Preference for Toxic and Non-toxic Artificial Sweeteners in Rodents[1]

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Abstract

Four species of deer mice (*Peromyscus*) were given choices of glucose and three artificial sweeteners: sodium saccharin and sodium or calcium cyclamate. Preferences for glucose *vs* saccharin were based on relative sweetness, with the sweeter of a pair being chosen. However, *Ss* preferred glucose to the cyclamates regardless of relative sweetness. Evidence is presented to suggest that aversion to the cyclamates may be based either on noxious stimuli associated with ingestion (according to recent toxicity studies of cyclamates) or on taste qualities in addition to sweetness that may be present in the cyclamates. Therefore, learning and physiological factors as well as taste are implicated in food preferences.

Introduction

Artificial sweeteners have been available as sugar substitutes for diabetics and dieters for many years. While saccharin has a bitter aftertaste to humans, it has been used extensively as an effective reward in many animal learning studies since 1933 (5). Squirrel monkeys, however, do not prefer saccharin (2). New artificial sweeteners, the cyclamate salts, were introduced in 1950 and rapidly surpassed saccharin in popularity, accounting for sales of $25 million in 1961. Perhaps because of the newness of the cyclamates, few studies with animals have been reported: Murray et al. (8) found rats to prefer water to all concentrations of Na cyclamate, but Smith and Ross (15) found C57 black mice to prefer maximally a 1% Na cyclamate solution to water.

The basis for sweet preference is usually ascribed to innate and/or learning factors. Glucose is sweet and directly utilizable for energy by the cells. These sweet preferences can be "unlearned" when paired with noxious stimuli (12). Nees and Derse (11) and Japanese researchers recently reported that cyclamates fed to rats and mice resulted in infertility, laxative effects and fetal deaths. It may be hypothesized that these toxic effects would result in a preference change similar to that reported above when sweet compounds were paired with noxious stimuli, although the chemical need not be ingested in sufficient quantities to produce toxic effects, since Nachman (9, 10) has shown laboratory rats to avoid LiCl once having experienced ill effects and Fregly (3) has found adult rats to avoid LiCl although never having experienced toxic effects. Gunther (1), on the other hand, finds that lactose fed to early posthatching chicks resulted in many deaths and that apparently there was no preference change to the less noxious stimuli available. Kare (6), however, reports that chicks and adult chickens are indifferent to lactose, suggesting an inability to discriminate a lactose solution from water.

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It was the purpose of this study to examine the preferences of several rodent species for artificial sweeteners either known to be non-toxic or suspected of having toxic effects.

Materials and Methods

Four species of deer mice—males and females of *Peromyscus leucopus* (N=7 and 4, mean weight 23.5 and 21.6 gm.), *Peromyscus eremicus* (N=4 and 3, mean weight 18.5 and 17.9 gm.), *Peromyscus maniculatus bairdii* (N=2 and 3, mean weight 20.4 and 15.5 gm.), and *Peromyscus floridanus* (N=3, mean weight 33.0 gm.)—were supplied by John A. King at Michigan State University.

Sweet substances used were anhydrous glucose, sodium saccharin,[3] and sodium and calcium cyclamate.[4]

All *S*s were housed individually with standard (Big Red) laboratory chow always present and water available except during testing. Using a paired-comparison procedure, each *S* was presented with pairs consisting of a 4, 8, or 16% glucose solution and either a 0.23% saccharin, 0.91% Na cyclamate or a 1.81% Ca cyclamate solution. These particular concentrations were chosen since Stellar (16) reported rats to prefer maximally a 0.23% Na saccharin solution, and Smith and Ross (15) found mice to prefer a 1% Na cyclamate solution. Saccharin-glucose pairs were presented between July 3 and 24, 1967. Glucose—0.45% Na cyclamate pairs were presented for 11 days from July 25 to August 5 under the mistaken assumption that saccharin might be only twice as sweet as cyclamate. (However, Schutz and Pilgrim (14) report that saccharin is ten times sweeter than Ca cyclamate to humans, when both are paired with sucrose.) The Na cyclamate concentration was doubled to 0.91%, approximating that of Smith and Ross, and paired with glucose for 15 days from August 7 to 22. For three days 0.91% Ca cyclamate was paired with each of the three glucose solutions. Because of the continuing rejection of the artificial sweetener the concentration was again doubled and the 1.81% Ca cyclamate-glucose pairs were presented for 15 days from August 26 to September 9, 1967. Due to the rather preliminary nature of the use of 0.45% Na cyclamate and 0.91% Ca cyclamate solutions, the intake for these pairs is not included in the data to be reported. Each glucose-artificial sweetener pair was repeated five times on a randomly cycled schedule. Pairs of solutions were presented for two hours per day. Solution positions were altered randomly to inhibit development of drinking patterns based on position rather than on sweetness. Analysis of variance was computed on intake corrected for body weight since there were wide variations in weight across species. The data are presented graphically as percent glucose preference, which is a method that not only takes body weight into account but also, by examining their relative preference rather than their absolute intake differences, tends to equate preferences for animals who drink small or large amounts.

[3] The sodium saccharin was provided by the Monsanto Chemical Company.

[4] The cyclamate salts were provided by Abbott Laboratories.

Results

An analysis of intake uncorrected for body weight showed that *Peromyscus floridanus* drank more and showed a greater absolute degree of preference and that *P.m. bairdii* males generally drank the least and showed indifference to the choices. (The greater intake by *P. floridanus* is probably linked to their greater size.) The fact that these generalizations are maintained in the percent-preference data attests to the stability of the correction factor used. All further data presented and analyzed are on the basis of the intake corrected for body weight rather than on the basis of raw intake.

The preference data are presented differently in Figure 1 from Figures 2 and 3 because for saccharin there are clear differences in preference depending on the glucose concentration. This glucose dependence is not seen for the cyclamates since in all cases glucose was chosen to a very great extent over the cyclamates regardless of concentration (the exception in all cases being *P.m. bairdii* males).

Na Saccharin

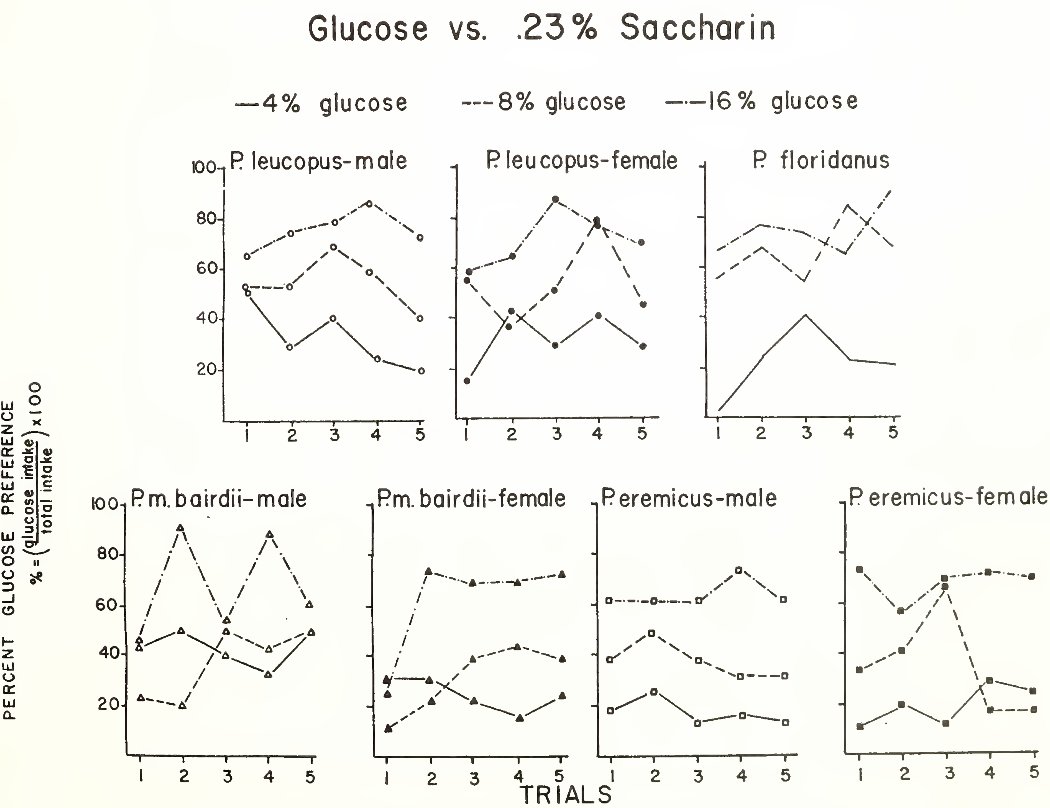

Figure 1. Relative Intake of Glucose and Sodium Saccharin in Peromyscus.

Only for the saccharin-glucose choice did most of the species prefer the artificial sweetener (See Figure 1). When given the 4% glucose vs. 0.23% saccharin all subjects preferred saccharin ($F=15.15$; $df=1/6$; $p<.001$), with *P. floridanus* showing the greatest preference and *P.m. bairdii* males being indifferent. *P.m. bairdii* females showed preferences similar to *P. eremicus*. *P. eremicus* showed a greater corrected intake than *P. leucopus* ($F=17.92$; $df=1/19$; $p<.005$) and a greater saccharin preference ($F=81.0$; $df=1/9$; $p<.001$). No sex differences were found for these last two species but sex differences were significant for *P.m. bairdii* ($F=16.76$; $df=1/4$; $p<.025$).

When 8% glucose was paired with saccharin, *P. floridanus* developed a marked glucose preference, *P. eremicus* and *P.m. bairdii* females showed a saccharin preference, and *P. leucopus* and *P.m. bairdii* males showed no consistent preferences ($F=5.58$; $df=6/24$; $p<.001$). Again, no sex differences were found for *P. leucopus* or *P. eremicus* although by examining these two species separately preference differences were found ($F=5.43$; $df=1/9$; $p<.05$). For *P.m. bairdii* sex was significant ($F=17.76$; $df=1/4$; $p<.025$).

All *S*s preferred 16% glucose to saccharin, and it is obvious from graphical inspection that there are no significant species differences.

Na Cyclamate

The various glucose concentrations were preferred to cyclamate except by *P.m. bairdii* males, who were inconsistent in their preferences as is seen in Figure 2. Generally, *P. floridanus* showed a greater degree of preference, and when these data are coupled with the *P.m. bairdii* male drinking pattern, there is produced a significant species $\times$ sweetener interaction ($F=31.78$; $df=6/24$; $p<.001$) for 16% glucose vs. Na cyclamate. It is also seen in Figure 2 that the 8% glucose combination produced a similar difference in species preference. *P. eremicus* and *P. leucopus* exhibited no species or sex-based differences in preference for glucose. *P.m. bairdii* sex differences were found for the 8% glucose combination ($F=29.24$; $df=1/4$; $p<.01$) and for the 16% glucose combination ($F=30.37$; $df=1/4$; $p<.01$).

In spite of the pronounced glucose preference, the mice still managed a total solid intake of from 0.04 gm Na cyclamate for the *P.m. bairdii* to 0.07 gm for *P. eremicus* females, to 0.08 to 0.10 gms for the remaining mice.

Ca Cyclamate

As is seen in Figure 3, for all pairs of solutions, *P. floridanus* drank more and showed the greatest glucose preference, *P.m. bairdii* males were inconsistent or preferred Ca Cyclamate, and the remaining animals were intermediate (for 4% glucose, $F=85.0$; $df=6/24$; $p<.001$, for 8% glucose, $F=63.0$; $df=6/24$; $p<.001$, and for 16% glucose, $F=6.37$; $df=6/24$; $p<.001$). The only sex-based intake differences in preference for *P. eremicus* and *P. leucopus* were found when the choice was a 4% glucose solution or Ca cyclamate. *P. eremicus* females maintained a consistent glucose preference while the other *S*s showed an initial glucose preference changing to indifference ($F=3.28$; $df=4/4$; $p<.05$). *P.m. bairdii* showed consistent sex differences for all three pairs of solutions (for 4%, $F=14.91$; $df=1/4$; $p<.025$, for 8%, $F=122.1$; $df=1/4$; $p<.001$, and for 16%, $F=118.6$; $df=1/4$; $p<.001$).

At the conclusion of the exposure to the three artificial sweeteners and glucose, the mice had a total solid cyclamate consumption of from 0.15 gms for *P.m. bairdii* to 0.21 gms for *P. leucopus* males.

If possible toxic effects would cause an increased aversion to cyclamates with extended experience, such aversion should be seen as the significant effect of "days" or "days by sweetener." Significant "day" and "day by sweetener" effects were found for 16% glucose vs. Na cyclamate and 4% glucose vs. Ca cyclamate, and "days" were significant for 8% glucose vs. Ca cyclamate. However, the significances were not caused by a consistent decline in cyclamate preference, but rather by marked day-to-day variation in sweetener preference by the several species. In addition, the already low initial preferences for cyclamates

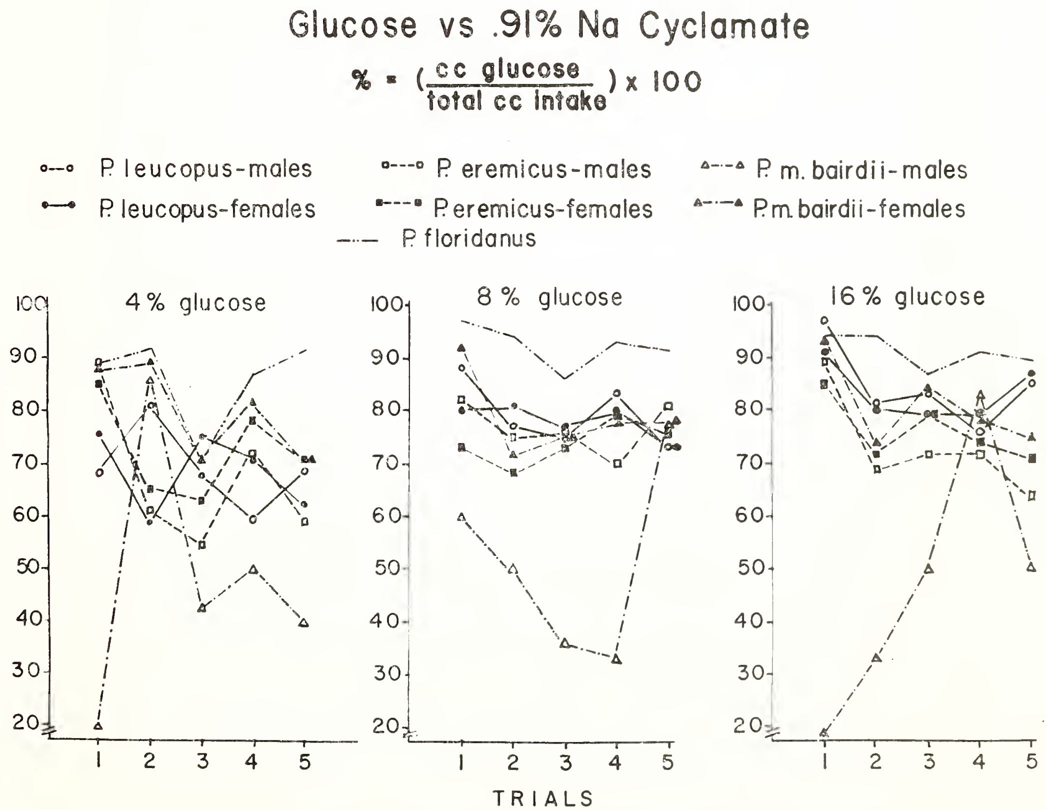

Figure 2. Relative Intake of Glucose and Sodium Cyclamate in Peromyscus.

makes it difficult for them to decrease to any appreciable extent. Consequently, an unequivocal answer is not provided to the hypothetical effects of toxicity on preference.

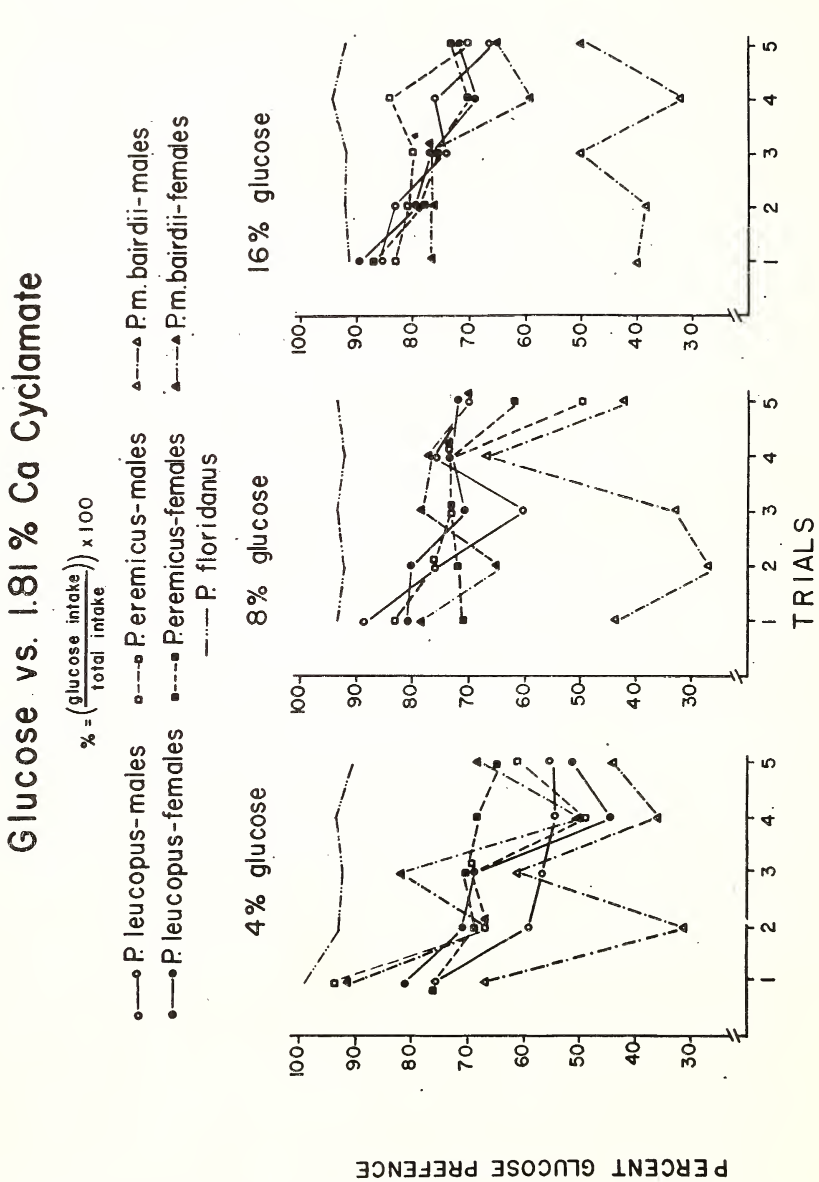

Figure 3. Relative Intake of Glucose and Calcium Cyclamate in Peromyscus.

Discussion

The species differences found here present further evidence of the more general phenomena referred to in the introduction (for species other than deer mice) and also reported for glucose preferences in deer mice (21). At present these differences cannot be explained in terms of the differing natural habitats. *P. floridanus* and *P. eremicus* live in xeric (dry) areas and *P. leucopus* and *P.m. bairdii* live in mesic (moist) areas, but the sweet preferences of these species living in similar areas are not consistently similar. In fact, there often seem to be similar preferences between species of quite different natural habitats. This phenomenon was also found for glucose-glucose preferences by Wagner and Rowntree (21).

The pronounced sex differences in preferences for glucose vs. saccharin in laboratory rats recently noted by Valenstein (18, 19) were not found for *P. leucopus* or *P. eremicus*, although *P.m. bairdii* also showed consistent sex differences in preferences for all three artificial sweeteners. However, where Valenstein found males to switch to glucose and females to continue preferring saccharin, here the male *P.m. bairdii* remained indifferent to or preferred the artificial sweetener while the females switched to a glucose preference. It is difficult to evaluate the species differences in preference for Na cyclamate in the other studies (8, 15) since the rats and mice were given a choice of cylamate and water and the rats were partially food deprived. As Stellar and McCleary (17) and Wagner and Rowntree (20) have emphasized, the type of choice available determines the preference (as most obviously does the presence of hunger).

The strong glucose preferences over the cyclamates are very interesting, especially when it is realized that the 1.81% Ca cyclamate is approximately as sweet as a 16% glucose solution (to these writers). This means that preferences (or aversions) for cyclamates are probably not based on sweetness *per se*. There is also literature suggesting that sugar of lead (lead acetate), while sweet, is avoided by rats due to its toxicity. However, this research is somewhat suspect in suggesting that some sweets are avoided; Mason and Safford (7) have reported that although this compound *may* be sweet it also has strong taste components of bitter and astringent or metallic which probably produce the aversion. While these taste components have not been reported for the cyclamates, many persons do report anecdotally a different, sort of "thin" or "metallic" character in the taste of artificially sweetened beverages.

It is not possible to show definitively that the cyclamate aversion in deer mice is based on toxicity or physiological discomfort. Nees and Derse (11) fed rats for one year on diets with 5% and 10% Ca cyclamate added. This amounted to between 312 gms and 780 gms of total solid cyclamate ingested or from slightly less than one gram to slightly more than two grams per day for females and males respectively. (These animals, it should be noted, were not given a choice of an alternative unadulterated diet.) With animals averaging over 400 gms this is equivalent to an intake of less than 0.1 gms in a 20 gm mouse. Additional recent findings by Tanaka in Japan (personal communication and translation from Nees) suggest that an acute LD_{50} for 20 gm mice is .10-.20 gm Na cyclamate and, further, that a more sensitive measure of fetal deaths, FLD_{50}, is 0.0036 gm! On the basis of these two studies it is quite possible that the deer mice could have taken sufficient cyclamate to experience toxic effects and could have developed an aversion to these solutions.

However, it is not even necessary to show that the animals are experiencing marked physiological discomfort in learning an avoidance since Fregly (3) found adult rats to avoid all concentrations of poisonous LiCl, even those too dilute to produce discomfort; Rozin (13) also found half-wild and domestic rats to subsequently avoid a Lithium-poisoned diet for a long time after having experienced the ill effects only once.

This study points up the need for more research to separate preferences based on taste or other receptors. Toxic sweet substances such as xylose, ribose, and dulcin may give clues to the basis for preferences or aversions. In addition, the study of various species and sex differences is needed to understand further the basic aspects of taste.

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