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Pharmacology of Flavor Preference Conditioning in Sham-Feeding Rats: Effects of Naltrexone

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YU, W.-Z., A. SCLAFANI, A. R. DELAMATER AND R. J. BODNAR. *Pharmacology of flavor preference conditioning in sham-feeding rats: Effects of naltrexone*. PHARMACOL BIOCHEM BEHAV **63**(4) 573–584, 1999.—Relatively little is known about the neurochemical and pharmacological mechanisms involved in flavor preference learning. The present study examined the ability of the opioid antagonist, naltrexone to alter the acquisition and expression of flavor preferences conditioned by the sweet taste of sucrose. This was accomplished by adding a novel flavor (the CS+) to a sucrose solution, and a different flavor (the CS−) to a less-preferred saccharin solution. Rats were trained to drink these solutions with an open gastric fistula (sham-feeding), which minimized postingestive actions. Food-restricted (Experiments 1 and 2A) and ad lib-fed (Experiment 2B) rats were given either limited (Experiment 1) or unlimited (Experiment 2) access to the CS+ and CS− solutions during one-bottle training. Preferences were assessed in two-bottle tests (with the CS+ and CS− flavors presented in mixed sucrose–saccharin solutions) following vehicle or naltrexone (0.1–10 mg/kg, SC) treatment. The rats displayed significant CS+ preferences following vehicle, particularly after unlimited access training. In four of five experiments, naltrexone significantly reduced total intakes during the two-bottle, sham-feeding tests. Except for one instance, however, the drug failed to block the preference for the CS+ flavor over the CS− flavor. The effects of naltrexone (0.1 mg/kg) on the acquisition of flavor preferences were studied in sham-feeding rats under limited (Experiment 3A) and unlimited (Experiment 3B) training access conditions. Rats treated with naltrexone during training displayed similar CS+ preferences as did saline-treated rats, even though they consumed less CS+ during training. The naltrexone-trained rats also displayed smaller reductions in total or CS+ intakes than did saline-trained rats when all rats were treated with a 2.5 mg/kg dose of naltrexone during testing. As in previous studies, these results show that naltrexone significantly reduces the intake of sweet solutions, yet it has little or no effect on the acquisition or expression of flavor preferences conditioned by sucrose in sham-feeding rats. © 1999 Elsevier Science Inc.

Conditioned flavor preference Sham-feeding preparation Naltrexone Opioids Acquisition studies Expression studies

BEHAVIORAL studies demonstrate that learning plays a major role in food preferences, although innate taste biases, particularly for sweet taste, are an important factor [see review, (34)]. The most common procedure used in the study of acquired food preferences in animals is the conditioned flavor-preference paradigm. In one version of this paradigm an arbitrary flavor (the conditioned stimulus or CS+) is paired with a nutritive source (e.g., sugar solution), and a second flavor (the CS−) is paired with a nonnutritive source (e.g., saccharin solution) during one-bottle training sessions. Preference learning is then assessed in a two-choice test with the two flavors presented in a common base (e.g., water or a sugar–saccharin mixture) to ensure that any differential intake can be attributed to a learned response to the two cue flavors. A variety of nutrients are effective as unconditioned stimuli in flavor-preference learning including glucose, sucrose, maltodextrin, corn oil, and ethanol [see review; (34)]. With some nutrients, the palatable flavor of the nutrient (e.g.,

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sweet taste of sugar) as well as the postingestive actions of the nutrients may serve as unconditioned stimuli. That a palatable flavor alone is sufficient to condition flavor preferences (flavor–flavor conditioning) is demonstrated by studies in which a CS+ flavor is mixed into a preferred saccharin solution and a CS− flavor is mixed in a less preferred saccharin solution or plain water (12,13). Other studies document that postingestive nutrient actions are sufficient to condition flavor preferences (flavor–nutrient conditioning) by pairing the CS+ flavor with intragastric (IG) nutrient infusions (34). Some data suggest that different processes may mediate these two types of flavor conditioning (12,28). For example, whereas flavor–nutrient conditioning is possible with delays between the CS and US of several minutes or more, the US flavor must be closely associated with the CS flavor for flavor–flavor conditioning (11,12,35).

Although conditioned flavor preferences have been characterized in some detail on a behavioral level, relatively little is known about the neurochemical and pharmacological mechanisms involved in flavor preference learning. The endogenous opioid system has emerged as one potential candidate to mediate conditioned flavor preferences. Mehiel (27) reported that the opioid antagonist, naloxone (4 mg/kg) disrupted both the expression and acquisition of a preference for a CS+ flavor added to a nutrient solution of either glucose or ethanol. Ramirez (31) found that naloxone (0.1–0.3 mg/kg) interfered with increased flavor acceptance conditioned by intragastric carbohydrate infusions. O'Hare ad co-workers (29) found that naloxone failed to affect an operant discrimination task in which different concentrations of sucrose were used to gain food, indicating that naloxone's effects upon sucrose were not affecting taste. Finally, Shide and Blass (39) found that naloxone (0.25 mg/kg) interfered with odor preferences conditioned by intraoral sucrose or corn oil infusions in rat pups. These findings are consistent with a much larger literature documenting the ability of general opioid receptor antagonists to reduce the intake of palatable foods and fluids [e.g., (9,20,25,26,36,40)].

Antidipsogenic effects of general opioid antagonists are more potent in inhibiting sucrose and saccharin intake relative to water intake (23). In particular, opioid antagonists appear to reduce the hedonic qualities of the sweet substances because they: (a) are more potent in inhibiting sucrose and saccharin intake than water intake (23), (b) block that portion of feeding driven by sweet taste during food restriction (24), (c) reduce positive hedonic properties of sucrose in a taste–reactivity paradigm (30), and (d) reduce sucrose intake in sham-fed rats (14,17,18,32). Indeed, sucrose intake is reduced in sham-fed rats in a manner that is behaviorally equivalent to the reduction of palatability obtained by diluting the test solution (18). Also, naloxone's effects can be reversed by increasing sucrose concentration within a sham-feeding test (18). Selective μ and κ, but not δ, opioid receptor subtype antagonists significantly reduced sucrose intake in sham-fed rats (22) to the same degree and with similar potencies as real-fed rats (4). Because both sham-fed and real-fed rats display identical magnitudes and potencies of inhibition, these data suggest that central μ and κ antagonists act on orosensory mechanisms supporting sucrose intake.

The present study further evaluated the role of the endogenous opioid system in food preference learning. Specifically, three experiments examined whether naltrexone affected the acquisition and expression of flavor preferences conditioned by the sweet taste of sucrose. Although Mehiel (27) reported that conditioned preferences for sugar are attenuated by opioid antagonists, his study did not distinguish between the taste and the postingestive reinforcing actions of sugar. Given the many findings suggesting that opioid antagonists reduce the palatability of sweet solutions, they may also reduce the flavor preference conditioning effects of sweet taste. To test this hypothesis, the rats in the present study were trained to sham-feed flavored sucrose and saccharin solutions. In the sham-feeding procedure, ingested fluid drains out an open gastric fistula, and thus the postingestive actions of the solution are minimized, although not completely eliminated (37). Consequently, the ingestive response to solutions in sham-feeding tests is controlled primarily by orosensory rather than postingestive stimuli (43). A parallel study (3) investigated the effects of opioid antagonists on flavor preference conditioned by the postingestive actions of sugar using the IG infusion technique.

EXPERIMENT 1: FOOD-RESTRICTED RATS RECEIVING LIMITED ACCESS DURING TRAINING: NALTREXONE AND EXPRESSION OF CONDITIONED FLAVOR PREFERENCES

Prior work indicates that rats learn to prefer a flavor mixed into concentrated sugar solutions over a flavor mixed into a saccharin solution (27,35). The taste as well as the postingestive nutritive effects of the sugar appear to reinforce this preference (35). Thus, although saccharin and sugar are both sweet, rats prefer concentrated sugar solutions to saccharin solutions in choice tests (8,49). Further, in sham-feeding tests, rats drink considerably more of the concentrated sugar solutions than they do of a 0.2% saccharin solution (38). Experiment 1 utilized the sham-feeding technique to determine if naltrexone alters the flavor preference conditioned by the taste of sucrose. Rats were trained to sham-feed a sucrose (16%) solution with one distinct novel flavor, and a saccharin (0.2%) solution with a second distinct novel flavor in separate daily one-bottle training sessions. Flavor preferences were subsequently measured in sham-feeding choice tests with the two flavors presented in mixed sucrose-saccharin solutions. During training, the rats were given restricted access to the training solutions (10 ml/30-min sessions) to limit differences in the amounts of sucrose and saccharin solutions consumed during sham-feeding training sessions. Intakes were unlimited in the two-choice, sham-feeding tests during which the animals were treated with naltrexone or vehicle.

Method

Subjects. Twenty male albino Sprague–Dawley rats (350–400 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages and maintained on a 12-h light/12-h dark cycle, with Purina rat chow and water available ad lib. Each rat was pretreated with chlorpromazine (3 mg/kg, IP) and anesthetized with Ketamine HCl (100 mg/kg, IM). Following a midline incision (4–7 cm) exposing the stomach outside of the skin and muscle, a stainless steel gastric fistula surrounded by mesh (Bard Marlex) was inserted into the greater curvature of the stomach, and was held in place by a pursestring series of sutures. The fistula was externalized through overlying skin and muscle, and an external stainless steel screw closed the fistula to prevent leakage of stomach contents. Two weeks of surgical recovery followed to allow for drug clearance.

Test solutions. The training solutions consisted of either 16% sucrose (Domino Sugar) or 0.2% sodium saccharin (Sigma Chemical Co., St. Louis, MO) flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White

Plains, NY). Half of the rats had a cherry flavor added to the sucrose solutions (CS+) and a grape flavor added to the saccharin solution (CS−); the two flavors were reversed for the remaining rats. In the two-choice preference tests, the CS+ and CS− flavors were each presented in a mixed 8% sucrose + 0.1% saccharin solution. For initial sham-feeding training, an 8% maltodextrin solution was used (BioServ, Frenchtown, NH) which has a distinctive taste to rats (33).

Procedure

Initial training. The rats were placed on a food-restriction schedule that maintained their body weights at 85–90% of their ad lib level. They were initially trained to drink an 8% maltodextrin solution from calibrated sipper tubes (100 ml, 1-ml gradations) under initial water-deprivation and subsequent ad lib conditions. Prior to each daily 30-min session, the rats' gastric fistulae were opened, and their stomachs emptied by repeatedly flushing warm water (10–20 ml). At the end of the session, their stomachs were again flushed with warm water to minimize nutrient absorption, and the fistulae were closed. This sham-feeding procedure was repeated daily until all rats approached the sipper tubes with short (<1 min) latency, typically within 5 days.

One-bottle training. The rats were given 10 one-bottle, sham-feeding training sessions (30 min/day) with the CS training solutions; intake was limited to a maximum of 10-ml/session. The CS− was presented on odd-numbered days, and the CS+ was presented on even-numbered days. Food was unavailable during all test times. On days 7–10, the rats received vehicle treatment (1 ml normal saline/kg body weight, SC) 30 min prior to the training session during which they had access to two sipper tubes, one containing the CS− or CS+ solution, and the other containing water. This acclimated the rats both to the injection procedure and the presence of two sipper tubes during the choice tests. Water intakes were negligible in these training trials. The position of the CS and water sipper tubes varied across days using a left–right–right–left pattern.

Two-bottle testing. Following training, the rats were given eight two-bottle sham-feeding test sessions (30 min/day) with unlimited access to the CS+ and CS− flavors presented in mixed sucrose (8%)–saccharin (0.1%) solutions. The positions of the two sipper tubes were counterbalanced as described above. On day 1, subgroups of rats received vehicle (1 ml/kg, SC, $n = 10$) or naltrexone (Sigma Chemical Company, St. Louis, MO) at doses of either 0.1 ($n = 5$) or 1 ($n = 5$) mg/kg 30 min prior to the test sessions. This pattern of treatments was systematically altered over the ensuing 3 days such that all 20 rats received two vehicle injections, and naltrexone at doses of 0.1 and 1 mg/kg. The pattern was then repeated on days 5–8 so that all 20 rats received two more vehicle injections and naltrexone at doses of 2.5 and 5 mg/kg.

Statistics

CS intakes were recorded to the nearest ml. Intakes during training were evaluated by a repeated-measures factorial analysis of variance with the CS− and CS+ conditions as one variable, and the five days of exposure as the repeated variable. Tukey corrected comparisons ($p < 0.05$) detected significant effects. Separate randomized-blocks analyses of variance evaluated alterations in CS+ and CS− intake as a function of pooled vehicle and naltrexone dose treatments, alterations in total intake as a function of vehicle and naltrexone treatment, and alterations in CS+ preference as a function of vehicle and naltrexone treatment. CS+ preference was defined as the percentage of CS+ intake/total intake.

Results

CS+ and CS− intake during limited access training. Significant differences in sham intakes were observed across training days, $F(4, 76) = 6.41$, $p < 0.0002$, and between CS+ and CS− conditions, $F(1, 19) = 87.50$, $p < 0.0001$, but not for the interaction between days and conditions. Overall, the rats drank nearly twice as much CS+ than CS− during training (8.5 vs. 4.5 ml/30 min) (Fig. 1A).

Naltrexone and conditioned flavor preferences. Naltrexone treatment significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 76) = 5.01$, $p < 0.0012$, but there were no significant differences among the different naltrexone doses. Intakes were suppressed by 20–41% (Fig. 1B). Overall, the rats consumed more CS+ than CS− solutions during these tests, $F(1, 19) = 8.86$, $p < 0.008$ (Fig. 1C). Because there was no reliable interaction between naltrexone dose and CS solution, $F(4, 76) = 0.63$, none of the naltrexone doses specifically reduced intake of the CS+ relative to CS− solution (Fig. 1C). Naltrexone treatment did not significantly alter the percent CS+ intake, $F(4, 76) = 0.96$, NS (Fig. 1D).

Discussion

This experiment revealed that rats develop a reliable preference for a flavor paired with sucrose over a flavor paired with saccharin during one-bottle sham-feeding sessions. Because the sham-feeding procedure minimized the postingestive actions of the sucrose solution, the CS+ preference is attributed to flavor–flavor conditioning. Consistent with prior reports (14,17,18,22,32), naltrexone significantly reduced overall intakes of the sweet solution consumed during sham feeding in the two-bottle tests. The degree of suppression (20–41%) was smaller than that obtained in some prior studies [50–80%: (18)], which may be related to the animals' state of food restriction. Rockwood and Reid (32) reported that naltrexone suppressed sham drinking less in fluid-deprived rats relative to nondeprived animals (27 vs. 50%). While suppressing total sham-fed intakes, naltrexone did not reliably alter the preference for the CS+-flavored solution over the CS−-flavored solution. Although higher naltrexone doses appeared to reduce percent CS+ intake (55.3–59.7%) relative to vehicle treatment (66%), this effect was not significant.

EXPERIMENT 2A: ROOD-RESTRICTED RATS RECEIVING UNLIMITED ACCESS DURING TRAINING: NALTREXONE AND EXPRESSION OF CONDITIONED FLAVOR PREFERENCES

Although significant, the percent CS+ intake (66%) obtained in Experiment 1 was not very strong. One possible explanation was that the CS flavors were presented in a palatable sucrose–saccharin mixture during the two-bottle choice test. One approach to increase the percent CS+ intake would be to use different concentrations of saccharin, and pair the flavors with each concentration (12). A limitation of this approach, however, is that the intakes during the choice tests would have been much lower because saccharin does not stimulate much sham feeding, and hence, drug effects might have been masked by low intakes. Thus, we chose not to use this approach.

Another factor that may have limited percent CS+ intake in the first experiment is that the CS intakes were limited dur-

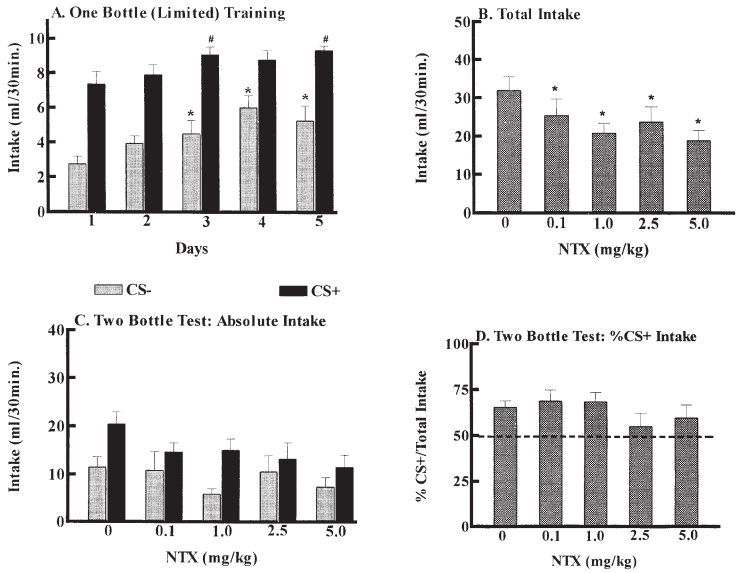

FIG. 1. Food-restricted rats receiving limited access during training: naltrexone and expression of conditioned flavor preferences. (A) Alternations in sham-feeding intakes (mean ± SEM) over 30 min of either a saccharin (0.2%) solution paired with a novel grape or cherry (0.05%) flavor (CS−) available on odd-numbered days or a sucrose (16%) solution paired with a novel cherry or grape flavor (CS+) available on even-numbered days in rats food-restricted to 85–90% of their normal body weight. Limited (10 ml) amounts of the solutions were available during training. The asterisks denote significant increases in sham-feeding intake relative to the first day of training, while the number signs denote significant differences between CS+ and CS− sham-feeding intake on paired days. (B) Alterations in sham-feeding total intakes (mean ± SEM) over 30 min of a combined saccharin (0.1%) and sucrose (8%) solution offered in two bottles with the CS+ and CS− flavor, respectively, following pretreatment (30 min) with either vehicle (mean of four tests) or naltrexone at doses of 0.1, 1, 2.5, or 5 mg/kg. (C) Alterations in sham-feeding intakes of the CS+-flavored and CS−-flavored solutions following pretreatment with either vehicle or naltrexone. (D) Alterations in the percentage of CS+ intake over total intake following pretreatment with either vehicle or naltrexone.

ing training. In particular, the rats presumably drank much less of the CS+/sucrose solution than they would have if intakes were unlimited. This may have resulted in a "frustration" effect that reduced their attraction to the CS+ flavor [e.g., (2)]. Experiment 2 avoided this potential problem by providing sham-feeding rats unlimited access to the CS+ and CS− solutions during one-bottle training.

Methods

Subjects and initial training. Twenty naive male rats were fitted with gastric cannulas as in Experiment 1. They were food restricted and given initial sham feeding as previously described.

CS−/CS+ training procedure. The rats were given 10 one-bottle training sessions with the CS+/sucrose and CS−/saccharin solutions as in Experiment 1, except that unlimited amounts (i.e., 80 ml) of the training solutions were available during the 30-min sessions.

Following training, the rats were given two-bottle preference tests with the CS+ and CS− presented in a sucrose-saccharin mixture as in Experiment 1. Rats were exposed to four vehicle tests and one test each following doses of 0.1, 1, 2.5, and 5 mg/kg according to the identical regimen described in Experiment 1. All rats were then subsequently tested following either vehicle or naltrexone at a dose of 10 mg/kg.

Results

CS+ and CS− intake during unlimited access training. Significant differences in sham-feeding intakes were observed across days, $F(4, 76) = 59.76$, $p < 0.0001$, between CS+ and CS− conditions, $F(1, 19) = 359.70$, $p < 0.0001$, and for the interaction between days and conditions, $F(4, 76) = 48.18$, $p < 0.0001$. Overall, intakes of the CS+ solution (39.2 ml/30 min) were five times higher than the CS− solution (7.7 ml/30 min) during one-bottle training (Fig. 2A).

Naltrexone and conditioned flavor preference. Overall, the rats consumed more CS+ than CS− during the two-bottle tests, $F(1, 19) = 36.56$, $p < 0.0001$ (Fig. 2C). Naltrexone treatment produced only small (8–12%), nonsignificant reductions in total intakes during these sham-feeding tests over the 100-fold dose range, $F(5, 95) = 1.73$, NS; (Fig. 2B). Because there was also no reliable interaction between CS solution and the naltrexone dose, $F(5, 95) = 1.59$, NS, none of the naltrexone doses specifically reduced intakes of the CS+ relative to CS− solution (Fig. 2C). The rats displayed slightly lower percent CS+ intakes following the three higher naltrexone doses (66–

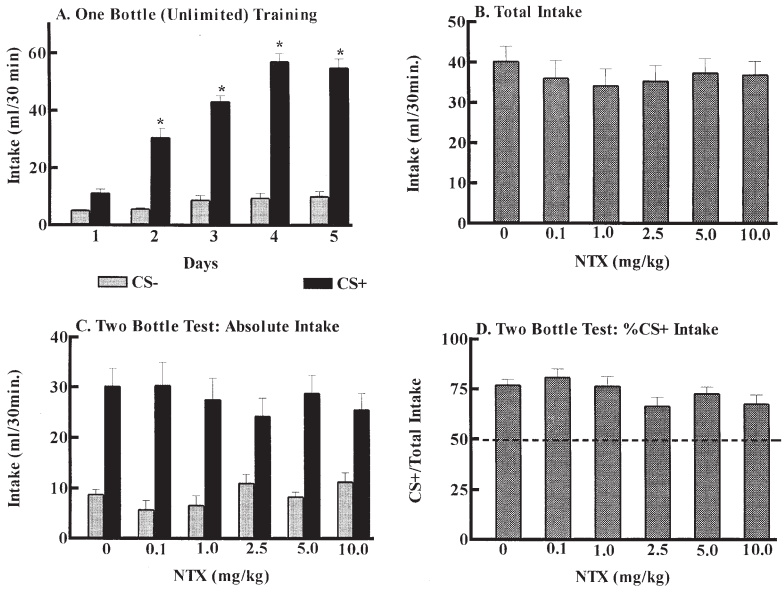

FIG. 2. Food-restricted rats receiving unlimited access during training: naltrexone and expression of conditioned flavor preferences. (A) Alternations in sham-feeding intakes (mean ± SEM) over 30 min of either a saccharin solution paired with a novel flavor (CS−) available on odd-number days or a sucrose solution paired with a novel flavor (CS+) available on even-numbered days in rats food-restricted to 85–90% of their normal body weight. Unlimited (80 ml) amounts of the solutions were available during training. The asterisks denote significant increases in sham-feeding intake relative to the first day of training and between CS+ and CS− sham-feeding intake on paired days. (B) Alterations in sham-feeding total intakes (mean ± SEM) over 30 min of a combined saccharin and sucrose solution offered in two bottles with the CS+ and CS− flavor, respectively, following pretreatment with either vehicle or naltrexone. (C) Alterations in sham-feeding intakes of the CS+-flavored and CS−-flavored solutions following pretreatment with either vehicle or naltrexone. (D) Alterations in the percentage of CS+ intake over total intake following pretreatment with either vehicle or naltrexone.

72%) relative to the vehicle test (77%), but these differences were not significant, $F(5, 95) = 2.07$, $p < 0.077$ (Fig. 2D).

Discussion

As expected, unlimited access to the solutions during one-bottle training resulted in markedly higher CS+/sucrose intake than CS−/saccharin intake relative to corresponding values observed in Experiment 1. Also, a stronger CS+ preference (77%) was obtained in this experiment under vehicle conditions than in the first experiment (66%). Increased exposure to the CS+ flavor during training may have contributed to the stronger CS+ preference obtained in this experiment. However, prior work indicates that flavor exposure per se does not completely account for preferences resulting from flavor–flavor conditioning (12).

Data from the first experiment suggested potential CS+ preference reductions following naltrexone (55–60%) relative to vehicle (66%) treatment following limited-access training, and that the failure to observe significant reductions could be attributed to the relatively low preference. Despite the higher conditioned flavor preference (77%) following unlimited-access training in the present experiment, naltrexone persisted in producing small and nonsignificant reductions in the CS+ preference (66–72%). These data suggest that opioid receptors are not strongly involved in the expression of a conditioned flavor preference regardless of the type of training (limited or unlimited access) or the magnitude of the preference.

However, naltrexone did not significantly reduce total intakes during the two-bottle preference tests. The minor (8–12%) reductions in sham intakes contrast with the 20–41% reductions observed in Experiment 1 as well as with the marked reductions in sucrose intake in sham-fed and mildly (6 h) deprived rats reported in other studies [50–80%: (17,22,32)]. Thus, the amount of solution consumed during training emerged as an important variable in naltrexone's anorectic potency. Indeed, there was a 4.6-fold increase in the amount of CS+ consumption in limited (8.5 ml) and unlimited (39.2 ml) access conditions, relative to only a 1.7-fold increase in the amount of CS− consumption in limited (4.5 ml) and unlimited (7.7 ml) access conditions. Hence, this suggests that extensive preexposure to the flavored solutions during training in food-restricted rats retard naltrexone's ability to reduce sucrose/saccharin intakes in sham-feeding conditions. Chronic food restriction, similar in duration and magnitude to that observed in the present studies, alters μ and κ opioid binding in both forebrain and parabrachial sites involved in ingestion (44,45), and also produces site-specific changes in levels of prodynorphin-derived peptides (6). Moreover, a number of diencephalic and limbic structures, including the bed nucleus of the stria terminalis and the central nucleus of the amygdala, display c-*fos* activity following naltrexone ad-

ministration in food-restricted rats, suggesting opioid-mediated inhibitory control (7). Because naltrexone is more sensitive in reducing intakes under ad lib conditions (23–26), the second phase of this experiment evaluated the ability of naltrexone to inhibit sucrose/saccharin intake and the expression of conditioned flavor preferences during unlimited training access in sham-fed, but ad lib-fed weight rats.

EXPERIMENT 2B: AD LIB-FED RATS RECEIVING UNLIMITED ACCESS DURING TRAINING: NALTREXONE AND EXPRESSION OF CONDITIONED FLAVOR PREFERENCES

Method

At the end of Experiment 2A, the rats were given ad lib access to food and water for 2 weeks. They were then given four retraining sessions with unlimited access to the CS+/sucrose and the CS−/saccharin solutions; water bottles were also available during these sessions. Following retraining, the rats were given two-bottle preference tests with the CS+ and CS− flavors presented in the sucrose–saccharin mixture. They received four vehicle injections, and one injection each of the 0.1, 1, 2.5 and 5 mg/kg dose of naltrexone according to the regimen described previously.

Results

CS+ and CS− intake during unlimited access training. Significant differences in sham-feeding intake were observed between CS+ and CS− conditions, $F(1, 18) = 77.22$, $p < 0.0001$. There was also a significant interaction between days and conditions, $F(1, 18) = 4.82$, $p < 0.042$, but total intake did not differ across days, $F(1, 18) = 0.34$, NS. Intakes of the CS+ solution (31.4 ml/30 min) were approximately threefold higher than the CS− solution (10.6 ml/30 min), and remained stable over the 2 days (Fig. 3A).

Naltrexone and conditioned flavor preferences. Overall, the rats consumed more CS+ than CS− during the two-bottle tests, $F(1, 18) = 17.54$, $p < 0.0006$ (Fig. 3C). Naltrexone significantly reduced total intakes, $F(4, 72) = 12.09$, $p < 0.0001$, by 28–43% relative to vehicle treatment; no significant differences were observed among the naltrexone doses (Fig. 3B). There was also a significant CS by dose interaction, $F(4, 72) = 2.94$, $p < 0.026$. CS+ intake was significantly reduced by each of the naltrexone doses, whereas CS− intake was only significantly reduced following the 1 mg/kg dose of naltrexone (Fig. 3C). It should be noted, however, that CS+ intake was significantly higher than CS− intake following vehicle and all, but the 5 mg/kg naltrexone conditions. However, naltrexone did not significantly alter percent CS+ intake, $F(4, 72) = 1.45$, NS. Thus, the reductions in CS+ preference from 66.4% following saline treatment to 55.7% following the 5 mg/kg dose of naltrexone were not significant (Fig. 3D).

Discussion

The present experiment confirmed that the use of ad lib feeding conditions was more sensitive in detecting naltrexone's ability to reduce sucrose/saccharin intakes in sham-fed rats. Like previous studies (23–26), all naltrexone doses reduced total intake of a combined sucrose/saccharin solution in sham-fed and ad lib weight rats.

The data appeared suggestive that a high (5 mg/kg) dose of naltrexone affected the expression of the conditioned flavor preference. When evaluating absolute intake (Fig. 3C), the intakes of the CS+ (8.4 ml) and the CS− (8.4 ml) were equivalent following the 5 mg/kg dose, indicating a loss of preference. However, CS+ preference, calculated as the mean of preferences of individual animals in each treatment group indicated a 55% CS+ preference (Fig. 3D), which did not differ significantly from saline treatment. It should be noted, however, that the three lower (0.1, 1, and 2.5 mg/kg) naltrexone doses failed to alter the proportion between CS+ and CS− intakes either in absolute terms or in terms of CS+ preference. Therefore, these data suggest that opioid receptors play a limited role at best in the expression of a conditioned flavor preference, and may only do so when the animals are at normal body weight under ad lib feeding conditions.

EXPERIMENT 3A: FOOD-RESTRICTED RATS RECEIVING LIMITED ACCESS DURING TRAINING: NALTREXONE AND ACQUISITION OF CONDITIONED FLAVOR PREFERENCES

Given the relatively limited role of naltrexone in modulating the expression of conditioned flavor preferences, the third experiment was designed to evaluate whether daily naltrexone administration during training would affect the acquisition of a conditioned flavor preference compared to rats receiving daily saline treatment during training. Further, the impact of naltrexone on the expression of flavor preferences was evaluated in rats trained under naltrexone or saline treatment. The rats were food restricted and received limited access to the CS solutions during training to reduce the intake differences expected between the naltrexone- and saline-treated groups.

Method

Subjects and initial training. Thirteen naive male rats, prepared with gastric fistulas and housed individually and maintained as described previously, were food restricted and underwent the same sham-feeding training regimen with a maltodextrin (8%) solution.

CS−/CS+ training procedure. The rats were trained in a minor modification of the sham-feeding procedure described in Experiment 1 over a 14-day period. Rats received either vehicle (1 ml 0.9% normal saline/kg, SC, $n = 6$) or naltrexone (0.1 mg/kg, SC, $n = 7$) 30 min prior to each training session. This dose of naltrexone was chosen because preliminary studies demonstrated that a higher (1 mg/kg) dose eliminated intakes during training (data not shown). On the first four training days, only 4 ml of the CS+ and CS− solutions were available during the 30-min sessions, whereas on the remaining 10 days of training, 6 ml of the CS+ and CS− solutions were available. Saccharin (0.2%, CS−) was paired with a cherry flavor in one-half of the rats in each group, and with a grape flavor in the remaining rats on odd-numbered days. Sucrose (16%, CS+) was paired with a grape flavor in the first group, and with a cherry flavor in the second group on even-numbered days. The use of a second sipper tube filled with water on days 7–14 proceeded as described previously. Sham-feeding intakes (±1 ml) were recorded on each of the seven CS− and CS+ training days.

Conditioned preference testing procedure. The two groups of rats then underwent a 6-day testing period under sham-feeding conditions in the absence of food during the 30-min testing trial in a manner identical to that described previously. Sham-fed rats were exposed to two sipper tubes (80 ml) containing a combined saccharin (0.1%) and sucrose (8%) solution paired with either the CS+ or the CS− flavor. Each rat received two injections each of either vehicle, naltrexone at a dose of 0.1 mg/kg, and naltrexone at a dose of 2.5 mg/kg in counterbalanced order.

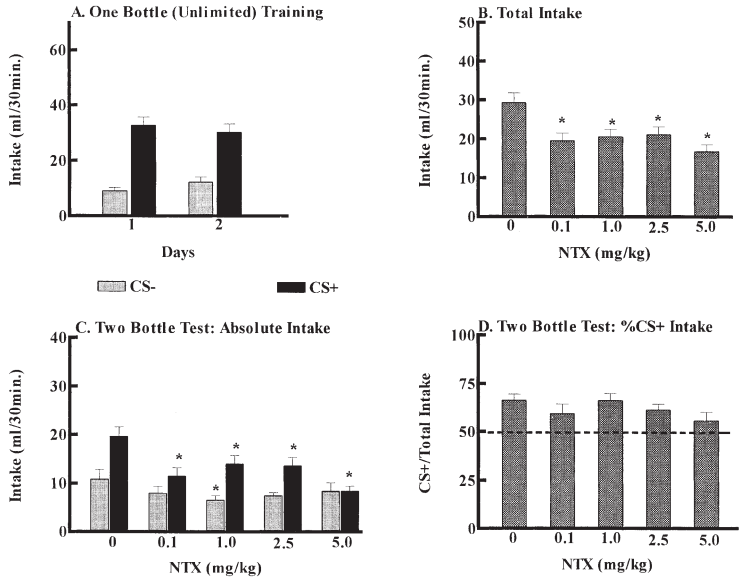

FIG. 3. Ad lib-fed rats receiving unlimited access during training: naltrexone and expression of conditioned flavor preferences. (A) Alterations in sham-feeding intakes (mean ± SEM) over 30 min of either a saccharin solution paired with a novel flavor (CS−) available on odd-number days or a sucrose solution paired with a novel flavor (CS+) available on even-number days in rats fed ad lib. Unlimited (80 ml) amounts of the solutions were available during training. (B) Alterations in sham-feeding total intakes (mean ± SEM) over 30 min of a combined saccharin and sucrose solution offered in two bottles with the CS+ and CS− flavor, respectively, following pretreatment with either vehicle or naltrexone. (C) Alterations in sham-feeding intakes of the CS+-flavored and CS−-flavored solutions following pretreatment with either vehicle or naltrexone. The asterisks in B and C denote significant decreases in the particular form of sham-feeding intake following naltrexone relative to corresponding vehicle values. (D) Alterations in the percentage of CS+ intake over total intake following pretreatment with either vehicle or naltrexone.

Results

CS+ and CS− intake during limited access training. Significant differences in sham-feeding intakes were observed across days, $F(6, 42) = 17.71$, $p < 0.0001$, and between CS+ and CS− conditions, $F(1, 7) = 6.60$, $p < 0.37$). The interaction between days and conditions was also significant, $F(6, 42) = 3.66$, $p < 0.005$. The main effect of training condition (vehicle or naltrexone) approached but was not significant, $F(1, 7) = 4.91$, $p < 0.062$. Overall, intakes during training were slightly higher in rats receiving the saline vehicle during training (3.7 ml/30 min) relative to rats receiving naltrexone during training (3.3 ml/30 min). Intakes of the CS+ solution (3.9 ml/30 min) were significantly higher than that of the CS− solution (3.1 ml/30 min) during training, and both forms of intakes remained stable over days (Fig. 4A).

Naltrexone training and naltrexone testing effects upon total intake. Significant differences in sham-feeding intakes during the two-bottle tests were observed among naltrexone testing conditions, $F(2, 12) = 15.28$, $p < 0.0005$, and for the interaction between training and testing conditions, $F(2, 12) = 4.38$, $p < 0.037$, but not between saline and naltrexone training conditions, $F(1, 6) = 0.13$, NS. Whereas rats receiving saline during training displayed significant reductions in total intake following the 2.5 mg/kg dose of naltrexone during testing (29%), rats receiving naltrexone during training failed to display significant reductions in total intake following naltrexone during testing (Fig. 4B).

Naltrexone training and conditioned flavor preferences. Although rats consumed more of the CS+ (19.5 mg/30 min) than the CS− (12.8 ml/30 min) solution, this variable was not significant, $F(1, 6) = 1.09$, NS. Neither naltrexone during training nor naltrexone during testing significantly affected CS+ or CS− intakes (Fig. 4C). Significant differences in percent CS+ intake were observed between training conditions, $F(1, 6) = 8.80$, $p < 0.025$, but not among testing conditions, $F(2, 12) = 1.76$, NS. The interaction between training and testing conditions was also not significant, $F(2, 12) = 0.06$, NS. Rats receiving naltrexone during training (75.7%) displayed significantly higher percent CS+ intakes than rats receiving vehicle during training (56.5%) (Fig. 4D).

Discussion

Any interpretations concerning the data in this experiment are compromised by the failure of vehicle-trained rats to display a reliable conditioned flavor preference (56.5%), an effect similar to that observed in Experiment 1. The small magnitude of conditioned flavor preferences in vehicle-trained rats may reflect frustration [see (2)] by these food restricted and sham feeding receiving very limited (4–6 ml) access to the CS+ sucrose solution. In contrast, naltrexone administered during training failed to prevent the acquisition of a conditioned flavor preference in sham-fed rats given the potent preference (75.7%) for the CS+ (23.8 ml/30 min) relative to

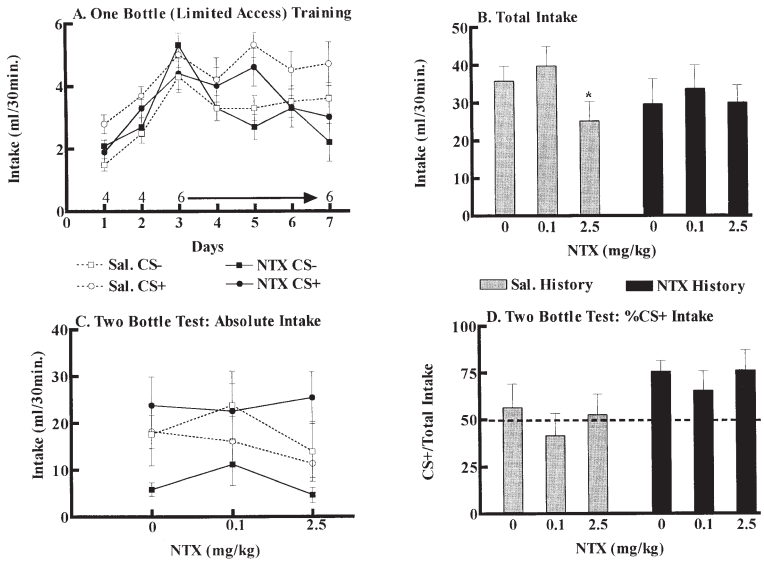

FIG. 4. Food-restricted rats receiving limited access during training: naltrexone and acquisition of conditioned flavor preferences. (A) Alternations in sham-feeding intakes (mean ± SEM) over 30 min of either a saccharin solution paired with a novel flavor (CS−, squares) available on odd-numbered days or a sucrose solution paired with a novel flavor (CS+, circles) available on even-numbered days in two groups of food-restricted rats receiving either daily injections of saline (open symbols) or naltrexone (0.1 mg/kg, closed symbols) 30 min prior to training. Limited amounts of the solutions were available during the first two pairs of days (4 ml) and the last five pairs of days (6 ml) of training. (B) Alterations in sham-feeding total intakes (mean ± SEM) over 30 min of a combined saccharin and sucrose solution offered in two bottles with the CS+ and CS− flavor, respectively, in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing. The asterisk in B denotes a significant decrease in sham-feeding intake following naltrexone testing in saline-trained rats. (C) Alterations in sham-feeding intakes of the CS+-flavored and CS−-flavored solutions in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing. (D) Alterations in the percentage of CS+ intake over total intake in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing.

the CS− (5.9 ml/30 min). Therefore, any comparisons made about the ability or inability of these manipulations to alter naltrexone's effects during testing must be done with the caveat that interactions may have occurred between the training treatment and frustration induced by limited access. Our earlier expression experiments demonstrating greater preferences following unlimited access during training (Experiment 2A) relative to limited access during training (Experiment 1) in food-restricted and sham-feeding animals indicate the ability of unlimited access during training to produce greater preferences. Therefore, the last phase of this experiment examined both training (acquisition) and testing (expression) effects of naltrexone upon conditioned flavor preferences in sham-fed rats with unlimited access to the CS+ and CS− flavors during training.

EXPERIMENT 3B: FOOD-RESTRICTED RATS RECEIVING UNLIMITED ACCESS DURING TRAINING: NALTREXONE AND ACQUISITION OF CONDITIONED FLAVOR PREFERENCES

Method

Subjects and initial training. Twelve of the male rats used in Experiment 3A were used in this phase of the study. They were housed individually and maintained as described previously, and were food restricted (85%).

CS−/CS+ training procedure. The rats were trained in a manner similar to the sham-feeding procedure described in Experiment 2A in which 80 ml of the solution was available to the animals during the 30 min training trial on each of the 10 training days. Rats received either vehicle (1 ml 0.9% normal saline/kg, SC, $n = 5$) or naltrexone (0.1 mg/kg, SC, $n = 7$) 30 min prior to each training session. Saccharin (0.2%, CS−) was paired with a novel unsweetened orange-pineapple (0.05%) flavor in one-half of the rats, and a novel unsweetened kiwi-lime (0.05%) flavor in the remaining rats on odd-numbered days. Sucrose (16%, CS+) was paired with the kiwi-lime flavor in the first group, and with the orange-pineapple flavor in the second group on even-numbered days. All other procedures were identical to those described previously.

Conditioned preference testing procedure. The two groups of rats then underwent a 6-day testing period under sham-feeding conditions in the absence of food during the 30-min testing trial in a manner identical to that described previously. Sham-fed rats were exposed to two sipper tubes (80 ml) containing a combined saccharin (0.1%) and sucrose (8%) solution paired with either the CS+ or the CS− flavor. Each rat received two injections each of either vehicle, naltrexone at a

dose of 0.1 mg/kg, and naltrexone at a dose of 2.5 mg/kg in counterbalanced order.

Results

CS+ and CS− intake during unlimited access training. Significant differences in sham-feeding intakes were observed between saline and naltrexone training, $F(1, 11) = 174.48$, $p < 0.0001$, across days, $F(4, 44) = 81.13$, $p < 0.0001$, between CS+ and CS− conditions, $F(1, 11) = 436.98$, $p < 0.0001$. All interactions between and among the main effects were also significant. Overall, intake of the solutions during training was significantly higher in rats receiving the saline vehicle (25.6 ml/30 min) relative to rats receiving naltrexone (10.9 ml/30 min). Intakes of the CS+ solution (29.8 ml/30 min) were significantly higher than that of the CS− solution (6.7 ml/30 min) (Fig. 5A). Whereas rats receiving naltrexone during training consumed similar quantities of the CS− solution as rats receiving saline during training, rats receiving naltrexone during training consumed significantly less of the CS+ solution during the first, second, third, and fifth days relative to rats receiving saline during training.

Naltrexone training and naltrexone testing effects upon total intake. Significant differences in sham-feeding intakes during two-bottle tests were observed among naltrexone testing doses, $F(2, 22) = 118.70$, $p < 0.0001$, and for the interaction between training and testing conditions, $F(2, 22) = 40.10$, $p < 0.0001$, but not between saline and naltrexone training, $F(1, 11) = 0.43$, NS. Whereas rats receiving saline during training displayed significant reductions in total intake following the 2.5, but not the 0.1 mg/kg testing doses of naltrexone, rats receiving naltrexone during training displayed significant reductions in total intake following both naltrexone doses during testing (Fig. 5B). The ability of naltrexone at a testing dose of 2.5 mg/kg to reduce total intake was significantly impaired in animals receiving naltrexone during training relative to animals receiving saline during training.

Naltrexone training and conditioned flavor preferences. Overall, rats consumed significantly more of the CS+ (32.2 ml/30 min) than the CS− (5.9 ml/30 min) solution, $F(1, 11) = 425.49$, $p < 0.0001$. Significant differences were also observed for interactions between the CS+ and CS− conditions and naltrexone testing, $F(2, 22) = 65.35$, $p < 0.0001$, and between

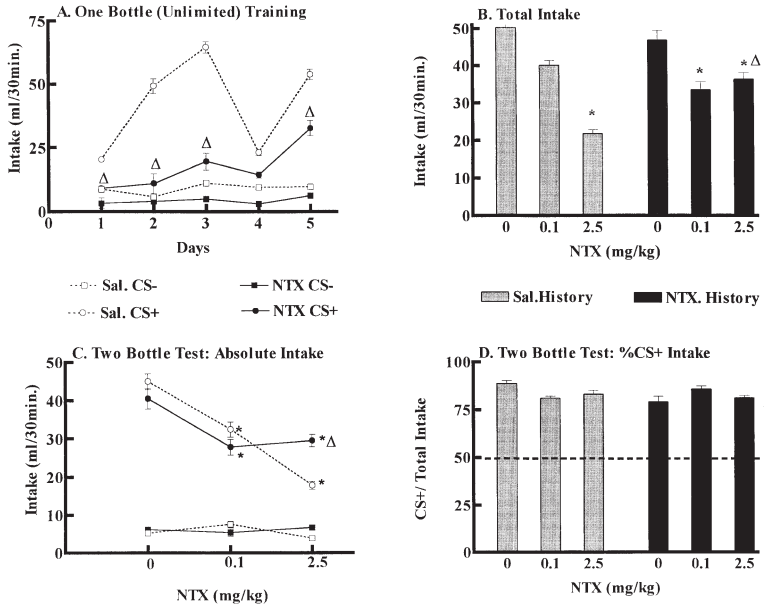

FIG. 5. Food-restricted rats receiving unlimited access during training: naltrexone and acquisition of conditioned flavor preferences. (A) Alterations in sham-feeding intakes (mean ± SEM) over 30 min of either a saccharin solution paired with a novel flavor (CS−, squares) available on odd-number days or a sucrose solution paired with a novel flavor (CS+, circles) available on even-numbered days in two groups of food-restricted rats receiving either daily injections of saline or naltrexone (0.1 mg/kg) 30 min prior to training. Unlimited (80 ml) amounts of the solutions were available during training. The triangles in this and subsequent panels denote significant differences between saline-trained and naltrexone-trained rats on that given response. (B) Alterations in sham-feeding total intakes (mean ± SEM) over 30 min of a combined saccharin and sucrose solution offered in two bottles with the CS+ and CS− flavor, respectively, in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing. The asterisk in B denotes a significant decrease in sham-feeding intake following naltrexone testing in saline-trained rats. (C) Alterations in sham-feeding intakes of the CS+-flavored and CS−-flavored solutions in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing. (D) Alterations in the percentage of CS+ intake over total intake in saline-trained and naltrexone-trained rats following pretreatment with either vehicle or naltrexone during testing.

naltrexone testing conditions and naltrexone training conditions, $F(2, 22) = 10.09$, $p < 0.0008$. CS+ intakes during vehicle testing were similar in both saline-trained (45 ml/30 min) and naltrexone-trained (40.5 ml/30 min) rats. Likewise, CS− intakes during vehicle testing were similar in saline-trained (5.3 ml/30 min) and naltrexone-trained (6.2 ml/30 min) rats. Both the 0.1 and 2.5 mg/kg doses of naltrexone administered during testing significantly and selectively reduced CS+, but not CS− intake in both saline-trained and naltrexone-trained rats relative to their corresponding responses following vehicle (Fig. 5C). However, naltrexone-trained rats displayed significantly smaller magnitudes in the inhibition of CS+ intakes than saline-trained rats following the 2.5 mg/kg dose of naltrexone administered during testing (Fig. 5C). The percent CS+ intake was quite pronounced during vehicle treatment in both saline-trained (88.7%) and naltrexone-trained (86.0%) rats, and naltrexone treatment during testing did not significantly alter percent CS+ intake in either saline-trained or naltrexone-trained rats (Fig. 5D).

Discussion

The present experiment clearly demonstrated that naltrexone administered daily during training failed to block the acquisition of a conditioned flavor preference in sham-feeding and food-restricted rats. Naltrexone-trained rats displayed a very strong preference, despite the fact that they drank significantly less of the CS+ solution than their saline-trained counterparts on 4 of the 5 training days, and despite the fact that they drank similar quantities of the CS− solution over all training days. Thus, although the dose of naltrexone administered daily during training was relatively low (0.1 mg/kg), it was producing expected inhibition of sucrose intake as described previously (23). Use of a higher (1 mg/kg) naltrexone dose in preliminary studies abolished intake, and use of such a higher dose would make it impossible to determine whether any subsequent effect upon acquisition of a conditioned flavor preference would be due to its pharmacological action or the failure to make discriminations between flavors due to low or nonexistent intake.

Chronic naltrexone exposure upregulates brain opioid receptors (19,42,46,47), and increases analgesic potencies of opiate agonists and both opioid-mediated and nonopioid-mediated stressors (1,41,42,46–48). Therefore, one might have expected increased sensitivity to opioid antagonists during testing. However, the present experiment also demonstrated that naltrexone treatment during training produced minor effects upon either naltrexone's inhibitory effects upon total intake during two-bottle testing, and naltrexone's effects upon the expression of a conditioned flavor preference during testing. Naltrexone administered during testing suppressed total sham-feeding intake in both saline-trained and naltrexone-trained rats, with the latter group displaying a smaller degree of inhibition following the 2.5 mg/kg test dose. Further, naltrexone-trained rats displayed a smaller degree of inhibition of CS+ intake following the 2.5 mg/kg test dose. However, the percent CS+ intake was similar in saline-trained and naltrexone-trained rats, both in the absence and presence of naltrexone during testing.

GENERAL DISCUSSION

The present results confirm and extend prior reports that rats acquire preferences for a sugar-paired flavor over a saccharin-paired flavor. The novel finding is that the sugar-conditioned preference was obtained in sham-feeding rats in which the ingested solutions drained out of an open gastric fistula. Thus, the US was primarily the sweet taste of the sucrose. However, sham feeding does not completely eliminate nutrient absorption (37), and thus some minimal amount of postgastric feedback may also have contributed to the CS+ preference. The present data also confirm prior reports that rats consume considerably more of sugar solutions (at moderate to high concentrations) than they do of saccharin solutions (at low to high concentrations) (33,38,43) under sham-feeding conditions.

In three of the five experiments, naltrexone reliably reduced the sham-feeding of combined sucrose and saccharin solutions, which is consistent with prior results (14,17,18,22,32). Naltrexone did not typically block the expression of the sucrose-conditioned CS+ preference in Experiments 1 or 3A in food-restricted rats. In Experiment 2B, ad lib-fed rats received unlimited access to the solutions during training. The sucrose-conditioned CS+ preference was blocked only by the high (5 mg/kg) dose of naltrexone when evaluating absolute intakes, but not when evaluating the data expressed as CS+ preference, which was the mean of preferences of individual animals in the group. In Experiment 3B, the sucrose-conditioned CS+ preference was selectively reduced by a test dose of naltrexone in saline-trained and naltrexone-trained animals when evaluating absolute intakes as the statistical measure. Again, however, the CS+ preference did not change significantly. This limited ability of naltrexone to alter expression of these preferences is in stark contrast to the ability of selective D_1 (SCH23390) and D_2 (raclopride) antagonists to potently block this identical flavor preference in sham-fed rats (50). Further, the third experiment showed that naltrexone failed to block the acquisition of the preference when the drug was given daily prior to each training session.

These data contrast with recent reports of naloxone suppressing flavor conditioning in adult rats (27,31), although the procedures of these earlier studies differ in important respects from the present work. In particular, Ramirez (31) examined flavor–postingestive nutrient conditioning because the nutrient US was delivered by IG infusions. Furthermore, flavor acceptance rather than preference was measured. Mehiel (27) also examined flavor–postingestive nutrient conditioning because the rats actually consumed the nutrient solutions of either glucose or ethanol. Thus, the failure of naltrexone to block flavor–flavor conditioning in the present experiment does not directly contradict the findings of Mehiel (27) and Ramirez (31), but suggests that minimization of postingestive consequences by sham feeding may be changing opioid antagonist effects. However, a parallel study conducted by our laboratories (3) demonstrated that naltrexone has minimal effects on flavor–nutrient conditioning postingestive using an IG training paradigm. Our findings also appear to agree with the failure of naloxone to alter sucrose-mediated operant discriminations for food reinforcers (29).

Shide and Blass (39) observed that naloxone blocked odor preference conditioning by intraoral sucrose and oil infusions in rat pups. Given the small amount of nutrients infused, it was presumed that the oral rather than the postingestive effects of the nutrients served as the US in the experiment. The sucrose and corn oil infusions also reduced isolation stress and elevated pain thresholds in rat pups. Therefore, it is possible that odor preference conditioned by these nutrients, which was measured by a place-preference technique rather than by intake per se, may represent a form of learned safety

rather than flavor–flavor conditioning, as typically measured in adult rats. Alternatively, it may be that flavor preference conditioning is more opioid dependent in neonatal rats than in adult animals. Further comparisons of neonatal and adult animals are needed to resolve this issue.

Although the present results do not directly contradict prior conditioning studies, they do appear inconsistent with the notion (10) that opioid antagonists reduce the hedonic response to sweet taste. One possible explanation is that once one establishes a CS+ preference by pairing a novel flavor with a sweet taste, the expression of that preference does not involve the opioid system. Thus, naltrexone treatment in Experiments 1 and 2 may have reduced the intakes of sucrose–saccharin solutions by reducing the attractiveness of their sweet taste, but left the CS+ preference intact. This model receives support from the observations that naltrexone alters the maintenance, but not the initiation of sucrose intake under both real-feeding (15,16) and sham-feeding (22) conditions. Furthermore, sham-feeding rats display a pattern of naltrexone-induced inhibition of sucrose intake that is behaviorally equivalent to diluting the sucrose concentration (18). More difficult to explain is why naltrexone treatment during initial training did not attenuate CS+ preference conditioning if it is assumed that the effective US was the palatable taste of sucrose, and naltrexone treatment reduced sucrose intakes during training. One possible explanation is that the naltrexone treatment attenuated the attractiveness of both the sucrose and saccharin solutions such that the difference in taste between the two solutions was still sufficient to condition the CS+ preference. It should be noted that the potencies of centrally administered naltrexone to reduce intakes by 40% were somewhat comparable when rats were exposed to either sucrose (6 nmol) or saccharin (29 nmol) (4,5). According to this interpretation, one should block conditioning by using a higher naltrexone dose during training. Pilot work revealed that rats treated with a 1.0 mg/kg dose of naltrexone during initial training drank very little of flavored saccharin solutions. Thus, a failure to obtain a subsequent CS+ preference would be difficult to interpret. It is important to note that the rats in the present study continued to drink substantial amounts under sham-feeding conditions even at with naltrexone doses up to 10 mg/kg. However, these rats were highly experienced with the flavors and were tested with a sucrose–saccharin mixture, which was presumably preferred to a plain saccharin solution. An alternative approach to study opioid involvement in flavor–flavor conditioning is to pair the CS+ and CS− flavors with qualitatively different US flavors such as a sweet flavor vs. a maltodextrin flavor. Naltrexone is also capable of blocking intake of a maltodextrin solution under both real-feeding (5) and sham-feeding (21) conditions, and it would be of interest to determine whether the opioid system is differentially involved in the flavor preferences conditioned by the tastes of sugar and maltodextrins.

In conclusion, the present study demonstrated that CS+ preferences can be obtained in sham-feeding rats either under food restricted or ad lib conditions, and thereby suggests that a sufficient US is the sweet taste per se, and not necessarily its postingestive consequences. Although naltrexone was effective in reducing overall intakes in sham-feeding animals, confirming its modulation of orosensory signals, it had no or limited effects on the acquisition or expression of conditioned flavor preferences.

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