 0.1% SSSH solution, whereas the SSSH-first group received 0.1% S solution.

2.1.3.2. Stage 2 (days 4–7): acceptance testing. Acceptance tests measured how much of a saccharin solution a rat would consume in a 30-min drinking session. Each rat received four acceptance tests across four days (one saccharin solution per day). For the S-first group the order of saccharin solution presentations was 0.1% S, 0.1% SSSH, 0.4% S, 0.4% SSSH, whereas the order for the SSSH-first group was 0.1% SSSH, 0.1% S, 0.4% SSSH, and 0.4% S.

2.1.3.3. Stage 3 (days 8–11): preference testing. Preference tests measured rats' preference for the four saccharin solutions relative to a 2% sucrose solution. Rats were given four 10-min two-bottle choice tests over four days. In each test one bottle always contained 2% sucrose, whereas the other bottle contained one of the four saccharin solutions. The positions of the bottles were exchanged after 5 min into each test. Tests were conducted in the same sequence of solutions as in Stage 1 for each group; for example, the S-first group received a preference test with 0.1% S on Day 8, 0.1% SSSH on Day 9, 0.4% S on Day 10, and 0.4% SSSH on Day 11. The starting position of the saccharin bottle was counterbalanced within each group, such that half the rats in the S-first group, for example, received 0.1% S on the left and 2% sucrose on the right on Day 8, while the other half received 0.1% S on the right and 2% sucrose on the left.

2.1.4. Data analysis

Data analyses was performed using SPSS 22.0 and the level of significance was set at $p < 0.05$. Data for each squad were analysed separately as they differed in age, strain, and sex. To examine potential differences in consumption in the acceptance tests in Stage 2, a 2 x (2) x (2) mixed-ANOVA was conducted on the amount of solution consumed, with Group (S first, SSSH first) as the between-subjects factor and Form of saccharin (S, SSSH) and Concentration (0.1%, 0.4%) as the within-subjects factors. Tests of simple effects, comparing intakes of S with SSSH at both high and low concentrations, followed when an interaction between Form and Concentration was detected.

For each preference test in Stage 3, the preference for a saccharin solution over a 2% sucrose solution was calculated as the intake of that saccharin solution as a percentage of total fluid consumed from both bottles in each drinking session. To examine potential differences in preference for a given saccharin solution over sucrose, as above, a 2 x (2) x (2) mixed-ANOVA was conducted on saccharin preference with Group (S first, SSSH first) as the between-subjects factor, and Form (S, SSSH) and Concentration (0.1%, 0.4%) as the within-subjects factors, with analysis of simple effects again following detection of Form by Concentration interactions.

2.2. Results

2.2.1. Acceptance tests

2.2.1.1. Female Sprague-Dawley rats. Intake data from the acceptance tests in female Sprague-Dawley rats are displayed in Fig. 1A. Analyses showed a main effect of Form [$F(1, 14) = 10.93$, $p = 0.005$], such that overall these rats drank significantly greater amounts of SSSH compared to S solutions, averaged over concentration. This was qualified by a Form x Concentration interaction [$F(1, 14) = 13.11$, $p = 0.003$]. Simple effects analysis revealed that at concentrations of 0.1%, these rats drank similar amounts of S and SSSH solutions [$F < 1$].