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Opioid mediation of starch and sugar preference in the rat

Kristine B. Bonacchi [b,d], Karen Ackroff [a,c], Khalid Touzani [a], Richard J. Bodnar [b,d], Anthony Sclafani [a,c,d,*]

[a] Department of Psychology, Brooklyn College, City University of New York, Brooklyn, NY, USA
[b] Department of Psychology, Queens College, City University of New York, Flushing, NY 11210, USA
[c] Cognition, Brain and Behavior Doctoral Subprogram, Graduate Center, City University of New York, New York, NY, USA
[d] Neuropsychology Doctoral Subprogram, Graduate Center, City University of New York, New York, NY, USA

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ABSTRACT

In our prior studies, administration of the opioid receptor antagonist naltrexone did not block conditioned preferences for a flavor paired with a preferred sugar solution over a flavor paired with saccharin. This may be because both training solutions were sweet, and their attractiveness was reduced by naltrexone. The present study compared the effects of naltrexone on preferences for flavors paired with sugar or starch drinks that have distinctive tastes to rats. Experiment 1 assessed naltrexone's effect on the preference for unflavored 8% cornstarch and 8% sucrose aqueous solutions/suspensions. The food-restricted rats displayed a significant sucrose preference which increased following systemic treatment with naltrexone (1 or 3 mg/kg) even though total intake of both solutions declined. In Experiment 2, rats were trained to drink flavored (cherry or grape) starch and sucrose solutions in separate one-bottle sessions. In a two-bottle choice test with both flavors presented in a sucrose–starch mixture, the rats significantly preferred the starch-paired flavor. Naltrexone treatment blocked the expression of this starch-conditioned preference. In Experiment 3, rats were treated with saline or naltrexone throughout one-bottle training with flavored sucrose and starch solutions. In a subsequent choice test, both the saline and naltrexone groups displayed significant preferences for the starch-paired flavor, indicating that opioid antagonism failed to alter the acquisition of this conditioned preference. In summary, novel outcomes of this study included the increased rather than the predicted decrease in sucrose preference produced by naltrexone. Also, starch unexpectedly conditioned the stronger flavor preference, although this can be explained by the differential post-oral reinforcing actions of starch and sucrose, and naltrexone blocked the expression, but not the acquisition, of this preference. These findings suggest that the reward value of starch in liquid form is more dependent upon opioid signaling than is that of sugar.

1. Introduction

There is extensive evidence documenting the role of brain opioid receptors in the control of feeding behavior. In particular, opioid agonist and antagonist drugs modify food intake, nutrient selection, and, of most relevance here, sweet taste preference (Bodnar, 2004; Cooper, 2007). For example, opioid antagonist drugs (naloxone, naltrexone) selectively reduce the intake of saccharin or sucrose solutions in rats more than plain water in one-bottle acceptance and two-bottle preference tests (Cooper, 1983; Le Magnen et al., 1980; Levine et al., 1982; Sclafani et al., 1982). Opioid antagonists also reduce the hedonic taste reactivity response to intraoral sugar infusions (Parker et al., 1992), operant responding for sugar rewards (Cleary et al., 1996), and sugar solution intake in sham-feeding tests that minimize post-ingestive factors (Kirkham and Cooper, 1988; Rockwood and Reid, 1982). Other studies report that naloxone or naltrexone reduced the intake of a high-sucrose diet more than a high-starch diet (Levine et al., 1995, 2002).

Rats are not only attracted to the sweet taste of sugar; they also acquire preferences for flavors associated with sweet taste as well as with the post-oral effects of sugars (Capaldi, 1996; Sclafani, 1991a). The converging data on opioid mediation of sweet preference suggest that opioid signaling should influence sugar-conditioned flavor preferences. An early study by Mehiel (1996) suggested that this was the case, although his experiment did not distinguish between the taste and post-oral reinforcing effect of sugar. Subsequent research in our laboratories, however, surprisingly indicated little or no opioid involvement in flavor preferences conditioned by the sweet taste or post-oral reinforcing actions of sugars. Our initial study focused on sweet taste conditioning by training rats with a flavor (the CS+, e.g., grape) added to a preferred sucrose solution and a different flavor (the CS−, e.g., cherry) added to a less preferred saccharin solution; the rats were sham-fed to minimize post-oral factors (Yu et al., 1999). Another study investigated sweet taste conditioning by

* Corresponding author. Department of Psychology, Brooklyn College, City University of New York, Brooklyn, NY, USA. Tel.: +1 718 951 5606; fax: +1 718 951 4824.
E-mail address: ASclafani@gc.cuny.edu (A. Sclafani).

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adding the CS+ and CS− flavors to preferred fructose and less preferred saccharin solutions that were real-fed (Baker et al., 2004). Post-oral conditioning was minimized in this case because fructose, unlike sucrose and glucose, has a minimal reinforcing effect when infused intragastrically (IG) (Sclafani et al., 1999). To investigate post-oral flavor sugar conditioning, in a third study rats were trained to drink flavored CS+ and CS− saccharin solutions that were paired with IG infusions of sucrose and water, respectively (Azzara et al., 2000). In all three studies, treating rats with systemic naltrexone during flavor training sessions did not block the learning of a CS + preference. In addition, following training, naltrexone injections had little or no effect on the expression of the learned CS+ preference (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). More recently, we observed that naltrexone microinjections into the nucleus accumbens did not block the expression of CS+ flavor preferences conditioned by the sweet taste of fructose or the post-oral actions of glucose (Bernal et al., 2010).

The failure of naltrexone to block flavor conditioning by the sweet taste of sucrose or fructose appears inconsistent with the many findings that implicate opioid signaling in the hedonic evaluation of sweet taste (Gosnell and Levine, 2009; Levine et al., 2003). It is possible, however, that our use of sugar in the CS+ training solution and saccharin in the CS− training solution obscured the effect of naltrexone on flavor conditioning. That is, the drug may have reduced the attractiveness of both the sugar and saccharin solutions such that the remaining palatability difference between the two solutions was sufficient to condition a CS+ flavor preference. The present study, therefore, reevaluated opioid involvement in flavor conditioning by using sweet (sucrose) and non-sweet (starch) training solutions. In view of reports that naltrexone is more potent in reducing sucrose diet than starch diet intake (Levine et al., 1995, 2002), we predicted that naltrexone would reduce the preference for a sucrose-paired flavor over a starch-paired flavor. Our results revealed a selective effect of naltrexone on flavor preference, but surprisingly, it was the intakes of starch and starch-paired flavor that were the most suppressed.

2. Experiment 1: sucrose vs. starch preference

In prior studies, rats strongly preferred pure sucrose or a high-sucrose composite diet to pure cornstarch or a high-starch composite diet (Levine et al., 2002; Sclafani et al., 1987; Weldon et al., 1996). However, in choice tests with sucrose and starch solutions, rats displayed sucrose, starch, or no preferences depending upon the concentration of carbohydrate solutions and the deprivation state of the animal (Ramirez, 1991a, 1993a; Sclafani and Ackroff, 1993). Therefore, before examining sucrose- and starch-conditioned flavor preferences, this experiment first determined the preference of rats for isocaloric 8% sucrose and 8% starch solutions and how this preference is altered by systemic naltrexone treatment.

2.1. Methods

2.1.1. Subjects

Male Sprague–Dawley rats (n = 23) bred in our laboratory from Charles River (Wilmington, MA) stock were used. The animals were 4 months old at the start of the experiment and were housed in wire-mesh cages in a vivarium maintained on a 12:12 h light:dark cycle (lights on at 0800 h) at 21 °C. The animals were given ad libitum access to water and restricted food rations (Lab Chow 5001, PMI Nutrition International, Brentwood, MO) that maintained them at 90% of free-feeding body weight. The experimental protocols were approved by the Brooklyn College Institutional Animal Care and Use Committee, certifying that all subjects and procedures were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.1.2. Test solutions

The test fluids contained food-grade sucrose (Domino Foods, Yonkers, NY) or cornstarch (ACH Food Companies, Memphis TN). Because cornstarch is not soluble in water, the cornstarch was prepared as a suspension using 0.3% xanthan gum (Sigma Chemical Co., St. Louis, MO) as described previously (Ramirez, 1991a). Xanthan gum was also added to the sucrose solution to control for the viscosity of the cornstarch + gum mixture. The carbohydrate + gum mixtures, hereafter referred to as solutions, were formulated on a weight/weight basis with tap water, blended in a food blender and cooled to room temperature before serving. The test solutions used in this and subsequent experiments are listed in Table 1.

2.1.3. Apparatus

One-bottle training and two-bottle test sessions were conducted in hanging wire-mesh cages (18 × 18 × 24 cm) housed in a separate test room. The sucrose and starch solutions were presented in graduated 100-ml glass bottles (Bio-Serv Inc., Frenchtown, NJ) with a drinking well that extended 4 cm into the cage. The drinking well had a round aperture with a diameter of 1.2 cm. Spillage, which was minimal, was collected in trays under the test cage. The bottles and spillage trays were weighed to the nearest 0.1 g at the start and end of the sessions.

2.1.4. Procedure

Prior to testing, the rats were familiarized with the test carbohydrates by giving them 10 ml of 8% sucrose or 8% starch in a jar placed in their home cages overnight. The order of presentation of the carbohydrates was counterbalanced across the two overnight periods.

Training consisted of alternate days of one-bottle access (30 min/day) to 8% sucrose and 8% cornstarch for a total of 6 days; the left–right position of the bottle alternated over sessions using an LRRL design. No injections were given prior to training sessions. The rats were then given a series of three two-bottle choice tests with 8% sucrose vs. 8% starch. Each test consisted of two 30-min/day sessions and the left–right position of the sucrose and starch alternated over sessions. The rats were then divided into two drug dose groups matched for their intakes during one-bottle training and the first two-bottle test. All rats were given a subcutaneous injection of saline 20 min prior to the second two-bottle test. The rats in the two drug dose groups were then given subcutaneous injections of 1 and 3 mg/kg naltrexone (Sigma Chemical Co.), respectively, 20 min prior to the sessions of the third two-bottle test.

2.1.5. Statistical analysis

Intakes during one-bottle training were averaged over three sessions with each carbohydrate, and compared using a *t*-test. Intakes during the saline and drug tests were averaged over the two sessions and entered into ANOVA with group (1 or 3 mg/kg) as a between factor and drug treatment (saline or drug) and carbohydrate (starch vs. sucrose) as within factors. Two-bottle preferences were expressed as percent sucrose intake (sucrose/total intake × 100). The percent

Table. 1

Exp #	Solution	
	1-bottle training	2-bottle testing
1	8% Starch	8% Starch
	8% Sucrose	8% Sucrose
2	CS+/8% Starch	CS + Sucrose/2% Starch + 2% Sucrose
	CS+/8% Sucrose	CS + Starch/2% Starch + 2% Sucrose
3	CS+/8% Starch	CS + Sucrose/2% Starch + 2% Sucrose
	CS+/8% Sucrose	CS + Starch/2% Starch + 2% Sucrose

All solutions were prepared as suspensions with 0.3% xanthan gum.
CS+ flavors = 0.1% cherry or grape.

data were evaluated by ANOVA with group as one factor and drug treatment (saline or drug) as a second factor.

2.2. Results and discussion

Overall, the rats consumed more sucrose than starch during the six one-bottle training sessions (24.4 vs. 20.7 g/30 min, t (22) = 2.23, $p<0.05$) as well as during the first two-bottle choice test (25.5 vs. 18.7 g/30 min, t (22) = 2.46, $p<0.05$). The results of the choice tests following saline and drug injections are summarized in Fig. 1. Overall, the 1 and 3 mg/kg groups did not differ in their carbohydrate intakes. In both groups, naltrexone suppressed total fluid intake compared to saline treatment ($F(1, 21) = 215.82$, $p<0.01$) (Fig. 1). There was also a main effect of test fluid and the rats consumed more sucrose than starch following saline and naltrexone injections ($F(1, 21) = 44.98$, $p<0.01$). In both groups, naltrexone tended to reduce starch intake more than sucrose intake but the drug × carbohydrate interaction was not significant. However, the percent sucrose intakes in both groups increased significantly from the saline test (65–67%) to the naltrexone test (79–82%; $F(1, 21) = 38.42$, $p<0.01$).

Inspection of the percent sucrose scores of individual animals revealed that while a majority of rats in each dose group preferred sucrose during the saline test, a substantial minority (9 of 23) did not. To determine if the drug effect on carbohydrate intake varied as a function of initial sucrose preference, we divided all the rats into a Sucrose Preferring group (sucrose preference>60%, n = 14) and a Sucrose Non-preferring group with weak or no sucrose preference (<60%, n = 9). As indicated in Fig. 2A, the Sucrose Preferring group had a 75% sucrose preference in the saline test which increased to 90% following naltrexone treatment (t (13) = 6.51, $p<0.01$). The Sucrose Non-preferring group had a 51% sucrose preference in the saline test, which increased to 66% in the naltrexone test (Fig. 2B; t (8) = 2.97, $p<0.01$). Analysis of the absolute intake data revealed that naltrexone reduced the intake of both sucrose and starch in the Sucrose Preferring group ($F(1,13) = 177.29$, $p<0.001$) whereas the drug reduced only starch intake in the Sucrose Non-preferring group (carbohydrate × drug interaction ($F(1, 8) = 6.89$, $p<0.05$).

Overall, the rats preferred sucrose to starch in the no-injection and saline tests. The preference was not very strong, however, and many rats did not prefer sucrose. However, irrespective of the initial preference, naltrexone significantly increased the preference for sucrose over starch. This is a robust finding which we replicated in two unpublished experiments using different training procedures. In one experiment, rats increased their sucrose preference from 58% to 78% and 86% when treated with 1 and 3 mg/kg naltrexone, respectively; in the other experiment sucrose preference increased from 55% to 80% with a 3 mg/kg naltrexone injection. These findings are in marked contrast to reports that naltrexone suppressed the intake of a sucrose-based diet more than that of a starch-based diet (Levine et al, 2002; Weldon et al., 1996). Possible reasons for these discrepant results are discussed in the General discussion.

3. Experiment 2: Expression of sucrose- vs. starch-conditioned flavor preferences

This experiment examined the effect of naltrexone on expression of the rats' conditioned preference for different flavors mixed in starch and sucrose solutions. Given the preference that the rats displayed for sucrose over starch in the first experiment, we expected that rats would learn to prefer the sucrose-paired flavor over the starch-paired flavor. Earlier studies showing that opioid receptor antagonism suppresses sweet taste preferences also led to the prediction that naltrexone would reduce the preference for the sucrose-paired flavor (Cooper, 1983; Le Magnen et al., 1980; Levine et al., 1982; Sclafani et al., 1982). However, the naltrexone-induced reduction in starch preference observed in Experiment 1 predicts just the opposite outcome, i.e., that the drug would reduce the preference for the starch-paired flavor.

To investigate these predictions, rats were given one-bottle training with distinctly flavored sucrose and starch solutions. Flavor preferences were then evaluated by giving the rats the choice of the two flavors presented in identical mixtures of sucrose and starch following saline and naltrexone injections. Note that the two-bottle tests were essentially extinction tests in that the flavors were no longer uniquely paired with sucrose or starch as they were in the training solutions. We used two procedures to evaluate whether any change in the flavor preference observed in the drug test was the result of an extinction process rather than to a drug effect, per se. First, flavor preferences after saline injection were evaluated both before and after the naltrexone test. Second, flavor preferences were evaluated in a control group that was given a series of three saline tests, i.e., they were not treated with naltrexone.

3.1. Methods

3.1.1. Animals

The 18 male rats used were born in our laboratory and housed and maintained as in Experiment 1. The rats were 3 months old at the start of the experiment.

3.1.2. Test fluids

A 2% Polycose (Ross Nutrition, Columbus, OH) solution containing 0.3% xanthan gum was used to adapt the animals to drink in the test cages; Polycose has a flavor different from that of starch and sucrose (Nissenbaum and Sclafani, 1987; Ramirez, 1991b). The rats were trained in one-bottle tests with 8% sucrose and 8% starch solutions

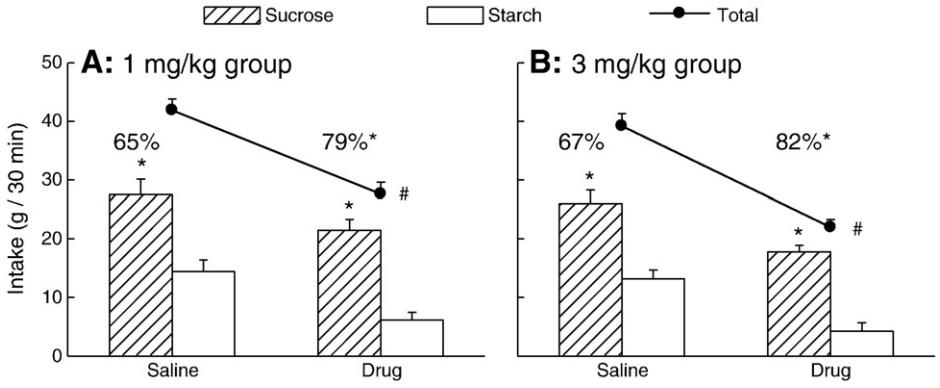

Fig. 1. Mean (+sem) 8% sucrose, 8% starch and total intakes in the groups tested with saline, 1 mg/kg (A) and 3 mg/kg (B) naltrexone in the two-bottle choice tests with saline and drug of Experiment 1. Percentages above bars indicate percent preference for sucrose relative to total intake. The asterisks and number signs denote significant ($P<0.05$) differences between sucrose and starch intakes or between percent sucrose intakes or total intakes in saline vs. drug tests.

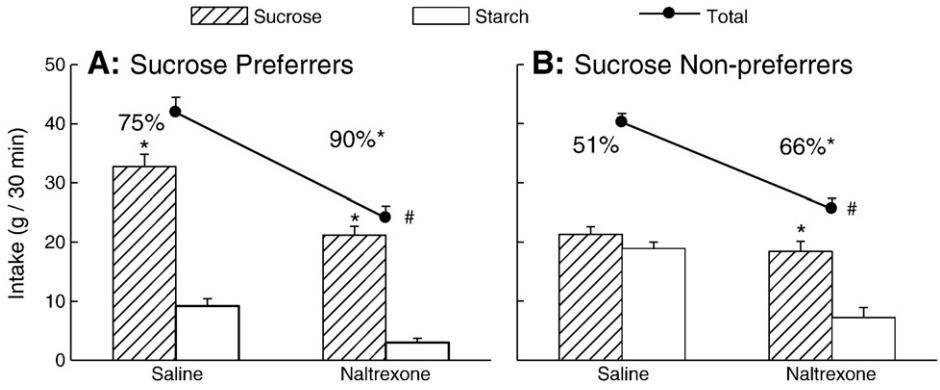

Fig. 2. Mean (+sem) 8% sucrose, 8% starch and total intakes in the Sucrose Preferring (A, n = 14) and Sucrose Non-preferring (B, n = 9) groups in Experiment 1. Percentages above bars indicate percent preference for sucrose relative to total intake. The asterisks and number signs denote significant ($P<0.05$) differences between sucrose and starch intakes or between percent sucrose intakes or total intakes in saline vs. drug tests.

prepared with 0.3% xanthan gum and flavored with the conditioned stimulus (CS); 0.1% grape or cherry unsweetened Kool-Aid (General Foods, White Plains, NY); see Table 1. Half of the animals were given grape–starch and cherry–sucrose; the flavors were reversed for the remaining animals. Training solutions are hereafter referred to as CS+/8% Sucrose or CS+/8% Starch. In two-bottle tests, the rats were given the choice of cherry- and grape-flavored solutions containing a mixture of 2% sucrose and 2% starch. The test solutions contained only 4% carbohydrate to enhance the salience of the Kool-Aid flavors. The flavor that was paired with 8% sucrose is referred to as the CS + Sucrose and the flavor that was paired with 8% starch as the CS + Starch.

3.1.3. Procedure

Animals were given home cage access to 20 ml of 2% Polycose + gum solution in a food cup for two consecutive overnight periods in order to familiarize them with the pre-training solution. They were then food-restricted and maintained at 90% of free-feeding body weight. The rats were familiarized with the test cages and procedures by giving them two 30 min/day sessions with two-bottle access to a 2% Polycose + gum solution vs. a gum only solution.

The rats were next trained to drink the CS+/8% sucrose and CS+/8% starch solutions during eight alternating one-bottle sessions (30 min/day). Conditioned preferences were then assessed over three consecutive two-bottle choice tests using the CS + Sucrose and CS + Starch flavors presented in the 2% sucrose + 2% starch mixed solutions; each test consisted of two 30 min/day sessions. In the first test, all rats were injected with saline 20-min prior to the sessions. The rats were then divided into two groups (n = 9 each) matched for their training and two-bottle fluid intakes. The Drug group was given naltrexone (3 mg/kg) prior to the second choice test and then saline prior to the third choice test. The Control group was given saline prior to all test series.

3.2. Results and discussion

Overall, intakes of the CS+/8% Sucrose and CS+/8% Starch solutions did not significantly differ during the one-bottle training sessions (15.0 vs. 13.8 g/30 min), indicating the similar acceptance of the two solutions. The rats were then given two-bottle tests with the two CS flavors presented in mixed 2% sucrose + 2% starch solutions. In the initial test following saline injection, the rats consumed more CS + Starch than CS + Sucrose flavor ($t(17) = 4.40$, $p<0.01$) with an overall preference of 70%; 15 of the 18 animals displayed CS + Starch preferences of 60% or greater. As shown in Fig. 3A, the Control rats, given two additional saline tests, continued to display significant CS + Starch preferences of about 70%. Analysis of their absolute intakes revealed that CS + Starch intake exceeded CS + Sucrose intake with no change over test trials ($F(1, 8) = 11.51$, $p<0.01$). In the Drug group, in contrast, intakes declined in Test 2 when the rats were

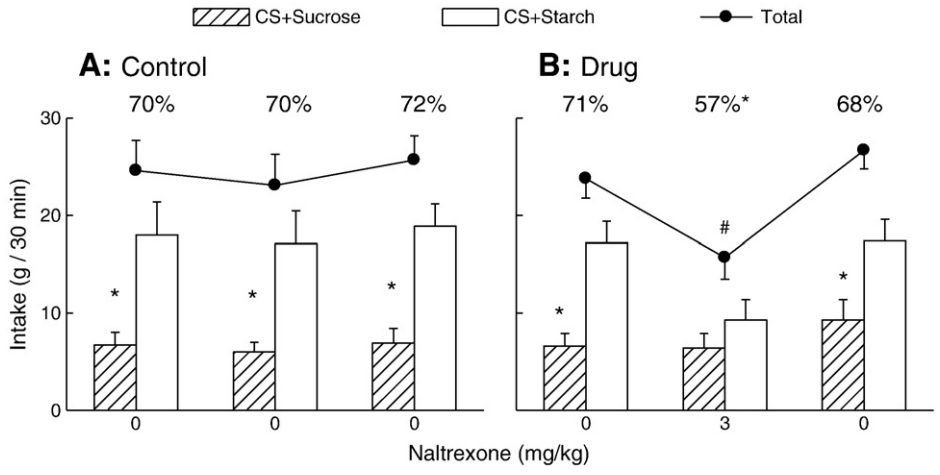

Fig. 3. Mean (+sem) CS + Sucrose, CS + Starch and total intakes in the Control (A) and Drug (B) groups during the two-bottle choice tests of Experiment 2. The Control group was treated with saline during the three sets of two-bottle tests whereas the Drug group was treated with saline in tests 1 and 3 but 3 mg kg naltrexone in test 2. Percentages above bars indicate percent preference for CS + Starch relative to total intake. The asterisks and number signs denote significant ($P<0.05$) differences between sucrose and starch intakes or between percent sucrose intakes or total intakes in saline vs. drug tests.

treated with naltrexone (Fig. 3B). Intakes during the pre- and post-drug saline tests did not differ and therefore were combined and compared to the intakes during the drug test. The ANOVA confirmed that the naltrexone reduced overall intake compared to the saline tests ($F(1, 8) = 63.33$, $p<0.001$). More importantly, the drug selectively reduced the intake of the CS + Starch and not the CS + Sucrose (CS × drug interaction, $F(1, 8) = 11.87$, $p<0.01$) solution such that the intakes of the two CS+ solutions did not differ in the drug test. In addition, naltrexone treatment significantly reduced the CS + Starch preference from 69% to 57% ($t(8) = 3.24$, $p<0.01$).

This experiment revealed two novel findings. First, the rats developed a significant conditioned preference for the starch-paired flavor over the sucrose-paired flavor. This CS + Starch preference cannot be attributed to a more preferred taste of starch since most rats in Experiment 1 preferred sucrose to starch or were indifferent to the two carbohydrates. Rather, as explained in the General discussion, the conditioned CS + Starch preference was most likely due to the differential post-oral reinforcing effects of starch and sucrose. The second new finding is that naltrexone treatment significantly altered the expression of the learned flavor preference, i.e., the rats lost their CS + Starch preference and consumed near-equal amounts of the two flavors. The selective decline in the CS + Starch intake cannot be attributed to an extinction of the learned flavor preference given that the Drug group recovered their CS + Starch preference in saline Test 3 after the drug test, and the Control group showed a persistent CS + Starch conditioned preference in all three sets of flavor tests. Rather, the naltrexone-induced suppression of the CS + Starch preference is consistent with the drug-induced reduction in starch preference observed in Experiment 1.

4. Experiment 3: acquisition of sucrose- vs. starch-conditioned preference

In view of the finding that naltrexone blocked the expression of CS + Starch preference in Experiment 2, this experiment determined if drug treatment during training inhibits the acquisition of the conditioned flavor preference. This was accomplished by treating rats with naltrexone during one-bottle flavor training sessions and then measuring their preference in the absence of the drug. Because naltrexone is reported to suppress sucrose intake much more in sugar-naïve than in sugar-experienced rats (Lynch and Burns, 1990), we first conducted a pilot study to determine the suppressive effect of the 3 mg/kg dose used in Experiments 1 and 2 on flavored sucrose and starch intakes in naïve rats. The effect was profound and the animals consumed less than 1 g/30 min session of either solution. A second pilot study revealed that a 1 mg/kg dose suppressed but did not eliminate intakes in rats trained in 60 min/day sessions, and these parameters were therefore used in the current experiment.

4.1. Methods

4.1.1. Animals

The 28 male rats used were purchased from Charles River Laboratories and maintained as in prior experiments. The rats were 2 months old at the start of the experiment.

4.1.2. Procedure

The rats were familiarized with an 8% Polycose + gum solution by giving them home cage assess to 20 ml for one night. They were then food restricted and given 60-min pre-training sessions with 8% Polycose vs. gum solution over 4 days. We used a more concentrated Polycose solution and four pre-training sessions in this experiment to establish a strong drinking response in the animals prior to the flavor training with naltrexone. On day 4 of pre-training, the animals were given a saline injection after the session to familiarize them with the subcutaneous injection procedure.

The animals were divided into two groups based on body weight and pre-training intakes. The rats were given one-bottle access (60 min/day) to grape- or cherry-flavored 8% sucrose (CS+/8% Sucrose) and flavored 8% starch (CS+/8% Starch) solutions for a total of 8 sessions (see Table 1). Saline and naltrexone (1 mg/kg) injections preceded the one-bottle sessions for the Control and Drug groups, respectively. Flavor preferences were then assessed in two-bottle choice tests (two sessions) with the CS + Sucrose and CS + Starch flavors presented in 2% sucrose + 2% starch mixtures as in Experiment 2. All rats were injected with saline prior to these tests.

4.2. Results and discussion

As indicated in Fig. 4A, the Drug group consumed significantly less of the flavored carbohydrate solutions during training than did the Control group ($F(1, 26) = 72.40$, $p<0.001$); overall, the drug-induced suppression in training intakes was 43%. However, within each group the one-bottle intakes of the CS+/8% Sucrose and CS+/8% Starch solutions were similar. In the two-bottle tests with the flavored sucrose + starch solutions (Fig. 4B), the Control and Drug groups both consumed more CS + Starch than CS + Sucrose ($F(1, 26) = 35.50$, $p<0.001$) and there were no group differences in the CS intakes. The Control and Drug groups also did not differ significantly in their CS + Starch preferences (68% vs. 63%). Thus, although naltrexone treatment blocked the expression of the CS + Starch in Experiment 2, drug treatment during training in Experiment 3 failed to prevent the acquisition of the CS + Starch preference even though it reduced the intake of training solutions by almost half.

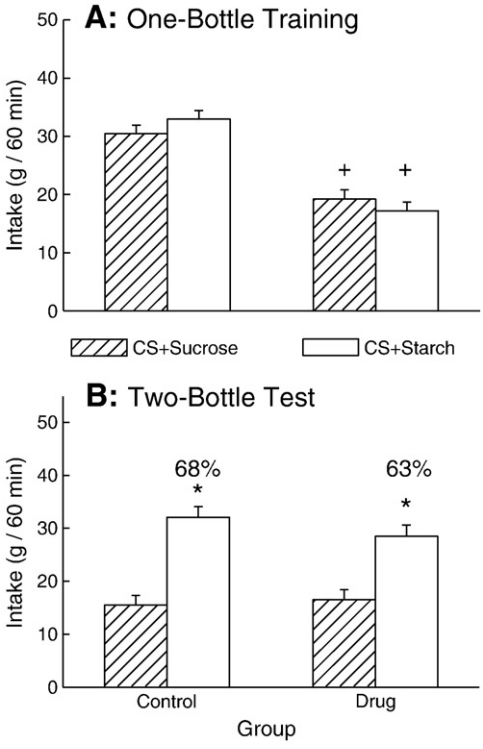

Fig. 4. Mean (+sem) CS + Sucrose and CS + Starch intakes during one-bottle training (A) and two-bottle tests (B) of Experiment 3. The Control and Drug groups were injected with saline and 1 mg/kg naltrexone throughout training; both groups received saline during two-bottle testing. Percentages above bars indicate percent preference for CS + Starch relative to total intake. The asterisks denote significant ($P<0.05$) differences between CS + Sucrose and CS + Starch intakes and the plus sign denotes significant ($P<0.05$) differences between Control and naltrexone groups in their intakes of CS + Sucrose and CS + Starch training solutions.

5. General discussion

In this study, we investigated the effect of opioid receptor antagonism with naltrexone on the conditioned preference for flavors paired with isocaloric sucrose and starch solutions. This was of interest because prior work in our laboratories revealed that naltrexone did not block the acquisition or expression of sugar-conditioned flavor preferences in rats (Azzara et al., 2000; Baker et al., 2004; Bernal et al., 2010; Yu et al., 1999). We hypothesized that flavor preference conditioning was unaffected by naltrexone because the animals were trained and tested with sugar and saccharin solutions so that drug-induced reductions in sweetener preference influenced both the CS+ and CS− flavors. We predicted that the drug would reduce the preference for a sucrose-paired flavor when it was compared to a starch-paired flavor. This prediction was not confirmed, but rather the present experiments revealed a selective effect of opioid receptor antagonism on starch-based preferences.

Sucrose vs. starch preference. The preference of rats for sweet foods and drinks is extensively documented (Levine et al., 2003). Of particular relevance here are reports by Levine and colleagues that rats consumed more of a high-sucrose than a high-starch diet in one- and two-jar tests and that naltrexone (or naloxone) preferentially reduced high-sugar diet intake (Levine et al., 1995, 2002). In view of these findings, we investigated naltrexone effects on the preferences for flavors paired with sucrose or starch. As in our previous conditioning studies, carbohydrate solutions rather than dry carbohydrates or complete diets were used as unconditioned stimuli paired with the CS flavors. In Experiment 1, we evaluated the effect of naltrexone on the preference for unflavored 8% starch vs. 8% sucrose solutions which served as the unconditioned stimuli in the subsequent experiments. The results revealed a significant, but modest 65% preference for sucrose over starch. Unexpectedly, rather than reducing the sucrose preference, naltrexone suppressed the percent intake for starch and thereby increased the percent intake of sucrose. This effect was observed in rats that strongly preferred sucrose relative to starch (Sucrose Preferrers) as well as in rats that were indifferent to sucrose relative to starch (Sucrose Non-preferrers). This is noteworthy because some previous studies of opioid modulation of nutrient preference (i.e., carbohydrate vs. fat) reported that drug effects were influenced by the animal's baseline nutrient preference (Glass et al., 1996; Gosnell et al., 1990).

The naltrexone-enhanced preference for sucrose observed in Experiment 1 is in marked contrast with prior reports that the drug selectively reduced sucrose intake in rats given one- or two-jar tests with diets rich in sucrose or starch (Levine et al., 2002; Weldon et al., 1996). The present and prior studies differed in several respects including the nature of the test diets used: pure carbohydrate solutions in Experiment 1 vs. dry, composite diets used in Levine's studies (Levine et al., 2002; Weldon et al., 1996). The liquid vs. dry form of the test diets may be of particular importance because adding water to pure carbohydrates (sugar, hydrolyzed starch, or starch) and high-carbohydrate diets enhances their flavor and increases consumption (Ramirez, 1987; Sclafani et al., 1988; Sclafani and Xenakis, 1984). This may be especially true for starch because of its insolubility in the saliva medium of the mouth.

Compared to sugar, starch is typically considered to be bland "tasting" (Sclafani, 1991b). However, hydrated starch (i.e., starch suspension or gel) is rather attractive to rats. In fact, at low concentrations, rats prefer starch to sugar solutions, and food deprivation enhances the relative preference for dilute (0.5–2%) starch over sugar (Ramirez, 1993a; Sclafani and Ackroff, 1993). Little is known about the orosensory properties of starch but, like sugar, it may include gustatory, olfactory and somatosensory features (Ramirez, 1991c; Ramirez, 1993b). Prior work in our laboratory indicated that rodents are very attracted to the taste of glucose polymers (Polycose) which we hypothesized mediates their attraction to pure starch via starch hydrolysis in the mouth (Sclafani, 1987). However, subsequent studies indicated that sugar, Polycose and starch have different "tastes" to rodents (Giza et al., 1991; Nissenbaum and Sclafani, 1987; Ramirez, 1991b, 1994; Sclafani et al., 2007; Treesukosol et al., 2009; Zukerman et al., 2009). There is as yet no recognized taste receptor for starch but the recent findings that starch preference is impaired by deletion of the taste signaling protein TRPM5 (Sclafani et al., 2007), and recent localization of the starch digestive enzyme amylase in taste cells (Merigo et al., 2009) supports the conjecture that taste cells can detect pure starch.

Thus, while much remains to be learned about starch "taste", there is now substantial evidence that starch, particularly in a hydrated form, is attractive to rodents. It is not surprising, therefore, that starch preference, like that for sugar, fat, and salt, would be modulated by the opioid reward system. The results of Experiment 1 suggest that the relative attractiveness of starch taste is even more dependent upon opioid receptor activity than is that of sugar. This may occur, in part, because sugar taste is more effective in activating the opioid system than is starch taste and, consequently, sugar reward is less disrupted than starch reward by partial opioid receptor antagonism. This interpretation remains speculative, and the neuropharmacology of sugar vs. starch preference requires further investigation.

Sucrose- vs. starch-conditioned flavor preference. Our original prediction was that rats would prefer a sucrose-paired flavor over a starch-paired flavor, and that naltrexone would suppress this sucrose-conditioned preference. The results of Experiment 2 did not support these predictions. First, despite the sucrose preference observed in the first experiment, the rats in the second experiment displayed a significant preference for the starch-paired flavor over the sucrose-paired flavor. Second, naltrexone preferentially reduced the expression of the preference for the CS + Starch flavor in the two-bottle test with both flavors presented in a common starch + sucrose mixture.

While not predicted, the starch-conditioned preference obtained in Experiment 2 can be explained by the differential post-oral reinforcing effects of carbohydrates. We previously observed that rats trained with CS flavored saccharin solutions paired with IG glucose and IG fructose infusions strongly preferred the glucose-paired flavor (Sclafani et al., 1999). This and other findings (Ackroff et al., 2001) showing differential conditioning by glucose and fructose are of relevance here because sucrose is a glucose + fructose disaccharide that yields only half as much glucose when hydrolyzed in the gut as does starch, a glucose polymer. The importance of this differential glucose yield is demonstrated by the findings that rats learn to prefer a flavor paired with IG infusions of maltose (a glucose + glucose disaccharide) over a flavor paired with IG sucrose infusion (Azzara and Sclafani, 1998). Furthermore, rats given 24 h/day two-bottle tests with 32% sucrose vs. 32% maltose, initially preferred sucrose but over days switched their preference to maltose (Ackroff and Sclafani, 1991). Thus, the rats in Experiment 2, even though they presumably preferred the taste of sucrose to starch, may have developed a significant preference for the starch-paired flavor because it was associated with a stronger post-oral glucose reinforcing action. A similar conditioning process may have contributed to the finding that sucrose was only mildly preferred to starch in Experiment 1. That is, the more preferred innate taste of sucrose was counteracted by the more reinforcing post-oral action of starch.

Although we originally hypothesized that naltrexone would selectively reduce the preference for a sucrose-paired flavor, the finding that the drug decreased starch preference in Experiment 1 led to the opposite prediction, which was confirmed in the second experiment. Flavor-nutrient conditioning studies indicate that during initial training, animals can form multiple CS–US associations (Delamater et al., 2006; Harris et al., 2000). That is, rats can learn to associate the CS flavor with the orosensory features of the nutrient, e.g., sweet taste or starchy taste, as well as with the post-oral reinforcing effect of the nutrients. Conceivably, naltrexone may have

eliminated the rats' preference for the CS + Starch flavor in Experiment 2 because the drug specifically blocked the expression of CS–US post-oral association. This interpretation is inconsistent, however, with the failure of naltrexone to block the preference for a CS + flavor conditioned by IG sugar infusions (Azzara et al., 2000). Instead, naltrexone may have attenuated the preference for the CS + Starch flavor because it reduced the reward value of the evoked orosensory representation of the starch flavor just as it reduced the evaluation of the actual starch flavor in the unflavored starch vs. sucrose choice test of Experiment 1.

In contrast to the results of Experiment 2, naltrexone did not block the expression of conditioned flavor preferences in our prior studies in which rats were trained with sugar and saccharin solutions. In these studies the CS + sugar and CS + saccharin flavors presumably evoked taste representations of similar quality, i.e., sweet, but of different intensity, and there is no reason for naltrexone to reduce the preference for the sweeter taste. Supporting this interpretation, we observed that the rats' preference for a concentrated saccharin solution over a dilute saccharin solution was not altered by naltrexone although the drug suppressed overall intake (unpublished findings, see Touzani et al., in press).

In view of the suppressive effect of naltrexone on the expression of the CS + Starch preference in Experiment 2, the third experiment determined if naltrexone treatment during original flavor-carbohydrate training would block the development of the CS + Starch preference. This did not occur. Although naltrexone treatment substantially reduced the training intakes of the flavored starch and sucrose solutions, it did not significantly attenuate the acquisition of the preference for the CS + Starch flavor as revealed in the subsequent two-bottle test. To the degree that the CS + Starch flavor preference is conditioned by the post-oral actions of the starch, Experiment 3 indicates that naltrexone does not disrupt flavor-nutrient conditioning. This is directly supported by our earlier findings that naltrexone does not block flavor preference conditioning by IG sugar infusions (Azzara et al., 2000). Taken together, the results of Experiment 2 and 3 suggest that flavor conditioning by starch is controlled by post-oral reinforcement while the expression of the previously conditioned CS + Starch preference is influenced by the memory of the starch flavor elicited by the CS+ at the time of testing.

In Experiment 3, naltrexone equally suppressed the intakes of the flavored starch and sucrose solutions during the one-bottle training sessions. Yet, in the two-bottle tests, the drug selectively reduced starch intake and CS + Starch intake in Experiments 1 and 2, respectively. These findings indicate that naltrexone is more effective in altering the relative attractiveness of starch vs. sucrose in choice tests than in reducing the acceptability of the two carbohydrates in one-bottle tests. Additional research is needed, however, to more fully evaluate the impact of opioid antagonists as well as agonists on sugar and starch preference and acceptance in rats. Of particular interest are sham-feeding studies that focus on the orosensory features of the two types of carbohydrates. Another area of interest is the effects of opioid drugs on the preferences for cornstarch vs. Polycose (hydrolyzed cornstarch), and sucrose vs. Polycose since these carbohydrates appear to differ in taste quality (Giza et al., 1991; Nissenbaum and Sclafani, 1987; Ramirez, 1991b; Ramirez, 1994; Sclafani et al., 1987, 2007; Treesukosol et al., 2009; Zukerman et al., 2009). Opioid drug effects on sugar vs. fat, which have been extensively investigated using solid diets (Taha, 2010), should also be reevaluated using sugar solutions and fat emulsions in light of recent findings suggesting the existence of fat taste receptors (Passilly-Degrace et al., 2009).

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