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Naltrexone suppresses the late but not early licking response to a palatable sweet solution: opioid hedonic hypothesis reconsidered

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Abstract

Opioid antagonists suppress the intake of sweet solutions, but typically have little effect on the initial rate of drinking. The lack of an early drug response was investigated in the present study because it questions the general idea that opioid antagonists reduce the hedonic response to sweets. The first experiment, which measured the rat's licking response to a sucrose + saccharin (S + s) solution, revealed that naltrexone suppressed S + s intake but not initial lick rates. Experiment 2A indicated that the drug's delayed behavioral effect was not due to the 10-min injection-test interval used. Increasing the interval to 20 min did not reduce the latency of drug action. Experiment 2B tested the idea that rats require several minutes to detect that naltrexone has reduced the hedonic value of the S + s solution. The S + s solution was presented either for 30 min without interruption or for 3 min followed, after a 6-min delay, by another 27-min access. In both test conditions, naltrexone did not suppress S + s licking until 7–9 min of drinking had occurred. However, the drug blocked an "appetizer effect"; a post-delay increase in licking rate produced by the split-session test procedure. Microstructure analysis indicated that in all cases, naltrexone reduced S + s licking by reducing the number of lick clusters rather than lick cluster size. In contrast to these drug effects, Experiment 2C showed that reducing the concentration of the S + s solution decreased initial lick rates. Together, these findings suggest that opioid antagonists do not affect all aspects of flavor hedonics, but may primarily alter the intake-maintaining action of palatable flavors.

Keywords: Sucrose+saccharin; Palatability; Lick microstructure; Oral habituation

1. Introduction

It is well documented that manipulations of brain opioid systems alter food and fluid consumption (Cooper et al., 1988; Reid, 1985; Glass et al., 1999b). This is demonstrated by the intake reductions produced by administration of opioid antagonists and increased intakes produced by opioid agonists. Although more than one mechanism may be involved in these effects (Glass et al., 1999a), there is considerable evidence suggesting that opioid drugs alter food and fluid consumption in part by modifying the palatability or hedonic response to flavor stimuli (Cooper and Kirkham, 1993; Kelley et al., 2002). Many studies report, for example, that general opioid antagonists (naloxone and naltrexone) decrease the intake of preferred foods and fluids more than the intake of less preferred items (Cooper and Turkish, 1989; Weldon et al., 1996; Giraudo et al., 1993; Sclafani et al., 1982). More specific evidence that these intake reductions involve an alteration in the hedonic evaluation of flavor stimuli is provided by taste reactivity and sham-feeding experiments. Naltrexone is reported to reduce the positive ingestive response to intraoral infusions of sucrose while having no effect on the negative responses to quinine infusions (Parker et al., 1992). Sham-feeding studies show that naloxone reduces the sham intake of sucrose in a manner analogous to reducing sucrose concentration (Kirkham and Cooper, 1988a,b, 1989; Rockwood and Reid, 1982). Complementary to these findings are reports that the consumption of palatable foods and fluids increases β-endorphin levels in the brain (Dum et al., 1983; Yamamoto et al., 2000). In particular, intake of both sucrose and saccharin solutions increased β-endorphin levels and this effect was blocked by gustatory nerve transection as well as by the prior formation of a conditioned taste aversion to sucrose (Yamamoto et al.,

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2000). The latter finding indicates that it is the hedonic rather than the sensory quality of sucrose that activates the brain endorphin system. Related to this point, naloxone treatment that reduces sucrose intake does not appear to impair sweet taste discrimination (Ohare et al., 1997). Taken together, these findings indicate an important role of brain opioid receptors in the hedonic response to food and fluids.

A major inconsistency with the general idea that the opioid system mediates food and fluid palatability is the common finding that opioid antagonists have a delayed suppressive effect on ingestion. For example, in sham-feeding tests naloxone injections did not reduce the rate of sucrose drinking until about 10 min into the test (Kirkham and Cooper, 1988a,b). Similarly, in taste reactivity tests, naltrexone failed to suppress ingestive responses to intraoral sucrose during 1-min intraoral infusion tests (Ferraro et al., 2002; Parker et al., 1992); ingestive responses were reduced only over 10-min test periods (Parker et al., 1992). Operant studies also indicate that naloxone does not affect initial rates of responding for food rewards but suppresses responding later in the session (Schwarz-Stevens et al., 1992; Kirkham and Blundell, 1986). There are some data showing an early effect of naloxone on food consumption (Higgs and Cooper, 1998), but these are the exceptions. Manipulations of palatability produced by altering sugar concentration or by aversion and preference conditioning are evident in the first few minutes of testing (Booth, 1985; Parker et al., 1992; Davis, 1973; Davis and Levine, 1977; Myers and Sclafani, 2001). Thus, the failure of opioid antagonists to reduce initial appetitive and consummatory responses suggests that the hedonic interpretation of drug action needs to be reconsidered.

The present study investigated in further detail the effect of the opioid antagonist naltrexone on the rat's early and late ingestive response to a palatable sweet solution. Licking rate and microstructure were analyzed based on prior studies showing that these are sensitive measures of solution palatability and drug effects (Davis, 1973; Davis and Levine, 1977; Davis and Smith, 1992). The first experiment, which compared the effects of naltrexone dose and deprivation state on the consummatory response to a sucrose + saccharin solution, revealed the drug's delayed suppressive effect on licking. The second series of experiments investigated possible explanations for this delayed response. These included a pharmacokinetic explanation, i.e., optimal drug levels occurred after the onset of drinking (Experiment 2A), and a reward desensitization explanation, i.e., that rats had a reduced sensitivity to reward devaluation as a result of extensive testing experience (Experiment 2C). The central hypothesis examined (Experiment 2B) was that it takes several minutes for naltrexone-treated rats to detect the reduced hedonic impact of the familiar sweet stimulus because the drug does not alter the taste intensity of the stimulus. This hypothesis was investigated by having the rats drink the test solution for 3 min followed by a short delay (6 min) before returning the solution for the remainder of the drinking session. Based on our hypothesis, it was predicted that naltrexone would immediately suppress the rate of licking when the solution was returned after the short delay.

2. Experiment 1

This experiment determined the time course over which naltrexone treatment suppresses the licking of a sweet solution. Food deprivation state and naltrexone dose were varied to determine the effective range of intake suppression produced by the drug. The test solution was a mixture of 2% sucrose and 0.2% saccharin because such dilute sugar–saccharin mixtures are highly palatable to rats while having minimal postingestive effects (Smith and Foster, 1980). We previously reported that naloxone was very effective in reducing 30-min intakes of a glucose + saccharin mixture, but the time course of this effect was not measured (Sclafani et al., 1982). The present experiment therefore analyzed licking behavior at a millisecond resolution to provide a detailed temporal description of the drug response. A sucrose + saccharin mixture was used because it is preferred by rats to a glucose + saccharin mixture (Sclafani et al., 1987).

2.1. Method

2.1.1. Subjects

Twelve adult female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were used. The rats were singly housed in a vivarium under a 12:12 light–dark cycle and were fed Laboratory Rodent Diet 5001 (PMI Nutrition International, Brentwood, MO) and tap water.

2.1.2. Apparatus

The animals were tested in eight plastic cages (23 × 24 × 31.5 cm) located in a room near the vivarium. The rats had access to one or two stainless steel drinking spouts through holes at the front of the cage. The spouts were attached to bottles fixed in a motorized retractor that automatically inserted and removed the spouts at the beginning and end of the test session. Small pans located below the spouts collected any spillage. Fluid intakes were measured by weighing the drinking bottles and spillage pans to the nearest 0.1 g before and after each test session. Licking patterns were recorded using electronic drinkometers and a microcomputer that stored the time of each lick to the nearest 1 ms.

2.1.3. Procedure

The rats were initially trained to drink in the test cages by placing them in a cage overnight with food and fluid. Water and a solution containing a mixture of 2% sucrose (commercial brand) and 0.2% sodium saccharin (Sigma, St. Louis, MO) were available for 30 min every hour using the bottle

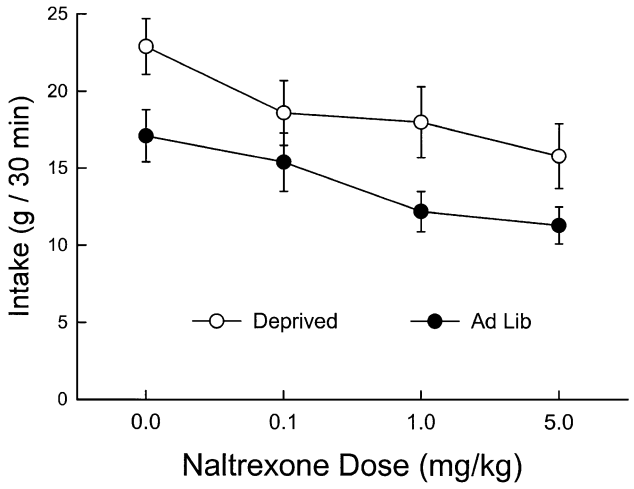

Fig. 1. Experiment 1. Mean (±S.E.M.) intake of the 2% sucrose+0.2% saccharin solution following injections of naltrexone at doses of 0 (saline), 0.1, 1 and 5 mg/kg during drinking tests conducted under food deprived and food ad lib conditions.

retractor. The rats were then food restricted and maintained at 85% of ad lib body weight and adapted to drink the 2% sucrose+0.2% saccharin solution (S+s) during six daily sessions. For each session, the rats were placed in the test cages and 10 min later the drinking spout was made available for 30 min. The rats were then returned to their home cages and given their food rations 1 h later. The left–right position of the drinking spouts was alternated daily.

The rats were next injected subcutaneously with isotonic saline (1 ml/kg body weight) 10 min prior to the start of the 30-min drinking session for five sessions. This was followed by naltrexone injections at doses of 0.1, 1 and 5 mg/kg, in that order. Successive drug sessions were separated by two saline sessions. The rats were then given ad libitum access to food for the remainder of the experiment. Following a day without testing, they were given five saline sessions and then naltrexone tests at 0.1, 1 and 5 mg/kg doses. The drug sessions were again separated by two saline sessions.

2.1.4. Statistical analysis

Solution intakes during the saline and drug sessions conducted under food restricted and ad libitum conditions were compared with analysis of variance (ANOVA; deprivation state vs. drug dose). The saline dose (0 mg/kg) represented the average intake in the saline sessions preceding each drug injection. The initiation and rate of licking were analyzed in terms of the latency to the first lick and lick rates averaged over 3-min periods during the course of the 30-min session. Lick microstructure was analyzed using the procedures of Davis (1990) (Davis et al., 1993). A lick cluster was defined as a period of sustained licking containing pauses no longer than 500 ms. Both mean lick cluster size and number were calculated and evaluated with ANOVA.

2.2. Results

Fig. 1 summarizes the intake results from the deprived and ad lib test series. Overall, the rats drank more S+s when food deprived than when nondeprived [$F(1,11)=19.01$, $P<.01$] and naltrexone suppressed intake relative to the saline treatment [$F(3,33)=48.46$, $P<.001$]. There was no drug × deprivation state interaction. Given the similar dose–response patterns obtained in the deprived and ad lib tests, further data analysis was restricted to the ad lib test data. Also, analysis is limited to the 1 and 5 mg/kg doses because the 0.1 mg/kg dose did not reduce ad lib S+s intake.

Fig. 2 presents the lick data for the 1 mg/kg naltrexone dose expressed as lick rates during successive 3-min periods during the 30-min tests. Overall, the 1 mg/kg dose reduced the rate of licking, relative to the saline baseline [$F(1,11)=33.40$, $P<.01$], lick rates declined over time [$F(9,99)=38.08$, $P<.01$] and there was a Drug × Time interaction [$F(9,99)=2.17$, $P<.05$]. In particular, 1 mg/kg naltrexone did not significantly reduce lick rates until the fourth 3-min period (10–12 min). The lick results obtained with the 5 mg/kg dose were very similar and are not presented in graphical form here. The high naltrexone dose did not significantly suppress 3-min lick rates until the fourth 3-min period and rates were suppressed for all remaining periods except for period 5 [Drug × Time interaction: $F(9,99)=3.67$, $P<.01$]. Consistent with the lack of effect on initial lick rates, naltrexone did not affect the latency to begin drinking relative to the saline baseline (8.7, 3.3 and 6.9 s at 0, 1 and 5 mg/kg doses, respectively).

The lick microstructure data are presented in Table 1. Overall, there was a main effect of drug treatment on lick cluster size [$F(3, 33)=3.40$, $P<.05$]. However, naltrexone did not alter cluster size relative to the saline baseline and the only significant difference was between the 0.1 and 5

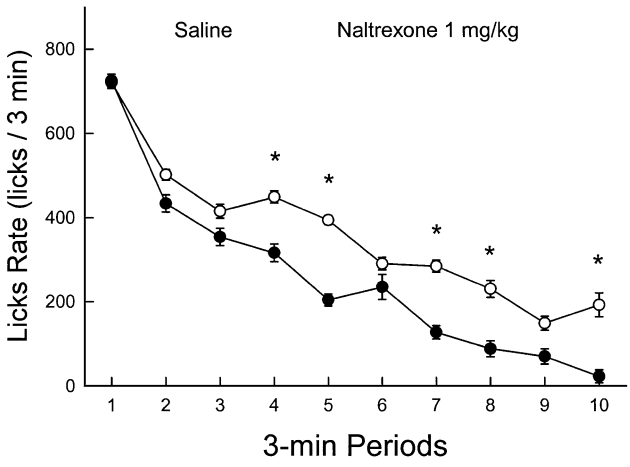

Fig. 2. Experiment 1. Mean (±S.E.M.) rate of licking in successive 3-min periods following treatment with saline or naltrexone (1 mg/kg). Asterisks indicate significant difference ($P<.05$) between naltrexone and saline lick rates.

Table 1
Lick microstructure as a function of drug, test condition and solution concentration

Experiment 1: ad lib	Saline	0.1-mg/kg NTX	1.0-mg/kg NTX	5.0-mg/kg NTX
Cluster size	46.8 (6.0)[abc]	51.3 (6.1)[ab]	48.1 (6.9)[abc]	41.7 (5.1)[ac]
Cluster number	81.9 (8.7)[a]	62.6 (4.8)[b]	60.7 (9.2)[b]	59.3 (6.9)[b]
Experiment 2A: 1 mg/kg	10-min saline	10-min NTX	20-min saline	20-min NTX
Cluster size	36.1 (2.6)	34.8 (3.0)	36.8 (3.4)	34.3 (3.4)
Cluster number	98.6 (9.6)[a]	72.7 (7.5)[b]	100.5 (10.1)[a]	84.5 (11.3)[b]
Experiment 2B: 1 mg/kg	Saline split	NTX split	Saline no-split	NTX no-split
Cluster size: 3 min	97.5 (19.5)	92.0 (17.8)	75.5 (12.5)	67.3 (12.0)
Cluster number: 3 min	10.9 (1.1)	11.7 (1.2)	11.5 (1.0)	12.0 (1.4)
Cluster size: 27 min	35.2 (2.6)	37.8 (5.0)	39.2 (3.8)	38.6 (4.4)
Cluster number: 27 min	93.4 (7.3)[a]	54.6 (9.2)[b]	86.3 (8.7)[a]	55.4 (8.5)[b]
Experiment 2C	2%+0.2% S+s 3 min	1%+0.1% S+s 3 min	2%+0.2% S+s 27 min	1%+0.1% S+s 27 min
Cluster size	106.4 (17.4)	74.0 (10.8)	39.4 (4.0)	39.8 (4.8)
Cluster number	10.9 (0.9)	12.2 (1.4)	85.3 (10.1)[a]	56.9 (6.6)[b]

Data represent Mean and (SEM). Cluster size refers to licks per cluster. Values with common superscript do not differ at the $P<.05$ level.

mg/kg doses. There was also a significant drug effect on the number of lick clusters [$F(3, 33)=6.38$, $P<.01$]. In this case, cluster number was reduced ($P<.05$), relative to saline baseline, by all doses of naltrexone, which did not did differ among themselves.

2.3. Discussion

These results confirm the ability of opioid antagonists to reduce the intake of sweet solutions (Rockwood and Reid, 1982; Sclafani et al., 1982; Kirkham and Cooper, 1988a,b). Food restriction increased total intake but did not alter the dose–response pattern. Similar results were obtained with water-restricted and nonrestricted rats tested with a glucose+saccharin solution and various doses of naloxone (Sclafani et al., 1982). The nondeprived rats were presumed to drink the sugar+saccharin mixture for its palatable sweet taste rather than to satisfy energy or hydrational needs. Therefore, data analysis in this and the subsequent experiment focused on results obtained with nondeprived animals.

Analysis of the lick data revealed that the 1 and 5 mg/kg doses of naltrexone did not alter the latency to start drinking and only began to suppress drinking, relative to the saline baseline, by about 10 min after the start of the session. This is consistent with prior findings obtained with rats sham-drinking a 10% sucrose solution after treatment with naloxone (1.25 mg/kg sc) (Kirkham and Cooper, 1988a,b). More recently, Higgs and Cooper (1998) reported that naloxone (3 mg/kg ip) suppressed licking for sucrose solutions during 1-min drinking tests. This finding is one of the few examples of an early opioid drug effect on ingestion. Analysis of the present lick microstructure data indicated that naltrexone suppressed drinking by reducing the number of lick clusters, but did not reduce mean cluster size. This confirmed the lick microstructure findings of Higgs and Cooper (1998) obtained with naloxone-treated rats. Since decreasing sucrose concentration, which is presumed to decrease palatability, reduces initial lick rates and lick cluster size (Davis, 1973; Davis and Levine, 1977; Davis and Smith, 1992; Higgs and Cooper, 1998), the present results obtained with naltrexone question the general idea that the opioid system mediates palatability.

A potential limitation of the present dose–response data is that the naltrexone doses were presented only in an ascending order. It is possible that different results would have been obtained if a random or counterbalanced dose schedule was used. Note, however, that a prior lick rate study (Siviy et al., 1982) found no effect of dose order on the drinking suppression produced by naloxone.

3. Experiment 2

This series of experiments investigated possible reasons why naltrexone treatment does not suppress the initial drinking response to a sucrose+saccharin solution. One simple explanation is that the 10-min interval between drug injection and the start of testing was too short to allow the drug to reach optimal levels at central receptor sites before the start of the drinking session. This seems unlikely because prior studies using longer injection-test intervals (15–30 min) have reported delayed suppressive effects on ingestive behavior (Siviy et al., 1982; Cooper and Holtzman, 1983; Kirkham and Blundell, 1986; Kirkham and Cooper, 1988a,b). Nevertheless, to exclude this possibility, Experiment 2A compared the effects of 10- and 20-min injection-test intervals on the drinking suppression produced by naltrexone. The 20-min interval was selected because Experiment 1 indicated that naltrexone began to suppress intake at 10 min after the start of drinking, or 20 min after the drug injection.

3.1. Experiment 2A

3.1.1. Method

Eighteen adult female Sprague–Dawley rats were initially trained to drink the S+s solution in the testing apparatus while food restricted as described in Experiment 1. They were then fed ad libitum and given additional training sessions. One rat was an inconsistent drinker when nondeprived and was removed from the study. The rats were divided into two subgroups and received injections of saline or 1 mg/kg naltrexone. Half of the rats were first tested with the injections 10 min prior to the drinking sessions, followed by a series with the injections 20 min prior to the sessions. The remaining subjects were given the injections in the reverse order. Each series included two treatments with 1 mg/kg naltrexone preceded by two or three saline sessions.

3.1.2. Results and discussion

Overall, 30-min intakes did not vary as a function of injection-test interval, and naltrexone reduced S+s intake relative to the saline baseline [$F(1,16)=51.39$, $P<.01$] under both test conditions. Intakes following saline and naltrexone were 15.4 and 10.4 g/30 min, respectively, in the 10-min interval tests, and 15.9 and 11.5 g/30 min, respectively, in the 20-min interval tests. Analysis of the lick rate data further revealed that naltrexone reduced the rate of licking relative to saline treatment [$F(1,16)=39.92$, $P<.01$], lick rates declined over session time [$F(9,144)=92.06$, $P<.001$] and there was an interaction between drug and session time [$F(9,144)=3.13$, $P<.01$] (Fig. 3). Most importantly, there were no differences between the two injection interval conditions (10 vs. 20 min) or significant interactions between interval and drug or time. Individual analysis of the 10- and 20-min interval tests revealed that naltrexone did not significantly reduce lick rate until the third and fourth 3-min periods, respectively (Fig. 3). The latency to initiate licking was also not affected by the injection-test interval or drug condition.

The lick microstructure analysis indicated that, overall, naltrexone reduced lick cluster number [$F(1,16)=8.12$, $P<.05$], but not lick cluster size relative to saline treatment (Table 1). There were no main or interactive effects of test condition and drug for either cluster size or number.

These data demonstrate that the delayed onset of naltrexone's suppressive action on S+s licking is not unique to the 10-min injection test interval. When the interval was extended to 20 min a similar delayed onset was observed; in fact, it was slightly longer with the 20-min injection-test interval. Additionally, the lick microstructure parameters were not affected by the injection-test intervals. In both conditions, naltrexone significantly reduced cluster number while having no effect on mean cluster size.

3.2. Experiment 2B

This experiment investigated an alternative explanation for the delayed suppressive effect of naltrexone on S+s drinking. Because opioid antagonists do not appear to alter taste perception per se (Ohare et al., 1997), it may take the naltrexone-treated rats several minutes to detect that the sweet taste of the S+s solution is no longer producing the same hedonic reward that they had come to expect. According to this view, providing naltrexone-treated rats with brief access to the solution followed by a period of no drinking may allow them time to experience the reduced hedonic impact of the S+s taste so that when the solution is returned a few minutes later their drinking response would be immediately suppressed. We evaluated this hypothesis by testing the rats under two conditions. In the continuous (no-split) session condition, they were injected with saline or naltrexone and 10 min later were presented with the S+s for a 30-min drinking session as in the prior experiments. In the split-session condition, the drinking tube containing the S+s was presented for 3 min and then automatically removed from the cage for 6 min before being returned for a final 27-min period. Thus, the drinking tubes were returned 9 min after the start of drinking session, which is

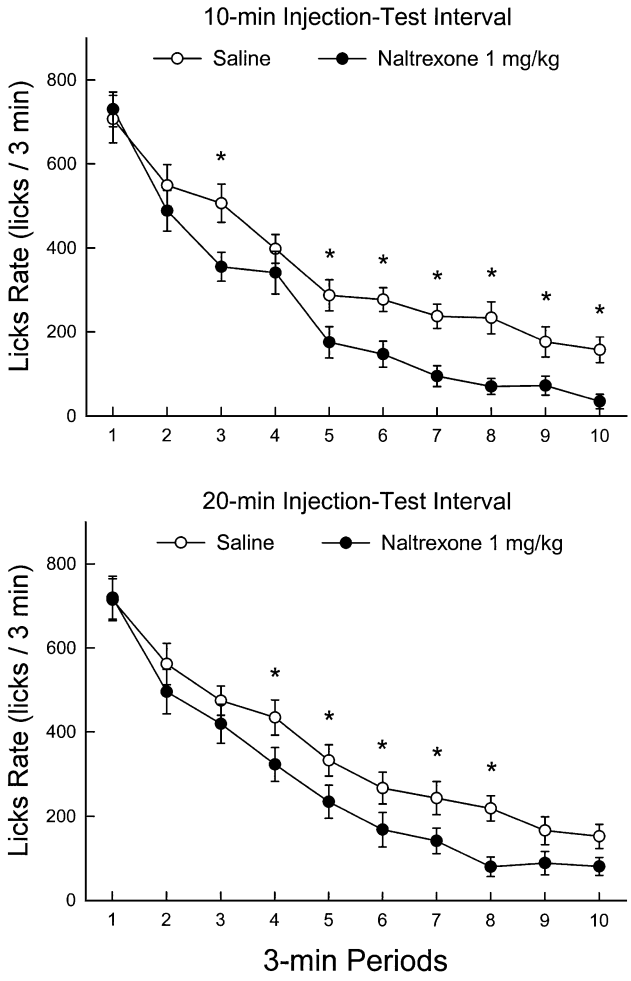

Fig. 3. Experiment 2A. Mean (±S.E.M.) rate of licking in successive 3-min periods following treatment with saline or naltrexone (1 mg/kg) 10 and 20 min prior to drinking session. Asterisks indicate significant difference ($P<.05$) between naltrexone and saline lick rates.

about the time the rats began to reduce their licking in Experiment 2A.

3.2.1. Method

The 17 rats from the previous experiment were divided into two subgroups. One subgroup was tested first with the split session procedure and then with the no-split session procedure; the second subgroup was tested in the reverse order. The animals were given three to five saline sessions to adjust to the test condition. They were then injected with 1 mg/kg of naltrexone followed by another two saline sessions and a second naltrexone injection. All injections were administered 10 min prior to the beginning of the drinking session. Data analysis was based on the means of the two naltrexone sessions and two preceding saline sessions under each test condition.

3.2.2. Results

Overall, S + s intakes did not vary as a function of test condition (split vs. no-split session) and naltrexone reduced intake to a similar degree (37%) in the two conditions [$F(1,16) = 103.54$, $P < .01$]. Intakes following saline and naltrexone were 17.1 and 10.8 g/30 min, respectively, in the no-split tests, and 18.0 and 11.4 g/30 min, respectively, in the split-session tests.

Analysis of the 3-min lick rate data, which are presented in Fig. 4, indicated that there was no main effect of test condition on lick rates but there were interactions between test condition and 3-min periods [$F(9,144) = 29.36$, $P < .001$] and drug and period [$F(9,144) = 7.72$, $P < .01$]. Therefore, separate analyses were performed for each test condition. In the no-split condition, naltrexone reduced lick rate as a function of period [$F(9,144) = 3.96$, $P < .01$]. Individual tests indicated that the drug reduced ($P < .05$) lick rates during periods 3 to 10, but not during periods 1 and 2. A similar Drug × Time interaction was obtained in the split test condition [$F(9,144) = 5.95$, $P < .01$] with naltrexone again reducing lick rates in 3-min periods 3–10, but not periods 1 and 2. This similar delay in drug action occurred despite the 6-min delay between the first and second 3-min periods of drinking in the split test condition. While not affecting the onset of the drug effect, the 6-min delay did alter the pattern of drinking. That is, in the split-session condition, unlike the no-split condition, the rats did not reduce their rate of licking from the first to the second 3-min period. Analysis of the pre- and post-split licking data revealed that the rats increased their licking rate from periods 1 to 2 when treated with saline, but not when treated with naltrexone [Drug × Period interaction: $F(1,16) = 4.69$, $P < .05$].

The lick microstructure data were evaluated separately for 1–3 and 4–30 min of drinking (Table 1). Naltrexone had no effect on lick cluster size or number during the first 3-min period. There was a main effect of test condition, however, with cluster size being larger in the first 3 min of drinking in the split-session condition than in the no-split

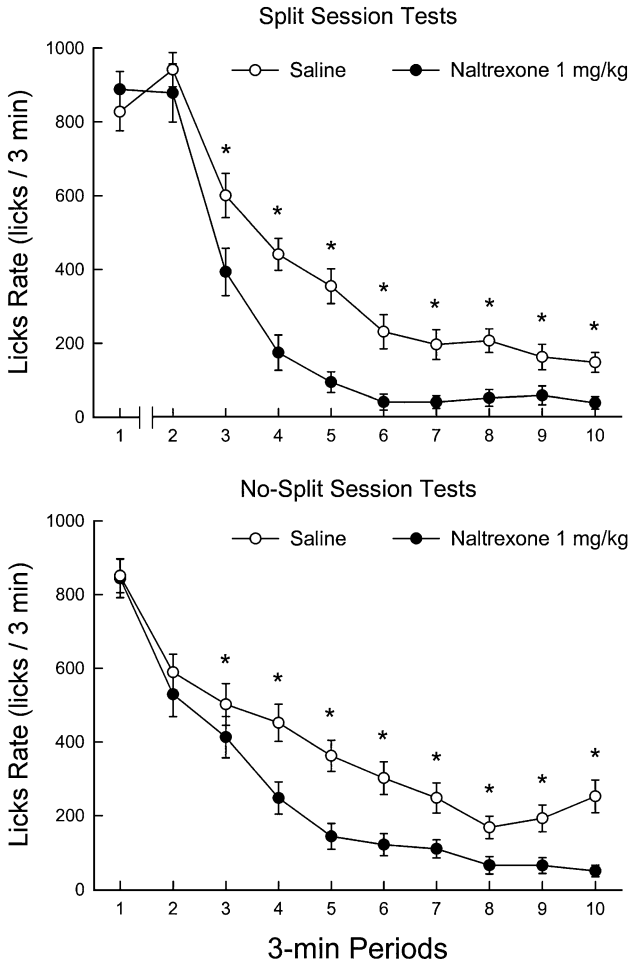

Fig. 4. Experiment 2B. Mean (± S.E.M.) rate of licking in successive 3-min periods following treatment with saline or naltrexone (1 mg/kg) in split-session and no-split session tests. In the split-session condition, the sucrose + saccharin solution was not available for 6 min between the first and second 3-min periods. Asterisks indicate significant difference ($P < .05$) between naltrexone and saline lick rates.

condition [$F(1,16) = 11.72$, $P < .01$]. Lick cluster size during 4–30 min of drinking was not affected by drug or test condition. However, naltrexone significantly reduced cluster number during the last 27 min of drinking under both test conditions [$F(1,16) = 45.92$, $P < .01$].

3.2.3. Discussion

These findings demonstrate that allowing rats to taste the S + s for 3 min followed by a 6-min period without drinking did not appreciably affect the onset of naltrexone's suppressive action on licking. Naltrexone treatment in both the split and no-split conditions did not significantly suppress licking until after 6 min of drinking had occurred. Thus, the results do not support the hypothesis that the drug's delayed suppressive action occurs because rats require time to appreciate that the hedonic impact of the sweet taste is suppressed by opioid receptor blockade. While the split-session procedure failed to alter the onset of licking suppression produced by naltrexone, it did reveal a more subtle

drug effect. That is, the rats increased their licking rate from the first 3-min period to the second period after the delay when treated with saline, but they did not do so when treated with naltrexone. The post-delay increase in licking rate observed in the saline sessions is similar to the increased appetitive and consummatory responding observed in other test situations, which has been described as a sensitization effect or more colloquially as an "appetizer" effect (Swithers, 1996; Yeomans and Gray, 1997). Naltrexone is reported to block this appetizer effect in human subjects and the present data suggest that it also blocks it in rats (Yeomans and Gray, 1997).

As in the preceding experiments, naltrexone suppressed licking by reducing lick cluster number rather than lick cluster size. This was true for both the split-session and no-split conditions. While not altered by drug treatment, cluster size was altered by test condition: cluster sizes were larger in the first three min of drinking in the split sessions than in the no-split sessions. Apparently, at the start of the split sessions the rats anticipated the impending withdrawal of the drinking tube and licked more persistently than they did at the start of the no-split sessions.

3.3. Experiment 2C

Prior work indicates that flavor palatability affects the immediate licking response to solutions (Davis, 1973; Davis and Levine, 1977; Higgs and Cooper, 1998). Thus, the consistent failure of naltrexone to suppress initial rate of licking in the preceding experiments suggests that opioid receptors may not mediate the initial hedonic response to sweet solutions. It may be, however, that with extensive training with the same sweet solution, the rat's drinking response becomes habitual and relatively insensitive to changes in palatability. If so, then the lack of a drug effect on initial lick rates would not be evidence against a hedonic interpretation of opioid receptor action. The present experiment determined whether the rats studied in Experiments 2A and 2B would respond to a decrease in the concentration of the sucrose + saccharin solution by reducing their initial rate of licking and lick cluster size.

3.3.1. Method

Four days after the end of Experiment 2B, the rats were given two test sessions with the 2% sucrose + 0.2% saccharin solution followed by a session with a 1% sucrose + 0.1% saccharin solution. This test sequence was then repeated. The rats were injected with saline 10 min prior to the drinking sessions, which were conducted using the 3–27-min split procedure of the previous experiment.

3.3.2. Results

The rats drank significantly less of the 1% sucrose + 0.1% saccharin solution than of the 2% sucrose + 0.2% saccharin solution [14.9 vs. 17.9 g/30 min: $t(16) = 3.97$, $P < .01$]. Lick rates were also lower with the diluted solution than with the original solution [$F(1,16) = 25.19$, $P < .001$] and declined over the course of the test sessions [$F(9,144) = 118.98$, $P < .001$]. There was also an interaction between solution and session time [$F(9,144) = 3.06$, $P < .01$]. As illustrated in Fig. 5, the rats licked at a lower rate ($P < .05$) for the diluted solution than for the original solution during the first three 3-min periods and again at the seventh 3-min period. The rats tended to increase their lick rate from periods 1 to 2 (i.e., before and after 6-min delay), but this difference was not significant.

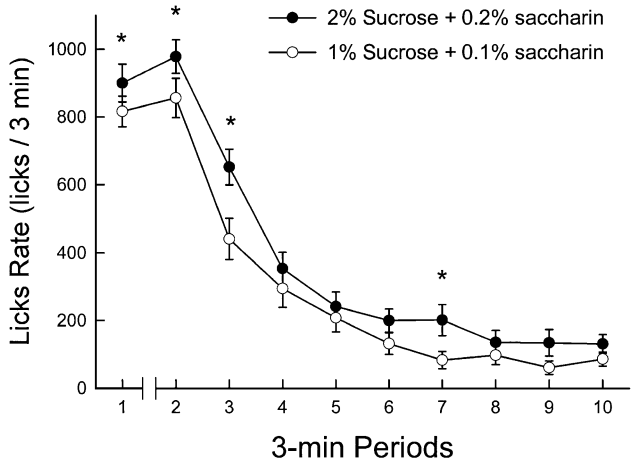

Fig. 5. Experiment 2C. Mean (± S.E.M.) rate of licking in successive 3-min periods of 2% sucrose + 0.2% saccharin and 1% sucrose + 0.1% saccharin solutions following treatment with saline. The solutions were not available for 6 min between the first and second 3-min periods. Asterisks indicate significant difference ($P < .05$) between naltrexone and saline lick rates.

Lick microstructure was also altered by diluting the S + s solution (Table 1). During the first 3-min drinking period, lick cluster size tended to decrease when the saccharin solution was reduced from 2% + 0.2% to 1% + 0.1% S + s, although this difference was only marginally significant [106.4 vs 74.0: $t(16) = 2.01$, $P = 0.06$]; lick cluster numbers were similar with the two concentrations. In contrast, during the post-split 27-min drinking period, cluster size did not differ with the two concentrations, but cluster number was lower with the 1% + 0.1% concentration relative to the 2% + 0.2% concentration [56.9 vs. 85.3: $t(16) = 3.76$, $P < .01$].

3.3.3. Discussion

These results indicate that the rats were sensitive to a change in solution concentration. They not only reduced their total intake but also their initial rate of licking when the S + s solution was diluted from a 2% + 0.2% concentration to a 1% + 0.1% concentration. In addition, they tended to reduce their lick cluster size during the initial 3-min drinking period when the S + s concentration was diluted by half. These findings contrast with the failure of naltrexone treatment to reduce initial lick rates or lick cluster size in preceding experiments. The present data indicate that the lack of a drug effect was not due to insensitivity to changes

in solution palatability as a result of extensive training experience. The different initial response patterns produced by naltrexone treatment and S + s dilution provide further evidence that the drug does not directly alter the perception of sweet taste stimuli (Ohare et al., 1997). Interestingly, naltrexone and S + s dilution were similar in that they both decreased lick cluster number rather than size in the post-split portion of the drinking session.

4. General discussion

This study investigated the impact of opioid receptor antagonism with naltrexone on the rat's consummatory response to a palatable sucrose + saccharin solution. The results were consistent in showing a lack of a drug effect on the rate of licking early in the test sessions (first 6 or 9 min) or on lick cluster size. Instead, naltrexone reduced solution intake by suppressing lick rates later in the session and by reducing lick cluster number. Experiment 2A revealed that naltrexone's delayed suppressive effect could not be explained by a submaximal drug action at the start of the drinking session due to the relatively short injection-test interval (10 min). Increasing the injection-test interval to 20 min did not reduce the latency for the drug-induced suppression of drinking. Experiment 2B showed that interposing a 6-min delay between the first 3 min of drinking and the remainder of the test session also did not appreciably change the action of the drug. In both the split and no-split sessions, naltrexone did not significantly reduce lick rate until after 6 min of drinking had elapsed. The final experiment (2C) revealed that the rats reduced their initial rate of licking when the concentration of the S + s solution was diluted by half. Thus, the lack of an early response to naltrexone could not be attributed to an insensitivity to changes in solution palatability as a result of extensive training. Since only one concentration of S + s was used in the naltrexone experiments, it is possible that an early drug effect on licking may be obtained with other S + s concentrations. Note, though, that variations in sucrose concentration did not reveal early effects of naltrexone on ingestive taste reactivity in other studies (Ferraro et al., 2002; Parker et al., 1992).

The delayed suppression in drinking produced by naltrexone treatment is consistent with many prior findings obtained with naltrexone or naloxone in consummatory or operant situations (Kirkham and Cooper, 1988a,b; Parker et al., 1992; Schwarz-Stevens et al., 1992). As previously noted, one exception to this general finding is the report by Higgs and Cooper (1998) that naloxone significantly reduced licking of a sucrose solution during 1-min test sessions in ad libitum fed rats. Although Higgs and Cooper used sucrose rather than a sucrose + saccharin solution, they tested a range of concentrations (1%, 3% and 10%) that likely overlapped in palatability with the S + s solution used in the present study. Perhaps, the most important difference between the present study and that of Higgs and Cooper (1998) is that their rats had only 1-min access to each solution and were tested with the three sucrose concentrations within a single session separated by 10-s intertrial intervals. This test protocol may have enhanced the rat's responsiveness to the licking suppressive effect of the opioid antagonist. This possibility warrants further investigation.

With respect to lick microstructure, the present findings agree with those of Higgs and Cooper (1998) in showing that opioid antagonists reduce licking by decreasing lick cluster number rather than cluster size. The failure of the drugs to reduce cluster size, like their failure to reduce initial lick rates, is not consistent with a hedonic interpretation of opioid drug action. Prior work indicates that both initial lick rates and cluster size vary as a function of sugar concentration and are presumed to represent changes in the hedonic evaluation of the solution (Davis and Smith, 1992; Higgs and Cooper, 1998). Higgs and Cooper (1998) hypothesized that palatability may have two components, hedonic evaluation and incentive salience, which are reflected by changes in lick cluster size and number, respectively. According to this view, opioid antagonists suppress intake by reducing the incentive salience but not the hedonic evaluation of palatable solutions. Berridge (1996) previously distinguished between hedonic evaluation and incentive salience components of food motivation, which he referred to as "liking" and "wanting" processes, respectively. Berridge (1996), however, hypothesized that the opioid system was primarily involved in the "liking" component whereas the dopamine system mediated the "wanting" component. Berridge's analysis of opioid function was based primarily on taste reactivity data and did not deal with initial lick rate or microstructure data. Clearly, more work is needed to integrate these various responses.

Another view of opioid function which focuses on the delayed suppressive effect of antagonists postulates that opioid receptors mediate processes that sustain ingestive behavior once it is initiated (Siviy et al., 1982; Glass et al., 1999a). This may operate by modulating the inhibitory actions of postingestive satiety signals or by modulating inhibitory (or stimulatory) actions of orosensory signals. Studies of sham-feeding animals, in which postingestive feedback is minimized by an open gastric fistula, indicate that opioid antagonists have a delayed suppressive effect on sucrose intake similar to that observed in "real-feeding" rats (Rockwood and Reid, 1982; Kirkham and Cooper, 1988a,b). Also, gastric preloads are reported not to alter the drinking suppressive effect of naloxone (Siviy et al., 1982). Taken together, these findings suggest that altering the response to postingestive satiety signals is not the primary mechanism of action of opioid antagonists.

Oral stimulation may also be a source of negative feedback as indicated by the decline in lick rates during sham-feeding tests and by the decrease in oromotor responding observed in extended intraoral infusion tests

(Swithers, 1996; Swithers and Martinson, 1998; Nissenbaum and Sclafani, 1987). This process has been referred to as oral "satiety" or habituation to distinguish it from the more extensively studied postingestive satiety processes (Swithers, 1996). Sham-feeding data indicate that the rate of oral habituation decreases as sucrose concentration increases (Nissenbaum and Sclafani, 1987). Thus, in addition to altering initial rates of licking, solution palatability may affect subsequent lick rates by reducing oral habituation and perhaps it is this component of taste hedonics that is mediated by central opioid receptors. This would explain why opioid antagonists have their primary action later in the ingestion bout. Opioid receptors are also implicated in the oral sensitization (appetizing) phase of ingestion, which occurs after the onset of ingestion (Yeomans and Gray, 1997). The present finding that naltrexone prevented the increase in licking rate produced by a 6-min delay in Experiment 2B is consistent with this interpretation. The intraoral infusion procedure is an effective way to study both oral sensitization and habituation and it will be of interest to examine the effect of opioid antagonists on this response measure (Swithers, 1996; Swithers and Martinson, 1998).

In the present study, as in most prior experiments, naltrexone's effect on fluid consumption and lick rates was evaluated in animals that were very familiar with the test solution. Lynch and Burns (1990) reported that treating nondeprived rats with daily injections of naloxone (1 mg/kg) beginning with their first exposure to a sucrose or saccharin solution almost completely suppressed solution intake. Although lick rates were not recorded, initial licking must have been suppressed given the very low intakes during the daily 35-min sessions. Following the cessation of drug treatment, sucrose intake and, to a lesser degree, saccharin intake rapidly increased. Subsequent naloxone injections again suppressed sucrose intake, but the suppressive effect with the now familiar solution was much less profound than that obtained at the start of the experiment. We have also observed a solution novelty effect with rats initially trained, as in the present study, to drink S + s (Sclafani and Frisina, unpublished findings). When these rats were injected with naltrexone (1 mg/kg) or saline, the S + s solution contained novel flavors (grape or cherry) and drinking was limited to 6-min sessions. Under these test conditions, naltrexone significantly reduced 6-min intake and licking rate compared to saline treatment. This contrasts with the failure of the drug to suppress licking during the first 6 min of testing with the familiar S + s solution in the present study. These findings indicate that opioid antagonists more effectively inhibit initial rates of ingestion when rats are tested with a novel solution than with a familiar solution. Therefore, in addition to their effects on sustaining the ingestive response to familiar foods and fluids, opioid receptors may be involved in the acquisition of the hedonic response to novel foods and fluids. The nature of this involvement requires further investigation because recent conditioning studies have revealed little effect of naltrexone treatment on flavor preference learning using different conditioning procedures (Azzara et al., 2000; Yu et al., 1999). That is, naltrexone treatment did not prevent rats from acquiring preferences for an arbitrary flavor that was paired with the sweet taste of sucrose or the postingestive actions of sucrose.

In summary, while there is considerable evidence supporting a hedonic intrepretation of opioid drug action (Cooper and Kirkham, 1993; Kelley et al., 2002), the present results along with other animal data indicate that opioid antagonists do not affect all aspects of flavor hedonics. Analyses of lick rates, taste reactivity and instrumental responding indicate that, with some exceptions (Higgs and Cooper, 1998), the initial responses to familiar sweet solutions are relatively unaffected by naloxone or naltrexone administration (present experiment; Ferraro et al., 2002; Glass et al., 1999a,b; Kirkham and Blundell, 1986; Kirkham and Cooper, 1988a; Parker et al., 1992; Schwarz-Stevens et al., 1992). Rather, the primary effect of the drugs may be to reduce the intake maintaining effect of palatable flavors and, perhaps, to block their sensitizing or "appetizing" effect. The significance of naltrexone's effect on lick microstructure (reduced cluster number, but not size) to flavor hedonics and incentive salience remains to be established. Another interesting question beyond the scope of the present discussion is how these rat data relate to reports of reduced pleasantness rating of palatable foods in humans treated with opioid antagonists (Arbisi et al., 1999; Drewnowski et al., 1992; Fantino et al., 1986; Yeomans and Gray, 1997).

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