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Naltrexone does not prevent acquisition or expression of flavor preferences conditioned by fructose in rats

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Abstract

The effects of the general opioid antagonist, naltrexone, on the acquisition and expression of flavor preferences conditioned by the sweet taste of fructose were examined. Food-restricted rats were trained over eight daily alternating one-bottle sessions (2 h) to drink an 8% fructose solution containing one novel flavor (CS+/F) and a less preferred 0.2% saccharin solution containing a different flavor (CS − /S). Four groups of rats were treated daily with either saline (control group) or naltrexone doses of 0.1, 1.0, or 5.0 mg/kg during training. Preferences were assessed in two-bottle tests with the CS+/S and CS − /S flavors presented in 0.2% saccharin solutions following saline injections. Naltrexone dose-dependently reduced fructose and saccharin intakes during training, confirming the drug's well-known suppressive effect on the intake of sweet solutions. Despite their reduced training intakes, the naltrexone groups displayed preferences for the CS+/S over the CS − /S (72–86%) that were similar to that of the control group (78%). The effect of naltrexone on the expression of the CS+/S flavor preference was evaluated by treating control rats with naltrexone (0.1–5 mg/kg) prior to CS+/S vs. CS − /S choice tests. The drug doses produced a dose-dependent reduction in CS+/S intake but did not significantly attenuate the CS+/S preference. These data are consistent with the relative inability of naltrexone to reduce flavor–flavor conditioning by sucrose in sham-feeding rats and flavor-nutrient conditioning in rats receiving intragastric sucrose infusions. In contrast, dopamine antagonists reduce both sucrose- and fructose-conditioned flavor preferences, which indicates the sensitivity of these conditioning paradigms to neuropharmacological manipulations. These data indicate that the endogenous opioid system, unlike the dopamine system, does not play a major role in either the acquisition or expression of flavor preference learning as measured in two-bottle choice tests.

Keywords: Flavor–flavor learning; Sweet taste; Fructose; Saccharin; Taste hedonics; Opioid antagonism

1. Introduction

Blockade of the endogenous opioid system with the general opioid antagonists naloxone and naltrexone potently reduces intake of palatable fluids, including sucrose and saccharin (e.g., Cooper, 1983; Levine et al., 1982; Lynch, 1986; Lynch and Libby, 1983; Siviy and Reid, 1983). Opioid antagonists appear to reduce the hedonic qualities of sweet substances because they (a) suppress intake of sweet solutions more than plain water (Cooper, 1983; LeMagnen et al., 1980; Sclafani et al., 1982); (b) block that portion of feeding that appears driven by sweet taste in food-restricted animals (Levine et al., 1995); (c) reduce sucrose's positive hedonic qualities in a taste reactivity (TR) paradigm (Parker et al., 1992); and (d) reduce sucrose intake in sham-fed rats (Kirkham, 1990; Kirkham and Cooper, 1988a; Rockwood and Reid, 1982) in a manner behaviorally equivalent to reductions in palatability obtained by diluting the test solution (Kirkham and Cooper, 1988b). Mu- and kappa-, but not delta-selective opioid antagonists also reduce sucrose intake in both real-feeding (Beczkowska et al., 1992) and sham-feeding (Leventhal et al., 1995) tests. Consistent with these results, central infusions of opioid agonists increase the intake of a saccharin solution, but not plain water (Zhang and Kelley, 2002), and consumption of sweet solutions increase brain β-endorphin levels more than does the consumption of plain water (Yamamoto et al., 2000).

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The opioid system has also been implicated in flavor preference conditioning by sweet taste. In particular, Mehiel (1996) trained rats to drink different flavors mixed into a preferred glucose solution and a less preferred saccharin solution and then measured their preferences for the flavors alone. Treatment with naloxone during the flavor + glucose training sessions prevented the rats from acquiring a preference for the glucose-paired flavor. However, these same rats were not treated with naloxone during the flavor + saccharin sessions, and thus they may have associated the glucose-paired flavor with mild aversive effects of the drug. More recently, Yu et al. (1999) trained rats with different flavors mixed into sucrose and saccharin solutions. One group was treated with naltrexone (0.1 mg/kg BW) during both sucrose and saccharin training sessions, while the second group was treated with saline throughout training. Although the naltrexone group consumed much less flavored sucrose during training than did the saline group, both groups displayed significant preferences for the sucrose-paired flavor over the saccharin-paired flavor. Naltrexone treatment during two-bottle testing also had little or no effect on the expression of the sucrose-conditioned preferences (Yu et al., 1999). The rats in these experiments sham-fed the sucrose and saccharin solutions throughout training and testing so that the learned flavor preferences were attributed to the sweet taste rather than the postingestive nutritive actions of sucrose. In another study, Azzara et al. (2000) trained rats with flavored saccharin solutions paired with intragastric infusions of sucrose or water. Significant preferences for the sucrose-paired flavor were observed in rats treated with naltrexone or saline throughout training. Thus, flavor conditioning by both the sweet taste and postingestive actions of sucrose was not blocked by the opioid antagonist.

In contrast to the failure of naltrexone to block flavor conditioning, other studies from our laboratory revealed that dopamine D1 and D2 receptor antagonism attenuated the expression of sucrose-conditioned flavor preferences in sham-feeding rats (Yu et al., 2000a,b). More recently, we observed that D1 and D2 antagonists blocked both the acquisition and expression of flavor preferences conditioned by the sweet taste of fructose (Baker et al., 2003). In the latter study, saline- and drug-treated rats drank matched amounts of flavored fructose and flavored saccharin solutions during one-bottle training sessions and were then given two-bottle choice tests with both flavors presented in saccharin solutions. The saline-treated control group displayed a significant preference for the fructose-paired flavor in the two-bottle tests which confirmed prior results (Sclafani and Ackroff, 1994). On the other hand, groups treated with D1 or D2 antagonists failed to acquire a preference for the fructose-paired flavor. The rats "real-fed" the solutions during training and testing, and were thus exposed to the postingestive nutritive actions of fructose as well as its sweet taste. Nevertheless, the learned flavor preference displayed by the control group was attributed specifically to the sweet taste of fructose. This interpretation was based on the results of other studies showing that intragastric fructose infusions, unlike glucose or sucrose infusions, do not condition flavor preferences in rats trained 30 min/day (Sclafani et al., 1999).

The dopamine drug effects obtained in the fructose conditioning study were more pronounced than those obtained in the sucrose conditioning studies; this may be due to procedural differences between the conditioning studies (e.g., real vs. sham feeding, matched vs. unmatched training intakes of sugar and saccharin solutions, 8% fructose vs. 16% sucrose sugar solutions, and use of mixed sucrose/saccharin solutions vs. only-saccharin solutions in two-bottle choice tests). These data suggest that the use of fructose as the unconditioned stimulus produces a conditioned flavor preference that is more sensitive to the effects of pharmacological perturbation of the putative neurochemistry that mediates the learning and expression of this behavior. Therefore, the present study used the fructose conditioning method to further investigate the impact of naltrexone on flavor conditioning by sweet taste. This was of interest because the failure of opioid antagonism to block sugar-conditioned preferences seems inconsistent with the ability of general and selective opioid antagonists to attenuate the intake of sweet solutions per se. An additional feature of the present study was that naltrexone's effect on sweet taste conditioning was evaluated at doses of 0.1, 1.0, and 5.0 mg/kg body weight, whereas only the 0.1-mg/kg dose was examined in our prior sucrose study. The combination of a more sensitive test method and expanded dose range was designed to increase the likelihood of obtaining a drug effect if, in fact, the opioid reward system is involved in flavor conditioning by sweet taste.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (250–325 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire-mesh cages and maintained on a 12:12-h light/dark cycle with water available ad libitum. All testing took place in the rat's home cage during the midlight phase of the light/dark cycle. Two weeks before testing began, the rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their ad-libitum level through the entire experiment by feeding them 12–15 g of Purina rat chow daily. The experimental protocol was approved by the Queens College Institutional Animal Care and Use Committee.

2.2. Test solutions

The training solutions consisted of 8% fructose (Sigma, St. Louis, MO) and 0.2% sodium saccharin (Sigma) flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft

Foods, White Plains, NY). Half of the rats in each group had the cherry flavor added to the fructose solution and the grape flavor added to the saccharin solution; the flavor–solution pairs were reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors were each presented in a 0.2% saccharin solution. The fructose-paired flavor is referred to as the CS+ and the saccharin-paired flavor as the CS− because 8% fructose is preferred to 0.2% saccharin (Sclafani and Ackroff, 1994). CS+/F represents the flavored fructose solution and CS−/S represents the flavored saccharin solution used during training. To determine if the animals developed a preference for the CS+ flavor in the absence of fructose, both the CS+ and CS− flavors were presented in saccharin solutions during the two-bottle choice test. CS+/S represents the saccharin solution containing the CS+ flavor that had been paired with fructose during training. An 8% maltodextrin solution (BioServ, Frenchtown, NJ) was used for training purposes because it has a flavor distinct from that of fructose and saccharin (Sclafani, 1987).

2.3. Procedures

Rats were initially trained (2 h/day) to drink the maltodextrin solution from calibrated bottles (100 ml, 1-ml gradations; Lab Products) while food and water restricted, and then while food was restricted with water available ad libitum except during the daily training sessions. The bottle was mounted on the front of the cage held by a spring, and was positioned so that the sipper spout entered the cage about 3–6 cm above the cage floor. This training procedure was repeated daily until all rats approached the sipper spouts with short (<1 min) latency, typically within 3 days. The limited food rations were given after each training session.

Four groups of rats were given eight consecutive daily one-bottle training sessions (2 h/day) with 24 ml of the CS+/F solution presented on odd-numbered days, and 24 ml of the CS−/S solution presented on even-numbered days. On Days 5–8, the rats had access to two bottles, one containing the CS+/F or CS−/S solution, and the other containing water. This acclimated them to the presence of two bottles during the choice tests. Water intake was negligible in these training trials. The position of the CS and water bottles varied across days using a left–right–right–left pattern. Intakes were measured to the nearest 1 ml at 0.5 and 2 h during each session.

The rats in the first group (control group, $n=20$) received a saline injection (1 ml normal saline/kg body weight sc) 30 min prior to each of the one-bottle training trials. The general opioid antagonist, naltrexone (NTX; Sigma) was administered subcutaneously 30 min prior to the one-bottle training trials to the three drug groups at doses of 0.1 (NTX 0.1 group, $n=10$), 1.0 (NTX 1.0 group, $n=10$), or 5.0 (NTX 5.0 group, $n=10$) mg/kg. Following training, all rats were treated with saline 30 min before being given two-bottle access (2 h/day) to unlimited amounts of CS+/S and CS−/S solutions on two consecutive days.

To determine the effect of naltrexone treatment on the expression of the CS+ preference, the control rats were given another seven 2-bottle test sessions following treatments with naltrexone or saline. Half of rats were injected with NTX at ascending doses of 0.1, 1.0, 2.5, and 5.0 mg/kg 30 min prior to the test sessions on the four odd-numbered days. The remaining rats were treated with the NTX doses in a descending order. All rats were given saline injections on the three even-numbered days.

2.4. Data analysis

Mean intakes averaged over the four 1-bottle training sessions with each CS were evaluated using a two-way analysis of variance for the control and three NTX groups as a between-subject variable and the CS+/F and CS−/S conditions as a within-subject variable. Mean intakes during the two posttraining CS+/S vs. CS−/S choice tests were similarly analyzed with groups and CS solutions as between and within factors, respectively. The effect of naltrexone on the expression of the CS preference was determined by analyzing the CS+/S vs. CS−/S intakes of the control rats following saline or drug treatment. Saline data were based on the average of the five saline tests. Preliminary analysis revealed no differences between the control rats tested with ascending and descending doses, and therefore only the combined data are presented. CS+ intakes during the two-bottle choice sessions were expressed as a percent of total intake, and these data were evaluated using analysis of variance.

3. Results

3.1. Naltrexone effects on training intakes

Fig. 1 presents the one-bottle training intakes of the CS+/F and CS−/S solutions of the four groups averaged over the four training days with each solution. Analysis of the 0.5-h data indicated that, overall, the groups differed in their CS intakes [$F(3,57)=67.07$, $P<.0001$], that CS+/F intakes were significantly higher than CS−/S intakes [$F(1,19)=31.46$, $P<.0001$], and that there was an interaction between groups and CS solutions [$F(3,57)=6.32$, $P<.001$]. Individual comparisons revealed that the CS+/F and CS−/S intakes of the control and NTX 0.1 groups were comparable and significantly higher than those of the NTX 1.0 group, which were in turn significantly higher than those of the NTX 5.0 group. CS+/F intake exceeded CS−/S intake only in the control group. Analysis of the 2-h data indicated that, overall, the groups continued to differ in their CS intakes [$F(3,57)=57.18$, $P<.0001$] and that there was an interaction between groups and CS solutions [$F(3,57)=2.83$, $P<.05$], but there was no difference between CS+/F and CS−/S intakes. Individual comparisons showed that the CS+/F and CS−/S intakes of the control and NTX 0.1

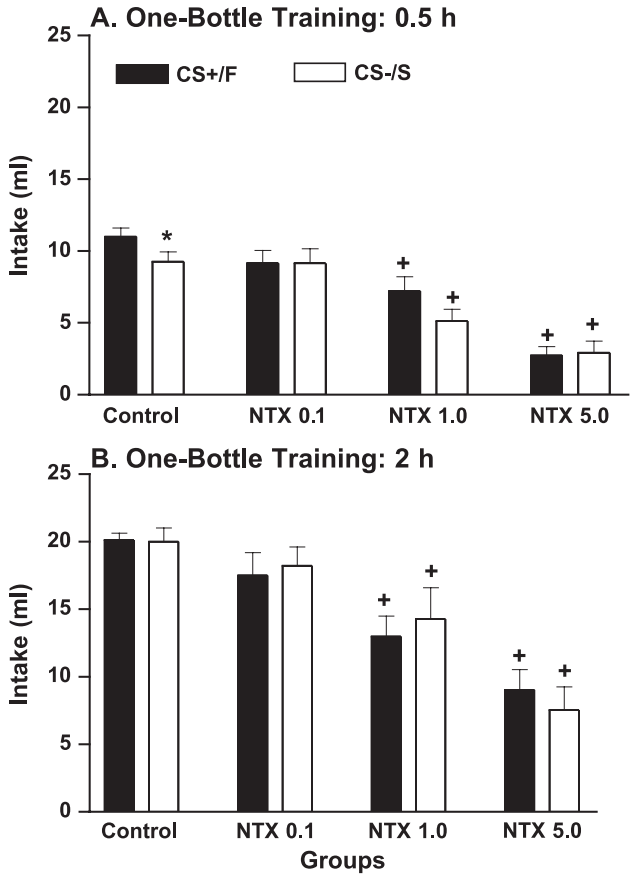

Fig. 1. Mean intakes (± S.E.M.) during one-bottle training sessions of flavored 8% fructose solution (CS+/F) and flavored 0.2% saccharin solution (CS − /S) after 0.5 h (A) and 2 h (B). The control group was injected with saline and the NTX 0.1, NTX 1.0, and NTX 5.0 groups were injected with naltrexone doses of 0.1, 1, and 5 mg/kg, respectively, 30 min prior to the training sessions. Significant differences between CS+/F and CS − /S intakes within a group are denoted by asterisks (* $P < .05$, Tukey comparisons). Significant differences in either CS+/F intake or CS − /S intake relative to the saline control training group are denoted by crosses (+ $P < .05$, Tukey comparisons).

groups were comparable and significantly higher than those of the NTX 1.0 group, which were in turn higher than those of the NTX 5.0 group.

3.2. Naltrexone effects on CS+ preference learning

The effect of the different drug treatments during training on preference conditioning was evaluated by comparing the two-bottle CS+/S vs. CS − /S intakes of the four groups following saline treatment (Fig. 2). Analysis of the 0.5- and 2-h data indicated that, overall, the rats consumed more CS+/S than CS − /S [0.5 h: $F(1,46) = 46.45$, $P < .0001$; 2 h: $F = 58.45$, $P < .0001$], and there were no group differences or Group × CS interactions. The four groups also did not significantly differ in their percent CS+ intakes (Fig. 2). The percent CS+ intakes of the control, NTX 0.1, and NTX 1.0 groups ranged from 72% to 80% and were slightly higher in the NTX 5.0 group (87%). Thus, despite the dose-dependent reduction in overall CS+ and CS − intakes during one-bottle training, the NTX 1.0 and NTX 5.0 groups displayed fructose-conditioned preferences indistinguishable from those of the control and 0.1 NTX groups.

3.3. Naltrexone effects on expression of CS+ preference

The effects of naltrexone treatment on the expression of the CS+ flavor preference were evaluated in a series of two-bottle tests conducted with the control rats. These were extinction tests in that the CS+ flavor was no longer paired with the fructose solution. A preliminary analysis revealed that half of the control rats ($n = 10$) displayed a robust CS+ preference that persisted from the first to the last two-bottle tests following saline treatment (90% to 83%). The remaining rats displayed weaker CS+ preference in the initial two-bottle test and lost their preference by the last saline test

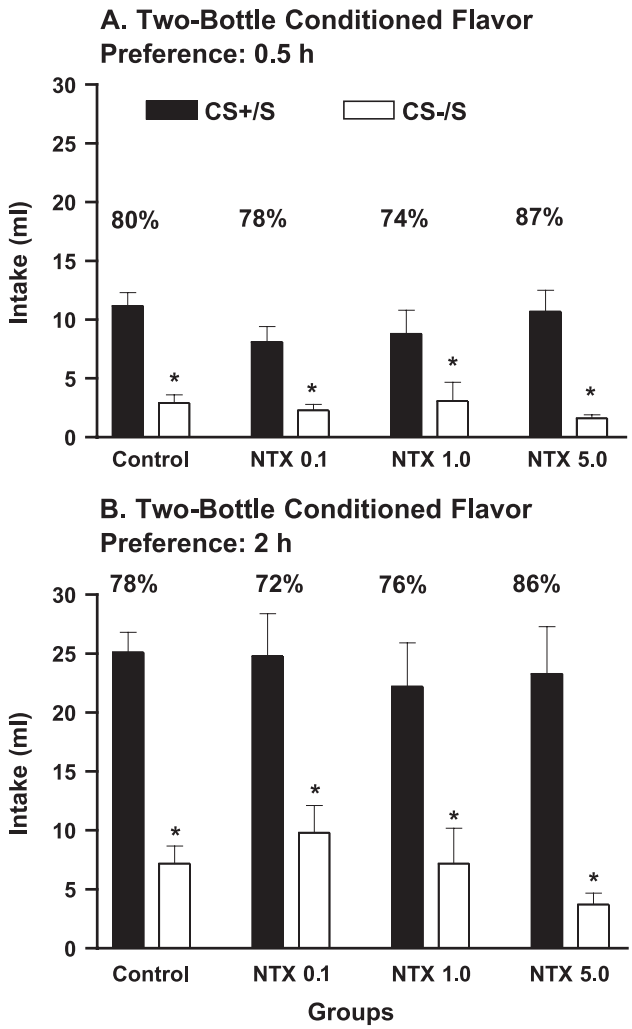

Fig. 2. Mean intake (± S.E.M.) after 0.5 h (A) and 2 h (B) of CS+/S vs. CS − /S solutions in the control, NTX 0.1, NTX 1.0, and NTX 5.0 groups during two-bottle preference tests. All groups were treated with saline prior to tests. Differences (Tukey comparisons, $P < .05$) between corresponding CS+ or CS − intakes are indicated by asterisks. The numbers atop the bars represent the percent of total intake consumed as CS+/S.

(68% to 53%). Only the subset ($n = 10$) of rats that showed a persistent conditioned flavor preference following saline treatment were included in the analysis of the drug effect on the expression of the CS+ preference.

As illustrated in Fig. 3, overall, the rats consumed more CS+ than CS − at the different naltrexone doses and the drug suppressed total CS intakes. Analysis of variance confirmed that there were CS main effects at both the 0.5- and 2-h time points [0.5 h: $F(1,9) = 60.467$, $P < .0001$; 2 h: $F(1, 9) = 76.464$, $P < .0001$] as well as a main effect of naltrexone dose on total intakes [0.5 h: $F(4,36) = 4.546$, $P < .0001$; 2 h: $F(4,36) = 9.944$, $P < .0001$]. There were also significant CS × Dose interactions at both time points [0.5 h: $F(4, 36) = 5.001$, $P < .001$; 2 h: $F(4,36) = 3.984$, $P < .01$]. This occurred because naltrexone reduced the intake ($P < .05$) of

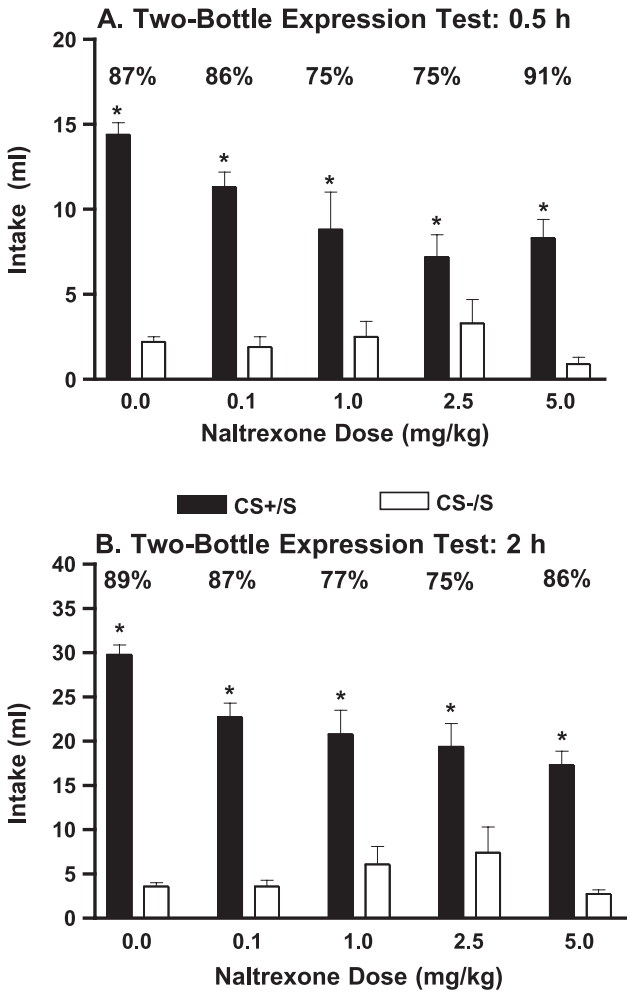

Fig. 3. Mean intake (± S.E.M.) after 0.5 h (A) and 2 h (B) of CS+/S vs. CS − /S solutions during two-bottle tests following treatment with saline and naltrexone (0.1 – 5 mg/kg) in the 10 control rats that displayed a robust CS+ preference that persisted from the first to the last two-bottle tests following saline treatment. Significant main effects for CS solution and drug dose as well as a CS × Dose interaction were observed. Significant differences between CS+/S and CS − /S intakes within a group are denoted by asterisks (* $P < .05$, Tukey comparisons). The numbers atop the bars represent the percent of total intake consumed as CS+/S.

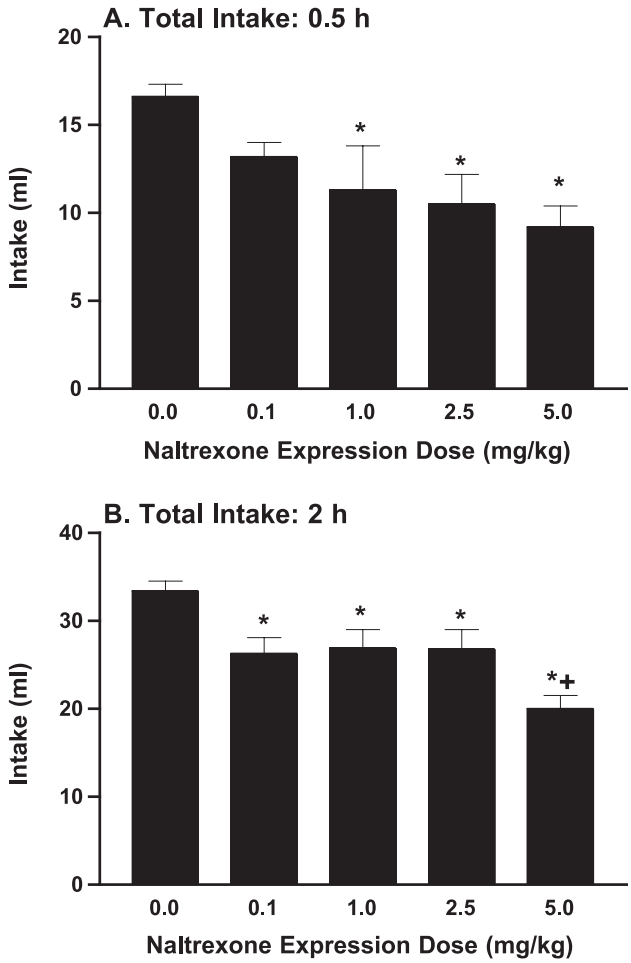

Fig. 4. Mean total intake (± S.E.M.) of CS+/S and CS − /S after 0.5 h (A) and 2 h (B) during two-bottle tests following treatment with saline (0 mg/kg) or naltrexone (0.1 – 5 mg/kg) in the control group. Asterisks denote significant intake differences between saline and all drug doses; crosses denote significant differences between 5-mg/kg dose and lower doses (Tukey comparisons, $P < .05$).

the CS+ but not the CS − . Nevertheless, CS+ intake exceeded ($P < .05$) CS − intake at all drug doses. During the first 0.5 h of testing, all doses of naltrexone suppressed ($P < .05$) total CS intake with respect to saline treatment, and intakes following the different doses did not differ from each other (Fig. 4). All drug doses also suppressed CS intake at 2 h and, in addition, intakes were lower ($P < .05$) after the 5.0-mg/kg dose compared to the lower naltrexone doses. Percent CS+ intakes fluctuated following the various naltrexone doses (Fig. 3), but there were no significant effects of naltrexone dose on this measure of CS+ preference at the 0.5- or 2-h time points.

4. Discussion

The sweet taste of sugar is a potent reward for rats and many other species, and there is a considerable evidence that the "sweet tooth" is mediated in part by the brain opioid

system. This was first suggested by reports that naloxone and naltrexone suppressed the intake of sugar and saccharin solutions in rats (LeMagnen et al., 1980; Sclafani et al., 1982), which is confirmed by the present results. Naltrexone reduced the intakes of the flavored fructose and saccharin solutions throughout training at 1- and 5-mg/kg doses, and reduced flavored saccharin intakes during two-bottle testing at doses of 0.1–5 mg/kg. As briefly reviewed in the Introduction and in greater detail elsewhere (Kelley et al., 2002; Levine et al., 2003; Yamamoto, 2003; Yeomans and Gray, 2002), a variety of findings indicate that the opioid system is specifically involved in the hedonic evaluation of sweet-tasting foods and fluids.

Sweet tastants, in addition to being primary rewards that elicit avid ingestive responses, can also condition preferences for associated flavors; that is, they can condition secondary rewards. This was first demonstrated by Holman (1975) who trained rats with distinctively flavored sweet (0.32%) and less sweet (0.065%) saccharin solutions. In subsequent choice tests with both flavors presented at the same saccharin concentration, the rats preferred the flavor that had been paired with the sweeter solution. Other studies have conditioned flavor preferences with the sweet taste of sucrose (Breslin et al., 1990; Myers and Hall, 2000; Yu et al., 1999). These later studies minimized the postingestive actions of sucrose as a conditioning factor by limiting the amount of sucrose consumed during training (Breslin et al., 1990; Myers and Hall, 2000) or by using a gastric sham-feeding procedure during training and testing (Yu et al., 1999). The present study used fructose rather than sucrose as the sweet reward because fructose, unlike other sugars, has little or no postingestive reinforcing action during short-term training sessions (Sclafani and Ackroff, 1994; Sclafani et al., 1999). In confirmation of prior reports, the control rats displayed a significant preference for the fructose-paired flavor (CS+) over the saccharin-paired flavor (CS−) when both were presented in saccharin solutions (Sclafani and Ackroff, 1994; Baker et al., 2003). This CS+ preference is attributed to rats associating the CS+ flavor with the sweet taste of the fructose solution consumed during training.

Like controls, the rats in the three naltrexone groups displayed significant preferences for the fructose-paired CS+ flavor although the drug, at the 1- and 5-mg/kg doses, suppressed the intake of the flavored fructose and saccharin solutions during training. Furthermore, pretreating the control rats with naltrexone (0.1–5.0 mg/kg) did not the block their expression of the CS+ preference during the two-bottle choice tests. That is, although naltrexone selectively reduced the intake of the CS+ during the preference tests, the rats continued to consume more CS+ than CS− at all dose levels. The percent CS+ preference was lower (75–77%) at some naltrexone doses, compared to the saline baseline (87–89%), but these differences did not achieve statistical significance. These findings confirm and extend the prior report of Yu et al. (1999) that a 0.1-mg/kg naltrexone dose did not prevent the establishment of a flavor preference conditioned by the sweet taste of sucrose in sham-feeding rats nor did the drug (0.1–5 mg/kg) block the expression of the sucrose-conditioned flavor preference when administered before the two-bottle tests.

The present results contrast with the report of Mehiel (1996) that naloxone (4 mg/kg) blocked the development of a preference for a flavor mixed into a glucose solution over a flavor paired with a less preferred saccharin solution. As noted in the Introduction, however, the rats in their experimental group were treated with naloxone only on CS+/sucrose training days and their lack of preference for the CS+ flavor may be related in part to an association between the CS+ flavor and potential aversive effects of the drug. Naltrexone (0.5 mg/kg) was also reported to block a sucrose-induced preference for an orange odor in 6-day-old rat pups as measured in a modified place preference paradigm (Shide and Blass, 1991). There was no CS− odor in this study and the possibility that an odor–drug association interfered with an odor–sucrose association cannot be ruled out. In adult rats, naltrexone (0.1–5.0 mg/kg) did not prevent the development of a sucrose-conditioned place preference when the drug was administered on training trials with the sucrose-paired place and water-paired place, but did attenuate the expression of an already formed place preference (Delamater et al., 2000). Another recent study reported that chronic naltrexone treatment inhibited the redevelopment of a sucrose-diet preference in animals offered the choice of a sucrose diet vs. starch diet but had little effect on the expression of an established preference (Levine et al., 2002). This latter study did not investigate flavor preferences conditioned by sweet taste, therefore the results, while interesting from a "diet" relapse perspective, are not relevant to the present conditioning data.

The failure of naltrexone to block sucrose- or fructose-conditioned flavor preferences appears to be inconsistent with the ideas that (a) opioid antagonists suppress the hedonic evaluation of sweet taste and/or that (b) sweet taste reinforces flavor preferences through a hedonic conditioning process. These apparent inconsistencies are discussed below.

While the opioid modulation of taste hedonics is well supported, other neurochemical systems are implicated in the hedonic response to sweet and other palatable tastants. In particular, several studies indicate that benzodiazepine receptors participate in the palatability evaluation of foods and fluids (see reviews: Berridge and Pecina, 1995; Cooper and Higgs, 1996). This may explain why opioid antagonists suppress but typically do not completely block the consumption of sweet solutions. A related point is that opioid antagonists typically do not reduce the initial response to sweet rewards during the first minutes of testing in experiments involving ingestive, operant, or TR measures (Beczkowska et al., 1992; Ferraro et al., 2002; Frisina and Sclafani, 2002; Kirkham and Cooper, 1988a,b; Leventhal et al., 1995; Schwarz-Stevens et al., 1992; but see Higgs and Cooper, 1998). These results suggest that the opioid system is not involved in all aspects of taste hedonics, but may be

primarily involved in the response-sustaining effect of palatable foods and fluids. Thus, naltrexone may fail to prevent flavor conditioning by sugar solutions because the drug has little effect on the animal's hedonic evaluation of the sugar and saccharin solutions early in the training sessions, which may be the basis for the conditioned flavor preference. Consistent with this view, intraoral infusion studies indicate that flavor preferences can be conditioned by small amounts of a sugar solution (Breslin et al., 1990; Myers and Hall, 2000). This interpretation may also explain why naltrexone has relatively little effect on the expression of the conditioned CS+ preference. That is, the rats' choice of the CS+ over the CS − in the two-bottle tests may be determined by their evaluation of the flavors early in the test session while their hedonic evaluation was unaffected by naltrexone.

The role of hedonics in flavor conditioning also requires consideration. According to Berridge (1996), food reward involves two separate processes: a hedonic component and an incentive salience component. The hedonic value of food is inferred by TR tests, which measure orofacial responses evoked by intraoral infusions of tastants, whereas the incentive value is inferred by instrumental approach responses to foods and fluids (Berridge, 1996). Berridge (1996) further proposes that opioids are primarily involved in the hedonic process, while dopamine systems are primarily involved in the incentive process. Manipulations that influence food reward typically impact on both food hedonics and incentive, but in some situations, only one or the other process may be affected. With respect to flavor conditioning, it is possible that conditioned preferences reflect increased incentive value in addition to, or instead of, increased hedonic value. Support for this view is provided by TR analysis of flavor preference conditioning by IG sugar infusions. In one study, rats were trained with flavored saccharin solutions as conditioned stimuli and they displayed a strong CS+ preference in two-bottle tests and an increased hedonic response to the CS+ in TR tests compared to the CS − (Myers and Sclafani, 2001). In a second study with bitter and sour CS solutions, rats also showed a strong CS+ preference in the two-bottle test, yet their TR responses to the CS+ and CS − did not differ (Myers and Sclafani, 2002). These data indicate that the conditioning of a strong flavor preference does not necessarily require a shift in the hedonic evaluation of the flavor as measured by TR responses. Whether a similar situation exists in the case of flavor preferences conditioned by sweet taste is not certain. Pairing a bitter or sour CS+ with intraoral infusions of sucrose has been reported to increase the hedonic TR response to the CS+ (Breslin et al., 1990). It is not known, however, whether this hedonic shift is necessary for the establishment or expression of a sweet-taste-conditioned CS+ preference. It is possible that treating rats with naltrexone during training with flavored fructose and saccharin solutions may have blocked the conditioning of a hedonic response to the CS+ flavor, yet the rats may have still preferred the CS+ in two-bottle tests because of a conditioned incentive response to the CS+. This interpretation can be tested by comparing the effects of naltrexone on CS+ preference and TR responses.

In contrast to the minimal effect of naltrexone on the acquisition and expression of the fructose-conditioned flavor preference observed here, we recently reported that D1 (SCH23390) and D2 (raclopride) antagonists completely blocked both CS+ preference acquisition and expression of fructose-conditioned flavor preferences (Baker et al., 2003). The same dopamine antagonists interfered with sucrose-conditioned flavor preferences in sham-feeding rats which are unaffected by naltrexone (Yu et al., 1999, 2000a,b). Together, these data indicate that flavor conditioning by the sweet taste of sugar is modulated by dopamine but not opioid receptors. Other findings suggest a similar receptor pharmacology of flavor preference learning produced by the postingestive actions of sugar. That is, dopamine antagonism, but not opioid antagonism, prevented rats from developing a preference for a CS+ flavor paired with IG infusions of sucrose (Azzara et al., 2000, 2001). In this case, however, only the D1 antagonist blocked preference learning. According to the model of Berridge (1996), these drug actions suggest that conditioned flavor preferences, at least as measured by two-bottle tests, are modulated by a dopamine incentive salience system rather than by an opioid-based hedonic system. This interpretation, as well as the role of other neurotransmitter systems in flavor learning, requires further investigation.

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