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Naltrexone fails to block the acquisition or expression of a flavor preference conditioned by intragastric carbohydrate infusions

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Abstract

The effects of naltrexone on the expression and acquisition of flavor preferences conditioned by the postingestive actions of carbohydrates were investigated. Food-restricted rats (Experiment 1) were given one-bottle training with one flavored saccharin solution (CS+) paired with intragastric (IG) infusions of 16% sucrose, and another flavored saccharin solution (CS −) paired with water infusions. In two-bottle tests CS+ was preferred to CS − , and naltrexone (1.0–5.0 mg/kg) reduced total intake but not CS+ preference. In Experiment 2 food-restricted rats that received naltrexone (0.1 or 1.0 mg/kg; NTX group) throughout one-bottle training consumed less CS+ and CS − than did saline-treated control rats. Yet, the NTX and control groups displayed similar CS+ preferences during two-bottle tests when treated with saline or naltrexone (0.1–5.0 mg/kg). In Experiment 3, rats were trained to accept more CS+ than CS − in one-bottle tests. Naltrexone (0.1–2.5 mg/kg) reduced the one-bottle intakes of both solutions, and the rats continued to consume more CS+ than CS − . We conclude that the opioid system modulates the consumption of flavored solutions, but is not critically involved in the acquisition or expression of flavor preferences conditioned by IG carbohydrate.

Keywords: Conditioned preference; Conditioned acceptance; Sucrose; Maltodextrin; Saccharin

Animals learn to prefer the flavor of foods based, in part, on the postingestive actions of nutrients (flavor–nutrient conditioning) [26]. This has been documented in our laboratory by training rats to consume a novel flavored solution, the conditioned stimulus (CS+), which is paired with an intragastric (IG) nutrient infusion, the unconditioned stimulus (US). On other trials, a different flavored solution (the CS −) is paired with an IG water infusion. After several training sessions, animals typically prefer the CS+ flavor over the CS − flavor in a two-bottle choice test. This flavor preference can be quite robust and resistant to extinction [9]. Furthermore, nutrient infusions can condition preferences for tastes that are normally avoided (e.g., bitter, sour) [9]. In addition to their postingestive actions, the flavor of some nutrients (e.g., sweet taste of sugar, oily texture of liquid fats) can serve as an US and condition a preference for a novel flavor (CS+) mixed with the nutrient (flavor–flavor conditioning) [26]. However, different processes may mediate these two forms of flavor preference learning, since flavor–nutrient learning is possible with longer CS–US delays than can support flavor–flavor conditioning [26].

Although a fair amount is known about flavor preference conditioning at the behavioral level, relatively little is known about the underlying neurochemical basis of such conditioning. The opioid system is one potential candidate to mediate flavor preference conditioning because of its long-recognized role in food reward processes [2,4,5]. There is a large body of research demonstrating that opioid antagonists suppress food and fluid intake. This suppression is greater for palatable sucrose and saccharin solutions than it is for water [3], indicating that opiate antagonism suppresses the hedonic response to sweet solutions. This conclusion is supported by the findings that opiate antagonism reduces positive facial reactivity to sucrose [23] as well as the sham intake of sucrose solutions [13]. Furthermore, opioid involvement

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in flavor–nutrient learning has been suggested by two recent studies. Mehiel [21] reported that the opioid antagonist naloxone attenuated the acquisition and expression of a preference for a flavor that had been mixed into glucose solutions. Ramirez [25] reported that naloxone attenuated the expression of a flavor acceptance conditioned by IG maltodextrin infusions. These results were taken as evidence that nutrient conditioning enhances the hedonic evaluation of the CS+ flavor by activating the opioid reward system.

The findings that naloxone attenuates flavor–nutrient preference conditioning are open to question, however. Note, in particular, that in Mehiel's acquisition experiment [21], in which rats were trained to drink a glucose solution containing one flavor (CS+) and a saccharin solution containing a different flavor (CS−), half the rats were treated with naloxone only on CS+ trials and half only on CS− trials. In the subsequent flavor preference test, both groups preferred the flavor that was paired with the saline injection over the flavor paired with the naloxone injection. This may have occurred because the drug had a mild aversive effect that was associated with the paired cue flavor. Recently, Yu et al. [30] trained rats to drink flavored sucrose (CS+) and flavored saccharin (CS−) solutions with one group being injected with the opioid antagonist naltrexone on all training trials and a second group being injected with saline on all training trials. In subsequent choice tests, both groups displayed reliable preferences for the CS+ flavor. Furthermore, injecting the rats with naltrexone prior to two-bottle choice tests did not attenuate the expression of the CS+ flavor preference. In the Yu et al. [30] study the rats were trained and tested with an open gastric fistula (sham-feeding procedure), which minimized the postingestive actions of the sucrose. Thus, the US that conditioned the CS+ preference was considered to be the sweet taste of sucrose (flavor–flavor conditioning), rather than the sugar's postingestive nutrient actions. In the Mehiel [21] experiment the rats "real-fed" the glucose solution so that both its sweet taste and postingestive effects may have contributed to the flavor conditioning.

In the present study, flavor–nutrient conditioning was explicitly investigated by pairing the CS+ flavor with an IG infusion of sucrose while the CS− flavor was paired with IG water infusion. Both flavors were presented in saccharin solutions so that the CS+ and CS− solutions were equally sweet and differed only in their cue flavors and postingestive consequences. Naltrexone effects on the *acquisition* and *expression* of flavor–nutrient preferences were measured. Preference expression was examined by training rats to associate the CS+ with the US and then treating them with the drug during the CS+ vs. CS− choice tests. Preference acquisition was investigated by treating separate groups of rats with naltrexone and saline throughout training and then comparing their CS+ preferences in a subsequent choice test.

1. Experiment 1A: effects of naltrexone on the expression of a conditioned flavor–nutrient preference in food-restricted rats

In prior studies investigating opioid antagonist effects on sugar conditioned flavor preferences, the rats consumed the sugar by mouth [22,30]. The present study focused on postingestive nutrient conditioning by training rats with the CS+ flavor paired with IG sugar infusions. Experiment 1 was conducted to determine if the expression of a sucrose-conditioned preference is dependent upon the endogenous opioid system.

1.1. Methods

1.1.1. Subjects

Twelve male Sprague–Dawley rats (375–400 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages maintained on a 12:12 light/dark cycle. Food (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and water were available ad libitum prior to surgery and during recovery.

1.1.2. Surgery

The rats were implanted with IG catheters by a method adapted from Davis and Campbell [7]. The animals were anesthetized with a 10:7 ketamine/xylazine mixture and a silastic catheter (0.04 in. i.d., 0.085 in. o.d.) was inserted into the fundus of the stomach and secured with sutures and polypropylene mesh. The catheter was routed subcutaneously to the head, where it connected to a Luer–Lok assembly that was secured to the skull with stainless steel screws and dental cement.

1.1.3. Apparatus

Testing was conducted in plastic cages ($23 \times 24 \times 31.5$ cm) with steel mesh flooring. Above the cage a counterbalanced lever held an infusion swivel connected, by plastic tubing, at one end to a syringe pump and at the other end to the rat's Luer–Lok assembly. The rats drank from one or two stainless steel drinking spout tubes that were accessible via two holes at the front of the cage. The spouts were attached to bottles fixed in a motorized retractor unit that automatically inserted and removed the spouts at the beginning and the end of a session. Licking was monitored by an electronic drinkometer connected to a microcomputer that activated the syringe pump as the animal drank. The infusion rate was 1.3 ml/min and the oral intake/infusion volume was maintained at approximately 1:1 by computer software.

1.1.4. Test solutions

The CS solutions consisted of 0.2% sodium saccharin (Sigma, St. Louis, MO) solutions flavored with 0.05% cherry or grape Kool-Aid (General Foods, White Plains, NY). The nutrient infusions were 16% w/v sucrose (Path-

mark Brand). Half of the rats received cherry as the CS+ paired with IG sucrose, and grape as the CS − paired with IG water; the flavor-infusion pairs were reversed for the remaining animals.

1.1.5. Procedure

After recovery from surgery, the rats were familiarized with unflavored 0.2% saccharin solution by giving them 24 h access to saccharin as well as water. Three rats with low saccharin intakes were given only saccharin for a second 24-h period. All rats were then food restricted and maintained at 85% of their post-recovery body weight.

The rats were next adapted to drink unflavored saccharin in the test cages during 30 min/day sessions. For the first six sessions, they were not attached to the infusion system; subsequently they were attached but not infused (six sessions) and finally infused with water as they drank the saccharin solution (six sessions). During this adaptation period some rats with low intakes were given a palatable 2% maltodextrin + 0.2% saccharin solution to stimulate drinking. All rats were drinking the 0.2% saccharin prior to the start of formal training.

Formal training consisted of 10 one-bottle training sessions (30 min/day) with the CS+ and the CS − solutions, paired with their appropriate infusions, presented on alternating days. The left–right position of the CS bottles was counterbalanced across days and animals. During the last four training sessions, a second bottle of unflavored water was available along with the CS solutions to familiarize the rats with a choice situation. Drinking from the water bottle was not paired with an infusion. Additionally, the rats were injected subcutaneously with isotonic saline (vehicle; 1 ml/kg body weight) 10 min prior to the start of the session to familiarize them with the injection procedure.

Following training, two-bottle preference tests were conducted with the CS+ and CS − solutions without IG infusions. Ten minutes prior to test sessions, the rats were injected with saline or naltrexone (Sigma) at doses of 1.0, 2.5, and 5.0 mg/kg of body weight. The order of presentation for the 2.5 and 1 mg/kg doses were counterbalanced and the rats received each dose once; all rats received the 5 mg dose at the same time. This dose was tested twice, on two sequential days, after tests with the lower doses. At least one vehicle session preceded each dose level.

1.1.6. Statistical analysis

CS intakes were corrected for spillage and measured to the nearest 0.1 g. Intakes of the CS+ and CS − solutions were averaged over one-bottle training sessions and analyzed with a *t* test. Intakes in two-bottle tests were analyzed using repeated measures analysis of variance (ANOVA), followed by tests of simple main effects and Newman–Keuls post hoc tests, where appropriate. Two-bottle intake data for the 5 mg/kg dose were averaged across the two testing days. The two-bottle data were also expressed as percent CS+ intake (CS+ intake/total intake × 100) and analyzed by ANOVA.

1.2. Results

The rats consumed identical amounts of CS+ and CS − solutions during the one-bottle training sessions (mean ± S.E.M.: 9.6 ± 1.3 and 9.6 ± 1.2, respectively).

The results of the preference tests appear in Fig. 1. Overall, the rats drank significantly more CS+ than CS − solution ($F(1,10) = 9.52$, $P < .05$). Naltrexone treatment reduced intake ($F(3,30) = 11.87$, $P < .0001$) relative to the vehicle treatment, but there were no significant intake differences among the naltrexone doses. There was also no interaction between dose and CS flavor. Percent CS+ intakes did not differ significantly among the four dose levels.

1.3. Discussion

This experiment confirms prior reports that rats learn to prefer a flavor paired with IG carbohydrate infusions over a flavor paired with IG water infusions [26]. The new finding here is that the expression of this preference was not attenuated by naltrexone treatment. The drug did suppress total CS intake, which is consistent with prior work demonstrating that opioid antagonists reduce saccharin intake [1,14,18]. However, this intake reduction did not attenuate the relative preference for CS+ over CS − solutions. These results indicate that a functioning opioid system is not necessary for the expression of a conditioned flavor–nutrient preference in food restricted rats. Experiment 1B inves-

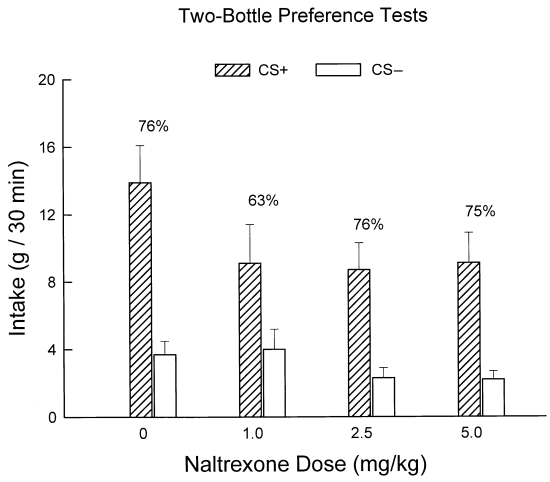

Fig. 1. Intakes (means + S.E.M.) of the CS+ and the CS − solutions during 30 min, two-bottle preference tests with food-deprived rats in Experiment 1A. Ten minutes prior to testing the rats were injected with 0 (vehicle), 1.0, 2.5, or 5.0 mg/kg of naltrexone. The CS solutions were grape- or cherry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS − with IG water infusions during training. The numbers atop the bars represent the percent CS+ intake at that dose.

tigated the effects of naltrexone when the rats were given ad libitum food. This was of interest because previous reports indicate that opioid antagonists are more effective in reducing intake in nondeprived rats than in deprived rats [15,20].

2. Experiment 1B: effects of naltrexone on the expression of a conditioned flavor–nutrient preference in ad libitum-fed rats

2.1. Methods

Ten of the rats from Experiment 1A served as subjects for this experiment. They were first given 6 one-bottle retraining sessions with the CS solutions from Experiment 1A, paired with their appropriate IG infusions as in Experiment 1A. Their food rations were gradually increased and by the fourth day of training food was available ad libitum except during the 30 min/day sessions. Next, the rats were given a series of two-bottle tests with the CS+ vs. CS − solutions. The rats were injected with vehicle (two sessions), naltrexone (2.5 mg/kg, two sessions), vehicle (four sessions), and naltrexone (5.0 mg/kg, two sessions), in that order, 10 min prior to the choice tests.

2.2. Results

As illustrated in Fig. 2, overall the rats drank more CS+ than CS − in two-bottle tests ($F(1,9)=8.14$, $P<.025$). Naltrexone treatment reduced intake ($F(2,18)=37.31$, $P<.0001$) and there was a significant dose × CS interaction ($F(2,18)=6.32$, $P<.01$). Simple main effect tests revealed that naltrexone reduced both CS+ ($P<.0001$) and CS − ($P<.025$) intakes. The rats consumed significantly more of the CS+ than of the CS − when vehicle treated ($P<.01$), but not when injected with naltrexone. However, the percent CS+ intakes did not significantly differ at the three dose levels.

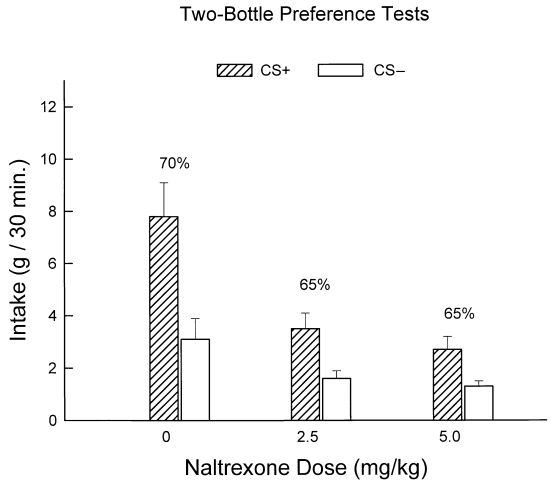

Fig. 2. Intakes (means + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests with food ad libitum animals in Experiment 1B. Ten minutes prior to testing the rats were injected with 0 (vehicle), 2.5, or 5.0 mg/kg of naltrexone. The CS solutions were grape- or cherry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS − with IG water infusions during training. The numbers atop the bars represent the percent CS+ intake at that dose.

2.3. Discussion

The absolute intake data indicate that naltrexone may inhibit the expression of a conditioned flavor preference in nondeprived rats. That is, the rats drank significantly more CS+ than CS − when vehicle treated, but not when treated with naltrexone. The percent CS+ intakes, however, did not differ between vehicle or drug conditions. A "floor effect" may have contributed to the lack of a significant difference in the absolute intakes of the CS+ and CS − after naltrexone treatment. The rats consumed very little (3.1 ml/30 min) of the CS − in the vehicle test, which did not allow for much reduction following naltrexone treatment.

3. Experiment 2A: effects of 0.1 mg/kg naltrexone on the acquisition and expression of a conditioned flavor–nutrient preference in food-restricted rats

The second experiment determined if opioid receptor antagonism during training impaired the acquisition of flavor preference conditioned by IG sucrose. It also provided further information on the effects of naltrexone on the expression of the conditioned flavor preference. The rats were initially trained with a low naltrexone dose (0.1 mg/kg) because pilot work revealed that rats treated with a 1.0 mg/kg dose at the start of training consumed very little of the CS solutions (~2 g/session) and thus had little opportunity to learn the flavor–nutrient association. The rats were subsequently trained with a 1.0 mg/kg dose.

3.1. Methods

3.1.1. Subjects

Twenty-eight male Sprague–Dawley rats (380–410 g) bred in our laboratory from Charles River stock were used. The rats were fitted with gastric catheters as in Experiment 1. Due to problems with their gastric catheters, three rats were removed from the study.

3.1.2. Procedure

Prior to surgery the rats were familiarized with sweet solutions by giving them ad libitum access to a 0.2% saccharin + 2% sucrose solution (2 days), followed by a 2% saccharin + 1% sucrose (2 days) and then a 0.2% saccharin solution (2 days). Food and water were also available. The extended exposure period was used because of the reluctance some rats displayed in Experiment 1 to drink the 0.2% saccharin solution. After recovery from the surgery, the rats were food deprived to 85% of their post-

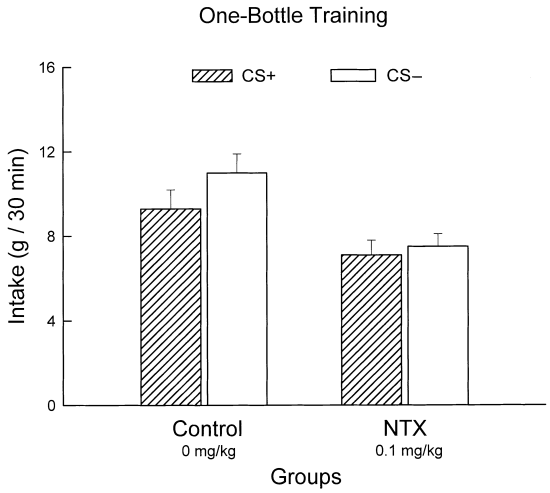

Fig. 3. Intakes (means + S.E.M.) of the CS+ and the CS – during 30 min, one-bottle training sessions with food-deprived animals in Experiment 2A. The NTX group was injected with 0.1 mg/kg naltrexone prior to each training session and the control group was injected with vehicle (0 mg/kg). The CS solutions were grape- or cherry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS – with IG water infusions during training.

recovery body weight. The rats were next adapted to the test cages and training procedure. They were trained to drink unflavored 0.2% saccharin during 30 min/day sessions first without being attached to the infusion system (three sessions), then while attached but not infused (three sessions), and finally while infused with water as they drank saccharin (five sessions). During the last three sessions, the rats were subcutaneously injected with 1.0 ml/kg saline.

The rats were divided into two groups equated for their saccharin intakes. The NTX group ($n = 13$) received 0.1 mg/kg naltrexone 10 min prior to the daily one-bottle training sessions, and the control group ($n = 12$) group received vehicle injections prior to training.

Formal training consisted of 10 one-bottle training sessions with the CS+ and the CS – paired with IG infusions of 16% sucrose and water, respectively. The CS solutions were grape- and cherry-flavored saccharin solutions, as in Experiment 1. Following training, two-bottle preference tests were conducted with the CS+ vs. CS – solutions without IG infusions. During preference testing both groups were treated identically and were given injections of vehicle and, in ascending order, 0.1, 1.0, and 5.0 mg/kg of naltrexone 10 min prior to the two-bottle sessions. Each naltrexone dose was presented for two consecutive sessions, and two vehicle sessions preceded each dose level.

3.2. Results

During one-bottle training the NTX rats drank significantly less of the CS solutions than did the control rats ($F(1,23) = 7.78$, $P < .02$) and there was no group by CS interaction (Fig. 3). Compared to the controls, the NTX rats drank 24% and 32% less, respectively, of the CS+ and CS – solutions. Consequently, these rats were infused with less sucrose than the control rats during the CS+ training sessions. Overall, the rats drank slightly more of the CS – than CS+ solution ($F(1,23) = 12.38$, $P < .01$).

As illustrated in Fig. 4, the rats in both groups consumed more CS+ than CS – in the two-bottle tests ($F(1,23) = 84.1$, $P < .0001$). There was also an overall effect of naltrexone dose ($F(3,69) = 63.74$, $P < .0001$) indicating that, in both groups, total intakes during the 0.1, 1.0, and 5.0 mg/kg tests were less than during the vehicle test. Most importantly, however, there were no group differences or significant interactions between any of the variables. Percent CS+ intakes were very similar for the NTX and control groups, and were not reduced by naltrexone treatment.

3.3. Discussion

These data demonstrate that treating rats with naltrexone at 0.1 mg/kg during training did not attenuate flavor

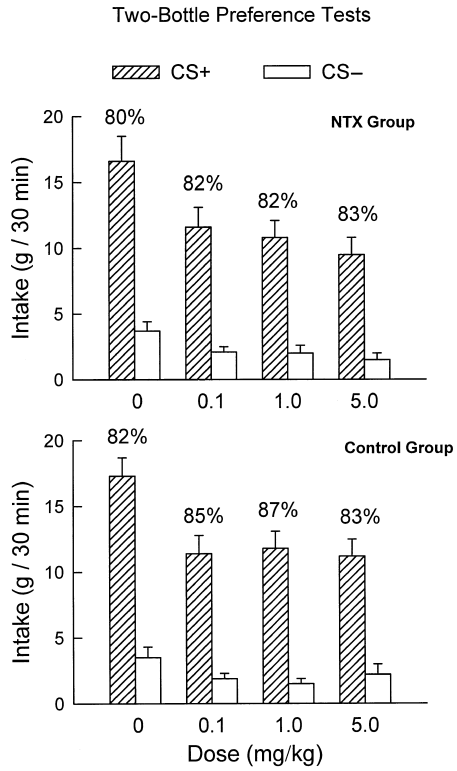

Fig. 4. Intakes (means + S.E.M.) of the CS+ and the CS – during 30 min, two-bottle preference tests with food-deprived rats in Experiment 2A. Ten minutes prior to testing the rats were injected with 0 (vehicle), 0.1, 1.0, or 5.0 mg/kg of naltrexone. The CS solutions were grape- or cherry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS – with IG water infusions during training. The top panel represents the data for the NTX group that was injected with naltrexone (0.1 mg/kg) during one-bottle training, and the bottom represents the control group injected with vehicle during training. The numbers atop the bars represent the percent CS+ intake at that dose.

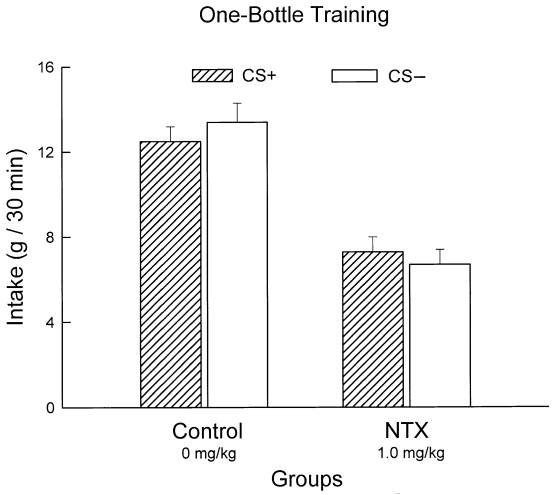

Fig. 5. Intakes (means + S.E.M.) of the CS+ and the CS – during 30 min, one-bottle training sessions with food-deprived animals in Experiment 2B. The NTX group was injected with 1.0 mg/kg naltrexone prior to each training session and the control group was injected with vehicle (0 mg/kg). The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS – with IG water infusions during training.

preference conditioning by IG sucrose infusions. The NTX rats acquired a CS+ preference similar to that of the control rats despite drinking less CS+ and being infused with less sucrose during training. The results obtained with the control group provide a replication of Experiment 1A, in that these rats were treated with naltrexone only during preference testing. As in the first experiment, naltrexone treatment prior to the two-bottle tests failed to attenuate the expression of the CS+ preference.

To determine if flavor–nutrient preference conditioning would be impaired if a higher naltrexone dose was used, the rats were retrained in Experiment 2B using 1.0 mg/kg naltrexone and new CS flavors.

4. Experiment 2B: effects of 1.0 mg/kg naltrexone on the acquisition and expression of a conditioned flavor–nutrient preference in food-restricted rats

The rats were redistributed into two new NTX and control groups. The new NTX group contained six rats from the former NTX group and seven rats from the former control group. The new control group contained six rats from the former NTX and six rats from the former control groups. The rats in these new groups were equated for their CS+ preferences and total intakes during the two-bottle tests of Experiment 2A.

The rats were trained as in Experiment 2A except that the CS solutions contained 0.2% saccharin flavored with orange and strawberry (Kool-Aid flavors), and the NTX group was treated with 1.0 mg/kg naltrexone throughout one-bottle training.

Following training, two-bottle preference tests were conducted with the CS+ and CS – solutions. The NTX and Control groups were treated identically. They were injected with vehicle prior to the first two sessions, 1.0 mg/kg naltrexone prior to the next two sessions, and vehicle prior to the last two sessions. Higher drug doses were not tested because no significant dose effect was obtained in Experiment 2A.

4.1. Results

During one-bottle training, the NTX rats drank significantly less of the CS solutions than did the control rats ($F(1,23) = 37.09$, $P < .0001$; Fig. 5). Compared to the controls, the NTX rats drank 41% and 51% less, respectively, of the CS+ and CS – solutions. Consequently, the NTX rats were infused with substantially less sucrose than were the Control rats during the CS+ training sessions. There were no differences between CS+ and CS – intakes within the two groups, and no interaction between group and CS intakes.

As illustrated in Fig. 6, the rats in both groups consumed more CS+ than CS – solution during the two-bottle

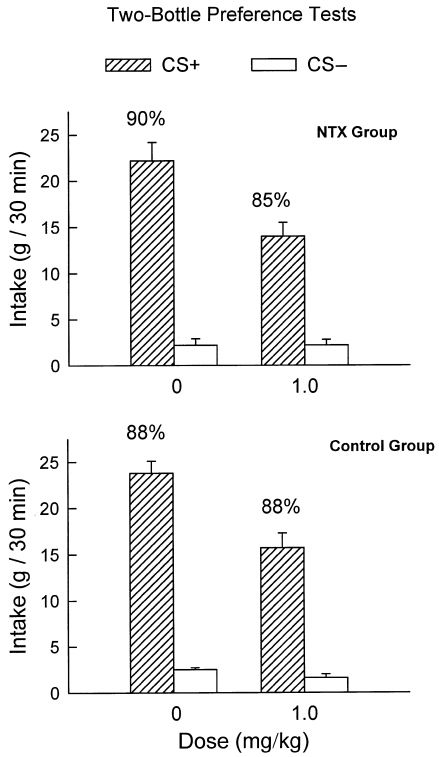

Fig. 6. Intakes (means + S.E.M.) of the CS+ and the CS – during 30 min, two-bottle preference tests with food-deprived rats in Experiment 2B. Ten minutes prior to testing the rats were injected with 0 (vehicle), or 1.0 mg/kg of naltrexone. The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS – with IG water infusions during training. The top panel represents the data for the NTX group that was injected with naltrexone (1.0 mg/kg) during one-bottle training, and the bottom represents the control group injected with vehicle during training. The numbers atop the bars represent the percent CS+ intake at that dose.

tests ($F(1,23)=132.8$, $P<.0001$). Naltrexone administered before testing significantly reduced total CS intake ($F(1,23)=130.7$, $P<.0001$). There was no group effect, nor were there significant interactions between group and CS or group and naltrexone dose. However, there was an interaction between dose and CS ($F(1,23)=38.2$, $P<.0001$), and further analysis revealed that naltrexone significantly ($P<.01$) reduced CS+ intake but not CS − intake in both groups. Nevertheless, CS+ intake exceeded ($P<.01$) CS − solution intake following both vehicle and naltrexone injections. The percent CS+ intakes of the two groups were very similar and were not altered by naltrexone treatment.

4.2. Discussion

These data extend the results of Experiment 2A and show that naltrexone at 1.0 mg/kg administered during training did not attenuate flavor preference conditioning by IG sucrose infusions. The strong CS+ preference displayed by the NTX rats is particularly impressive given that their CS+ intakes and sucrose infusions were 40% less than that of the control rats during training. As in Experiments 1A and 2A, when treated with naltrexone prior to the two-bottle tests they continued to consume more CS+ than CS −, and the percent CS+ intakes were not reduced relative to the saline test. However, naltrexone did selectively reduce CS+ intake without affecting CS − intake. These data are difficult to interpret because of a possible floor effect on CS − intakes. Even in the saline tests, CS − intakes were quite low.

Experiment 2C further examined the influence of naltrexone on the expression of the conditioned flavor preference. Following the rationale of Experiment 1B, the rats were tested while in a nondeprived state.

5. Experiment 2C: effects of naltrexone on the expression of a conditioned flavor–nutrient preference in ad libitum-fed rats

Twenty-four of the animals from Experiment 2B (NTX group $n=12$, control group $n=12$) were used; food was available ad libitum except during the 30 min/day sessions. The animals were given 4 one-bottle retraining sessions with the CS+ and CS − solutions used in Experiment 2B, paired with their appropriate infusions. In this retraining, all animals received saline injections, as naltrexone during training did not affect flavor preference learning in Experiment 2B.

Following training, two-bottle preference tests were conducted with the CS+ and CS − solution, as in Experiment 2B, following treatment with vehicle and 1.0 mg/kg naltrexone.

5.1. Results

Due to their differing drug history, the data from the NTX and control groups were analyzed separately although the rats were treated identically in this experiment. As illustrated in Fig. 7, both the NTX and control rats consumed more CS+ than CS − solution ($F(1,22)=54.4$, $P<.0001$) and there was no group effect or group interaction with dose or CS. Naltrexone treatment reduced CS intake ($F(1,22)=51.5$, $P<.0001$) and there was a significant dose × CS interaction ($F(1,22)=25.3$, $P<.0001$). Further analysis indicated that naltrexone reduced ($P<.01$) CS+ intake, but not CS − in both groups. However, CS+ intake exceeded ($P<.05$) CS − intake at both the 0 and 1.0 mg/kg doses, and there was no drug effect on percent CS+ intakes.

5.2. Discussion

These results are similar to those obtained in Experiment 1B and 2B in that naltrexone treatment produced a greater decrease in CS+ solution intake than CS − solution intake during two-bottle testing. However, unlike Experiment 1B, the ad libitum-fed rats in both groups drank significantly more CS+ than CS − even when drug treated. This residual CS+ preference may have occurred in the present experiment, but not in Experiment 1B, because of the greater CS+

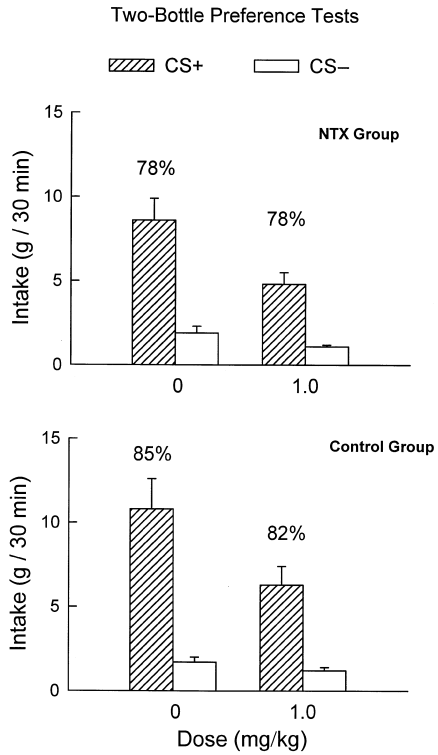

Fig. 7. Intakes (means + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests with ad libitum fed rats in Experiment 2C. Ten minutes prior to testing the rats were injected with 0 (vehicle), or 1.0 mg/kg of naltrexone. The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was paired with IG sucrose and the CS − with IG water infusions during training. The top panel represents the data for the NTX group that was treated with naltrexone (1.0 mg/kg) during one-bottle training, and the bottom represents the control group treated with vehicle during training. The numbers atop the bars represent the percent CS+ intake at that dose.

preference in the present experiment. It is also the case that higher drug doses were used in Experiment 1B, but this does not readily explain the different results because no dose effects were observed in any of the experiments of this series. Note that in both experiments naltrexone did not significantly decrease the percent CS+ intakes. As in previous experiments, the selective reduction in CS+ intake produced by naltrexone is difficult to interpret because of a possible "floor effect" on CS − intakes.

6. Experiment 3: effects of naltrexone on conditioned flavor acceptance

In addition to conditioning an increase in the intake of a CS+ solution relative to a CS − solution, which is measured in two-bottle tests, IG nutrient infusions may also condition an increase in the absolute intake of the CS+ solution intake, which is measured in separate one-bottle tests with the CS+ and CS − solutions. Increased acceptance is more difficult to obtain, however, in part because the satiating action of nutrient infusions counteract their intake stimulating effect [25]. Furthermore, conditioned flavor acceptance appears to extinguish more rapidly than conditioned flavor preference suggesting that different neurobehavioral mechanisms may mediate these conditioned responses [9,24]. While Experiments 1 and 2 provide little evidence for opioid involvement in nutrient-conditioned flavor preferences, a recent report by Ramirez [25] implicates the opioid system in the mediation of conditioned flavor acceptance. Using a between group design, Ramirez reported that rats drinking a saccharin solution paired with IG infusions of a dilute carbohydrate solution (6% maltodextrin) consumed more solution than did rats drinking a saccharin solution paired with IG water. Following training, naloxone (0.1 or 0.3 mg/kg) decreased solution intake more in rats drinking saccharin + IG carbohydrate than in rats drinking saccharin + IG water. In view of these results, the present experiment investigated whether naltrexone reduces the conditioned acceptance of a CS+ solution in one-bottle intake tests. To maintain comparability with Experiments 1 and 2, a within-group design was employed using the same CS flavors as in Experiments 2B and 2C. As in the Ramirez study [25], the CS+ was paired with IG infusions of dilute carbohydrate (6% maltodextrin) and dilute saccharin solutions (0.05%) were used. The rats were initially trained 20 h/day with the CS flavors because we observed that this is a particularly effective way of conditioning increased flavor acceptance [24]. For drug testing, 30 min/day sessions were conducted with the animals minimally (~ 95%) food-deprived.

6.1. Methods

6.1.1. Subjects

Twelve male Sprague–Dawley rats (331–357 g; Charles River Laboratories) started the experiment although one rat was excluded due to problems with its gastric catheter. These rats were used in a previous acceptance study that did not involve drug treatments, and used different CS flavors and carbohydrate infusions.

6.1.2. Apparatus

The rats were tested in plastic cages described in Experiment 1 except that peristaltic pumps replaced the syringe pumps to accommodate the larger infusion volumes required for the 20 h/day sessions. The pump rate remained at 1.3 ml/min.

6.1.3. Test solutions

The CS solutions consisted of 0.05% saccharin solutions flavored with 0.05% orange and strawberry Kool-Aid. The nutrient infusion was a 6% w/v maltodextrin solution (Maltrin M500, Grain Processing, Muscatine, IA). For half the rats, orange was the CS+ solution paired with IG maltodextrin, and strawberry was the CS − solution paired with IG water; flavor–nutrient pairs were reversed for the remaining rats.

6.1.4. Procedure

At the start of the experiment the rats were housed in the training cages and adapted to a feeding schedule in which lab chow and water were available for 2 h each day, followed by 2 h of no food or fluid, and then 20 h access to fluid only (which included the 12-h dark period). Initially, water paired with IG water was available during the 20-h access period. The rats were then given alternating one-bottle access (20 h/day) to the CS+ solution paired with IG maltodextrin infusions and the CS − solution paired with IG water for a total of 8 days. This was followed by a two-bottle test with the CS+ vs. CS − solutions for two 20-h/day sessions. During this test, intake of the CS+ solution was paired with IG maltodextrin; CS − intake, which was expected to be very low, was not paired with infusions because of apparatus limitations. The rats were next given one-bottle access to the CS solutions, each paired with their appropriate infusions, during alternating 30 min/day sessions. One hour after the daily 30-min sessions, water was provided ad libitum and a food ration was given that maintained the rats at approximately 95% of their free-feeding body weight.

After adapting to the 30 min/sessions for 4 days, drug testing began. During these one-bottle tests, intake of the CS+ and CS − solutions remained paired with their respective infusions, and the order of presentation was counterbalanced so that on a given day half of the rats drank the CS+ solution while half drank the CS − solution. The rats were injected with vehicle and, in ascending order, 0.1, 1.0, and 2.5 mg/kg naltrexone, 10 min prior to the daily sessions. Each drug dose was tested for two consecutive sessions (i.e., one CS+ session and one CS − session) and at least two vehicle tests separated each pair of drug tests.

6.2. Results

Over the course of the 20-h one-bottle training sessions, the rats substantially increased their intake of the CS+ solution relative to the CS − solution. During the last 4 training days, the rats consumed 129.4 ± 12.2 and 44.6 ± 5.1 g of the CS+ and CS − solutions, respectively ($t(10) = 7.36$, $P < .0001$). In the 20-h two-bottle test they drank substantially more CS+ than CS − solutions (95.8 ± 8.2 vs. 1.5 ± .1, $t(10) = 11.6$, $P < .001$). The rats continued to drink more CS+ (12.7 ± 0.9 g) than CS − solution (6.9 ± 0.4 g) during the first four 30-min/day one-bottle sessions ($t(10) = 5.37$, $P < .001$).

A preliminary analysis of the test data revealed that intakes during the vehicle test sessions preceding the 2.5 mg/kg naltrexone test were higher than those in the other vehicle tests. Therefore, the 2.5 mg/kg naltrexone data were analyzed separately from the 0.1 and 1.0 mg/kg data.

Analysis of the one-bottle intakes from the vehicle and the 0.1 and 1.0 naltrexone mg/kg tests revealed a significant flavor acceptance effect with the animals drinking more CS+ than CS − solution ($F(1,10) = 43.29$, $P < .001$; Fig. 8). The drug effect was significant and CS solution intakes were reduced in the 0.1 and 1.0 mg/kg tests compared to the vehicle test ($F(2,20) = 25.83$, $P < .001$). There was also a significant drug × CS interaction that indicated that naltrexone reduced CS+ intake more than CS − intake ($F(2,20) = 5.32$, $P < .05$). However, simple main effect tests revealed that intakes of both CS solutions were reduced ($P < .05$) by the 0.1 and 1.0 mg/kg doses, and at both doses the rats consumed more ($P < .05$) CS+ than CS − . When expressed as a percentage of the vehicle test intakes, CS+ and CS − intakes at the 0.1 mg/kg dose were 69% and 65% of vehicle baseline, and at the 1.0 mg/kg dose were 51% and 68% of baseline, respectively; these differences were not significant.

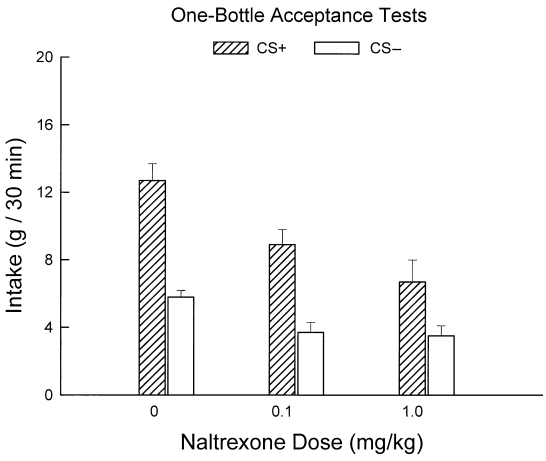

Fig. 8. Intakes (means + S.E.M.) of the CS+ and the CS − during 30 min, one-bottle acceptance tests with food-restricted rats in Experiment 3. Ten minutes prior to testing the rats were injected with 0 (vehicle), 0.1 or 1.0 mg/kg of naltrexone. The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was paired with IG maltodextrin and the CS − with IG water infusions throughout training and testing.

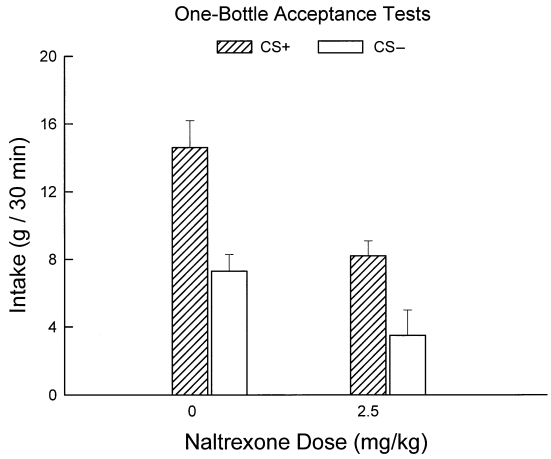

Fig. 9. Intakes (means + S.E.M.) of the CS+ and the CS − during 30 min, one-bottle acceptance tests with food-restricted rats in Experiment 3. Ten minutes prior to testing the rats were injected with 0 (vehicle), or 5.0 mg/kg of naltrexone. The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was paired with IG maltodextrin and the CS − with IG water infusion throughout training and testing.

The rats drank more CS+ than CS − solution in the vehicle and 2.5 mg/kg naltrexone tests ($F(1,10) = 49.75$, $P < .001$; Fig. 9). Naltrexone reduced overall CS solution intake ($F(1,10) = 36.05$, $P < .001$) and there was a significant drug × CS interaction ($F(1,10) = 6.17$, $P < .05$) although individual tests revealed that the drug reduced ($P < .01$) the intake of both CS+ and CS − solutions. Also, when expressed as a percentage of vehicle test intakes, the intakes of the CS+ and CS − solutions were similar at 59% and 55% of baseline. Finally, the rats drank more ($P < .05$) CS+ than CS − solution during both the vehicle and 2.5 mg/kg naltrexone tests.

6.3. Discussion

Confirming previous results [24], the rats consumed substantially more of the CS+ solution paired with IG carbohydrate infusions than of the CS − flavor paired with IG water during one- and two-bottle 20-h/day tests. They continued to overconsume the CS+, relative to the CS − , during the subsequent 30-min/day one-bottle tests. Evidence that this overconsumption represents a conditioned increase in the acceptability of the CS+ flavor, rather than a direct response to the nutrient infusions, is provided by prior data showing that CS+ intakes remain elevated during initial extinction tests when water rather than nutrient is infused [24,25].

Naltrexone treatment reduced the intakes of both CSs during the one-bottle tests, although CS+ intake was suppressed more than CS − intake as indicated by the significant dose × CS interaction. This partially replicates the finding of Ramirez [25] that naloxone decreased solution

intake more in rats drinking a saccharin solution paired with IG carbohydrate than in rats drinking a saccharin solution paired with IG water. Although Ramirez used unflavored saccharin solutions and a between group design, his maltodextrin- and water-paired saccharin solutions can be considered to be a "CS+" and "CS−" comparable to the CS solutions in the present experiment. The findings of the two experiments differ in that Ramirez reported that the lowest drug dose (0.1 mg/kg) decreased only "CS+" intake, but in the present experiment the 0.1 mg/kg decreased the intake of both the CS+ and CS− solutions. Furthermore, naltrexone did not suppress CS+ intake more than CS− intake when the data are expressed as a percent of the vehicle baseline intakes.

There are many differences between the present experiment and the Ramirez study that may account for the discrepant results. Of particular note, the vehicle baseline intakes of the "CS−" solution were lower in the Ramirez study than in the present study ($\sim$4 vs. $\sim$6.5 ml/30 min), which could explain why he observed a more specific drug effect on "CS+" intake. Ramirez [25] rejected a "floor effect" interpretation because he found that the dopamine antagonist pimozide suppressed "CS−" intake. However, opioid antagonists, unlike dopamine antagonists, typically do not suppress licking rates during the first several minutes of a drinking bout ([13,29]; but see Ref. [12]). Therefore, if baseline bout size is low, rats may stop drinking before the drug's intake-reducing actions are expressed.

7. General discussion

The present findings confirm prior reports that rats learn to prefer flavors paired with IG carbohydrate infusions and that opioid antagonists suppress the intake of sweet solutions. The new findings are that naltrexone treatment did not block the acquisition of a sucrose-conditioned flavor preference, and had minimal detectable effects on the expression of a learned flavor preference and acceptance.

In the five different two-bottle tests conducted in Experiments 1 and 2, naltrexone consistently suppressed total CS intakes but did not reduce percent CS+ intakes. In three of these tests, the drug suppressed CS+ intake more than CS− intake, but in only one case did the rats fail to consume more CS+ than CS− following drug treatment (Experiment 2B). This may have been due to a "floor effect"; however, as CS intakes in this test were lower than in the remaining four tests. Overall, these data indicate that a fully functioning opioid system is not critical for the expression of a flavor preference conditioned by IG carbohydrate infusions. Nevertheless, the drug × CS interaction observed in several of the experiments indicates that a role for the opioid system in conditioned flavors preferences cannot be ruled out. An inherent difficulty in evaluating this issue is that low CS− intakes during two-bottle tests make it difficult to observe nonselective decreases in CS intakes. As discussed below, theoretical considerations also preclude eliminating opioid involvement in the expression of learned flavor preferences.

In contrast to the present findings, several studies have reported that opioid antagonists suppress the preference for saccharin and sugar solutions that might suggest that different neurochemical systems mediate learned and unlearned flavor preferences. However, there are important methodological differences between these studies that limit comparisons. Note in particular that some of the data cited as evidence that naloxone reduces saccharin preference actually show decreased saccharin acceptance rather than decreased preference per se [18–20]. That is, although the nondeprived rats in these studies were offered the choice between saccharin and water, their water intakes were virtually nil and were not reported. More compelling evidence for naloxone-induced reduction in saccharin preference comes from studies of water-deprived rats given saccharin vs. water tests in which water consumption was measurable. In these experiments, naloxone reduced saccharin intake and water intake remained unchanged or even increased [3,14,28]. This outcome may be related to the fact that the rats were motivated by thirst to drink water and by taste to drink saccharin, and opioid antagonists are most effective in suppressing taste-motivated drinking [27]. Note that water restriction reduces the expression of a learned preference for a carbohydrate-paired CS+ flavor over a water-paired CS− flavor [9]. Thus, it may be inappropriate to use water-restricted rats to evaluate drug effects on nutrient-conditioned flavor preferences.

Opioid antagonists have also been found to alter preferences for solid foods in food-restricted rats. In particular, two studies observed that naloxone (0.3–3 mg/kg) or naltrexone (0.1–5 mg/kg) reduced the intake of a preferred food while the intake of the less preferred food remained the same or even increased [6,11]. The intake and preference reductions observed in these experiments were more pronounced than those observed in the present study. This may be due to differences in test substances (solid foods versus flavored saccharin solutions) and/or deprivation conditions (overnight food deprivation versus chronic food restriction). In addition, the choice foods used in the prior experiments, high-fat and high-carbohydrate semisynthetic diets [11] or chocolate cookie and lab chow [6], differed in flavor, nutrient composition, and caloric density, whereas the CS solutions used in the choice tests of the present study differed only in their cue flavor and training history. Another potentially important difference is that only nutritive choice items were used in the prior experiments whereas the CS solutions used in the present study were paired with nutritive and nonnutritive infusions. It may be that the all-or none nature of the nutrient reinforcement used in the present study, and the strong preferences it produced, obscured more subtle effects of opioid antagonism on flavor preferences. This possibility can be addressed by training rats with two CS+ solutions paired with different nutrient concentrations (e.g., 8% maltodextrin and 16% maltodex-

trin), which condition more moderate flavor preferences (i.e., CS+ 16% preferred to CS+ 8%) [16]. Another approach is to pair the CS+ solutions with different nutrients (e.g., isocaloric carbohydrate and fat infusions) that also condition moderate flavor preferences (i.e., CS+ carbohydrate preferred to CS+ fat) [17]. The use of different nutrients is also of interest in view of reports of nutrient-specific effects obtained with opioid antagonists and agonists [10].

In Experiment 3, drug effects on carbohydrate-conditioned flavor acceptance were investigated using one-bottle tests and the data were similar to the conditioned preference results of the first two experiments. Naltrexone decreased the absolute but not percent intake of the CS+ relative to the CS−, and the rats continued to consume more CS+ than CS− in the one-bottle tests. As previously noted, these results differ somewhat from those reported by Ramirez [25], but different vehicle baseline intakes may account for the discrepancy.

The minimal effects of naltrexone on the expression of a CS+ preference and acceptance in this study does not necessarily argue against the hypothesis that flavor–nutrient learning involves an opioid-mediated shift in hedonic evaluation [21,25]. It is conceivable, for example, that nutrient conditioning enhances the CS+ preference and acceptance in a way analogous to increasing the sweetness of the CS+ solution (recall that both the CS+ and CS− solutions contained saccharin). Naltrexone may attenuate the hedonic response to both CS solutions such that the relative difference between the CS+ and CS− remain about the same and the rat therefore continues to drink more CS+ than CS−. As a simple test of this idea, we determined the effects of naltrexone on the preference rats display for a 0.2% saccharin solution over a slightly less sweet 0.15% solution (Azzara and Sclafani, unpublished findings). Naltrexone (1.0, 2.5, and 5.0 mg/kg) significantly reduced 0.2% saccharin intake without reducing the already low intake of 0.15% saccharin, but the rats continued to consume more 0.2% saccharin than 0.15% saccharin in the two-bottle tests. Furthermore, the percentage of total intake consumed as 0.2% saccharin was not significantly reduced by the drug; percent intakes were 86% in the vehicle test, and 74% to 85% in the drug tests. These findings mirror the present results obtained with the CS+ and CS− solutions.

While the naltrexone expression results are not incompatible with an opioid mediation hypothesis, the acquisition data challenge the idea that the opioid system is critically involved in flavor preference learning. In Experiments 2A and 2B treating rats with naltrexone prior to the one-bottle training sessions had no effect on the magnitude of the CS+ preference they displayed in subsequent two-bottle tests. Furthermore, the NTX group responded like the control group to naltrexone injections during the two-bottle tests. The failure of naltrexone treatment during training to reduce subsequent CS+ preference is particularly noteworthy because the drug reduced the rats' exposure to the CS and US during training. These results indicate that the ability of IG carbohydrate infusions to condition a CS+ flavor preference is not mediated by opioid receptor activity. Although Mehiel [21] hypothesized that opioid activity is involved in carbohydrate conditioned flavor preferences, his results are difficult to interpret because the animals were treated with naloxone only on CS+ or CS− training sessions. Note that Mehiel also proposed an opioid mediation of ethanol-conditioned flavor preferences. The present data are based on carbohydrate conditioning only and thus it remains possible that the opioid system has an important role in the conditioning effects of other nutrients including ethanol.

In apparent contrast with the present results, Lynch [18] reported that naloxone blocks the normal acquisition of a saccharin preference in rats. Lynch [18] observed that daily naloxone injections prevented the gradual increase in saccharin intake displayed by saline treated rats. Although his rats had access to both saccharin and water, water intakes were not reported because the nondeprived rats drank virtually no water. Furthermore, saccharin vs. water preference was not measured following the end of drug treatment. In a subsequent experiment, Lynch and Burns [19] observed that daily naloxone injections almost completely inhibited sucrose and saccharin intake over 10 training sessions, but when subsequently tested without the drug sucrose and saccharin intake rapidly increased. In fact, the naloxone treatment appeared to stimulate subsequent sucrose intake. Water was available during these tests but intakes were not reported because they were so low. Lynch's data show that naltrexone suppressed the acceptability of the saccharin and sucrose solutions during drug treatment, but did block the preference for these solutions in subsequent drug-free solution vs. water tests. These results are not much different from the present findings: naltrexone treatment during one-bottle training limited the intake of flavored saccharin solutions, but did not suppress CS+ intake or preference in the two-bottle vehicle tests (Experiment 2).

In view of the extensive evidence linking the opioid system to affective aspects of reward, the failure of naltrexone to influence the acquisition of sucrose-conditioned flavor preferences in the present study suggests that this type of conditioning may not involve a shift in the hedonic evaluation of the flavor. Berridge [2] has recently proposed that food reward can be subdivided into "wanting," which is related to incentive motivation, and "liking," which corresponds with hedonic evaluation and palatability. He further argues that the opioid system is primarily involved in the liking component of reward, while the dopamine system is the primary mediator of the wanting component of reward. The minimal effects obtained in the present study with opioid antagonists suggest that flavor–nutrient learning may involve a change in dopamine-mediated incentive motivation ("wanting"). This is an interesting possibility that is under investigation. It should be noted, though, that the neuropharmacology of food reward is not fully understood, so that behavioral

characterizations of flavor preference conditioning based on the effects of drugs remain tentative.

The present results complement our recent observations that naltrexone has minimal effects on flavor–flavor preference conditioning by the sweet taste of sucrose [30]. In our prior study rats were trained to sham-drink flavored sucrose and saccharin solutions which drained out their gastric fistula. Naltrexone treatment during training or prior to choice testing did not block the acquisition or expression of a preference for the sucrose-paired flavor. In a third study, however, we observed a drug effect on sucrose-reinforced place preference conditioning [8]. In that study, food-restricted rats trained to associate one test chamber with sucrose and a second chamber with water displayed a preference for the sucrose-paired chamber in subsequent choice tests. Naltrexone injected prior to choice testing significantly reduced this place preference. Interestingly, drug treatment during training did not block or attenuate the acquisition of this place preference. Thus, at least one aspect of sucrose reinforcement appears to require the activation of opioid receptors.

In summary, the present experiments demonstrated that the opioid antagonist naltrexone did not suppress the acquisition of flavor preferences conditioned by IG carbohydrate infusions, and had minimal effects on the expression of carbohydrate-conditioned flavor preference and acceptance. Nevertheless, naltrexone reduced the total intakes of the saccharin-sweetened CS solutions, which confirms prior findings obtained with unflavored saccharin and sugar solutions. These findings indicate that opioid activity modulates the consumption of palatable flavors but does not specifically mediate carbohydrate-based flavor preference learning.

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