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THE PHARMACOLOGY OF FLAVOR PREFERENCES CONDITIONED BY INTRAGASTRIC SUCROSE: EFFECTS OF OPIOID AND DOPAMINE RECEPTOR ANTAGONISM

by

Anthony V. Azzara

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, City University of New York

2000

This manuscript has been read and accepted for the Graduate Faculty in Experimental Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

August 31, 2000
Date

Anthony Sclafani
Chair of Examining Committee

Sept 1, 2000
Date

Executive Officer

Karen Ackroff, Ph.D.

Richard Bodnar, Ph.D

Andrew Delamater, Ph.D.

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

THE PHARMACOLOGY OF FLAVOR PREFERENCES CONDITIONED BY INTRAGASTRIC SUCROSE: EFFECTS OF OPIOID AND DOPAMINE RECEPTOR ANTAGONISM

by
Anthony V. Azzara
Advisor: Professor Anthony Sclafani

Food preferences are influenced by learned associations between the food's flavor and postingestive nutritive effects. This dissertation research explored the neuropharmacology of conditioned flavor preferences (CFP). The role of the opioid system was determined using the general opioid antagonist naltrexone. Naltrexone administration did not block the expression of a preference for a flavor (CS+) paired with intragastric (IG) sucrose over another flavor (CS-) paired with IG water (Experiment 1). Additionally, naltrexone administered during training did not block the acquisition of a CS+ preference. Naltrexone-treated rats displayed preferences similar to controls, despite drinking less during training (Experiment 2). Experiment 3 revealed that naltrexone did not block the increased intake (acceptance) of the CS+, relative to the CS-, as measured in one-bottle tests. The role of the dopamine D_2 receptor in CFP was investigated with the antagonist raclopride. Raclopride did not block the expression of an established CFP (Experiment 4). When given throughout training (Experiment 5), raclopride suppressed CS training intakes and CS+ preference in subsequent choice tests, relative to the control group. Experiment 6 added a yoked control group to control for the training intake reductions caused by raclopride. Raclopride treatment suppressed training intakes but did not block the acquisition of a preference, as the drug, control, and yoked control groups all preferred the CS+ to CS-. Experiment 7 examined the role of the D_1 dopamine receptor in preference learning using the antagonist SCH23390. Unlike the control and yoked control groups, the SCH23390 treated rats failed to acquire a CS+ preference. Yet,

SCH23390 treatment during two-bottle testing did not block the CS+ preference in the control or yoked groups, except at doses that greatly suppressed intake. Together, these results indicate that the opioid system is not critically involved in the acquisition or expression of a flavor preference conditioned by the postingestive actions of sucrose. D_1, but not D_2 dopamine receptors are important for the acquisition of a sucrose- conditioned flavor preference, but both receptor subtypes have a more limited role in preference expression. The differential involvement of opioid and dopamine receptors in flavor conditioning is consistent with recent neurochemical models of motivation.

Acknowledgements

I would like to take this opportunity to thank the people who made this accomplishment possible. My warmest thanks go out to Andy Delamater, Karen Ackroff, Rich Bodnar, and Gerry Smith. They served as an excellent committee, and each of them has contributed greatly to my intellectual development and my arrival at this point in my career.

Special thanks are extended to Tony Sclafani, my mentor, for whom I have the ultimate respect and admiration. He truly lead by example, and helped me train my hands and my mind for a career in science. Without him, this work would not have been accomplished. Thank you Tony, for always knowing when to push me, and almost always knowing when not to. All kidding aside, I'm glad to have had you as a mentor, and to have you as a friend and colleague.

I would also like to acknowledge my father, who was always there for me, and always pushed me to reach my highest academic goals, while never letting me forget his motto – "Education is no substitute for intelligence". Sometimes I think that he is even smarter than he realizes.

Eileen Fisenne tolerated me through the trials and tribulations of writing this dissertation, and helped to keep me sane. She sometimes even seemed interested in what I was writing about. She is patient, caring, kind, and beautiful. These are only a few among the many reasons why I love her.

Finally, I must thank all of the people whom were there along the way: my fellow students, the faculty at Brooklyn College, my coworkers in the lab. Thanks for all of the laughs, thanks for all of the talks, and the fun we had. I will miss almost all of you. It was a great five years.

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Introduction

Behavioral research has demonstrated that food choice in animals, including humans, is guided by both innate and learned factors. Among the learned factors are social and cultural influences, however these are beyond the scope of the present discussion (see Rozin, 1996). Innate biases in many animals include an attraction to sweet taste and an avoidance of bitter taste (Jacobs, et al., 1977). Animals learn to prefer nutritious foods and avoid toxic ones by associating the flavor (i.e., taste, odor, and texture) of the food with the postingestive consequences of the food (Rozin & Kalat, 1971; Sclafani, 1990; Sclafani, 1995; Sclafani, 1999). The avoidance of flavors formerly paired with toxicosis is referred to as conditioned flavor aversion (CFA) or conditioned taste aversion (CTA) if a pure taste stimulus is used. This phenomenon has been well described and summarized elsewhere (Garcia, 1989). If the ingestion of a food is followed by a positive postingestive outcome, then a conditioned preference for the flavor of that food may be formed (Sclafani, 1995; Capaldi, 1996).

While conditioned flavor preference has been well described at a behavioral level, the neurochemical mechanisms supporting this type of learning remain to be identified. This dissertation research examined, through the use of antagonist drugs, the roles of the endogenous opioid and dopamine systems in flavor preferences conditioned by intragastric carbohydrate infusions.

The first set of experiments examined the role of the opioid system in flavor preferences conditioned with intragastric sucrose infusions. Experiment 1A determined what effects opioid antagonism with naltrexone has on an established flavor preference in food-deprived rats. Experiment 1B addressed the same issue in non-deprived rats.

Experiments 2A and 2B evaluated the effects of opioid antagonism on the development of a flavor preference, using two doses of the antagonist. Experiment 3 examined the effects of naltrexone on the conditioned increase in the intake of a flavor which had been paired with an infusion of a dilute maltodextrin solution.

The second set of experiments determined the role of the endogenous dopamine system in flavor preferences conditioned by intragastric sucrose infusions. Experiment 4 examined the effects of D_2 dopamine receptor antagonism on an established flavor preference. Experiments 5 and 6 determined the effects of D_2 dopamine blockade on the establishment of a flavor preference. The final experiment used a multiple group design to assess the effects of D_1 dopamine receptor blockade on the development of a preference, as well as on the expression of an established preference. The results of these experiments provide new information about the role of the opioid and dopamine systems in various aspects of flavor preference learning.

Conditioned Flavor Preference

Generally speaking, most food preferences are learned, rather than innate. The conditioned flavor preference paradigm has been employed in the laboratory to study how animals come to prefer novel foods. Conditioned flavor preference is thought of as occurring via Pavlovian learning mechanisms, and is described in classical conditioning nomenclature. So, for example, in flavor preference learning a cue flavor (conditioned stimulus, or CS+) is paired with a positive unconditioned stimulus (US), such as the sweet taste of sucrose or the intragastric infusion of a sugar, while another cue flavor (CS-) is paired with a non-sweet substance, or an infusion of water, or nothing. After being trained, the animals are presented with a choice between the two CS solutions. If

the animal has learned to associate the CS+ with the reinforcing properties of the US, then the animal should preferentially consume the CS+ (Sclafani, 1991; Sclafani, 1995; Sclafani, 1999). There are many variations on the basic conditioned flavor preference paradigm, but such experiments generally fall into two classes, those in which the flavor of the US is presumed to be the reinforcing stimulus (flavor-flavor conditioning), and those in which postingestive consequences of the US are regarded to be the reinforcer (flavor-nutrient conditioning).

Flavor-Flavor Conditioning

The palatable flavor of a nutrient can act as a US and condition a preference for another flavor. Flavor-flavor conditioning occurs when a novel cue flavor (CS+) is mixed into a preferred solution, such as a sucrose solution (US), and another flavor (CS-) is mixed into a less preferred solution, such as water. The animals are given repeated exposures to the flavored solutions, followed by a preference test. Preference testing is typically conducted with the cue flavors presented in a common or neutral base solution. Rats will acquire a preference for a novel flavor that has been mixed into an already preferred flavor (Rozin, 1990) such as the taste of sucrose.

Results from flavor-flavor conditioning studies are often difficult to interpret. On one hand, the flavor of the oral solutions may be the reinforcing agent. For example, it has been demonstrated that rats form a preference for a flavor mixed into a non-nutritive saccharin solution over another flavor which has been mixed into less concentrated saccharin (Holman, 1975). Other studies have demonstrated preferences for flavors mixed into glucose, sucrose, or starch solutions (Booth et al., 1972; Capaldi et al., 1987; Fedorchak & Bolles, 1987; Mehiel & Bolles, 1988).While the flavor of the noncaloric

saccharin was clearly the reinforcing agent in the Holman (1975) study, in the other studies using palatable nutritive solutions, it is possible that postingestive stimuli, rather than (or in conjunction with) flavor are acting as the US. For example, Elizalde & Sclafani (1988) blocked the acquisition of a preference for a flavor mixed into Polycose (a starch-derived polysaccharide) by inhibiting the digestion of Polycose with the intestinal amylase inhibitor acarbose, which indicates that it was the postingestive reinforcing qualities of Polycose, rather than its flavor, that conditioned the preference.

Another method by which postingestive contributions to flavor preference can be minimized is the sham feeding preparation, in which consumed fluid drains out of a gastric fistula. This maximizes the possibility that any association between the CS and US is based on flavor alone (but see Sclafani & Nissenbaum, 1985). Using a sham-feeding preparation, Yu et al. (1999, 2000, submitted) have demonstrated that rats learn to prefer a flavor previously mixed into a sucrose solution over a second flavor previously mixed into a less preferred saccharin solution, when the two flavors are presented in a common sucrose-saccharin base.

Flavor-Nutrient Conditioning

The form of flavor preference conditioning which was investigated in the present series of experiments uses the intragastric (IG) infusion conditioning paradigm, referred to as flavor-nutrient conditioning, and described in detail in Elizalde & Sclafani (1990). In flavor-nutrient conditioning, the animals are trained with a CS+ flavor paired with the IG infusion of a caloric substance, such as sucrose, and another flavor, the CS-, is paired with the infusion of water. Testing involves a choice between the two CS flavors, under

reinforced or extinction conditions. In extinction testing, the IG infusions are not administered, or the consumption of both CSs is paired with a water infusion. This ensures that any preferences expressed are based upon what has been learned about the CS solutions, rather than a direct response to the infusions. Note that this paradigm removes all oral stimulation provided by the US. Further, it may be assumed that the CS flavor, typically Kool-Aid, sometimes sweetened with saccharin, has few post-oral effects. Therefore, the oral flavors are equally sweet novel cues, and the US is purely the postingestive actions of the nutrients in question. The typical result is that, in two-bottle tests, the rats preferentially consume the CS+. The preference is presumed to arise from the association of the CS+ flavor with the positive postingestive effects of the infused nutrient.

Nutrient conditioned flavor preference can be quite robust and resistant to extinction (Drucker, et al., 1994). This method has been used to condition preferences for flavors paired with a variety of nutrients, including carbohydrates, fats, and protein (see Sclafani, 1999 for a review). This training procedure can condition preferences for tastes such as bitter or sour, which are normally avoided by rats (Drucker et al., 1994).

The Pharmacology of Conditioned Flavor Preference

Conditioned flavor preference has become a well described phenomenon in recent years, however, relatively little is known about the underlying neurochemical basis of such conditioning. Both the opioid and dopamine systems are implicated in the control of feeding behavior, and have been implicated in reward processes (Rodgers & Cooper,

1988; Smith, 1995). The present series of experiments was designed to assess the role of these systems in flavor preference learning.

Why the opioid system?

The opioid system has long been implicated as a mediator of reward, as rats will self administer opioid agonists (Olds, 1979), and opioid antagonism reduces responding for electrical self stimulation (Stein & Belluzzi, 1979). More relevant to the present investigation is the finding that opioid antagonism results in a suppression of food and fluid intake (Cooper et al., 1988; Levine et al., 1985). This suppression is greater for palatable sucrose and saccharin solutions than it is for water (Cooper & Kirkham, 1993), indicating that opiate antagonism suppresses the hedonic response to sweet solutions. The classic opioid agonist morphine, on the other hand, increases the consumption of sucrose solutions (Czirr & Reid, 1986).

Much attention has been given to the mechanisms by which opioid antagonists reduce intake. Evidence suggests that the intake reduction is due to a reduction in palatability. Rockwood & Reid (1982) utilized a sham feeding paradigm, in order to more directly evaluate the effects of opioid antagonism on palatability when post-ingestive feedback was minimized. They examined the effects of naloxone on the feeding of 10% sucrose and water in rats with their gastric fistula closed or open. The drug treatment reduced the consumption of the sweet solution regardless of whether the fistula was open (sham feeding) or closed (real feeding). The sham feeding data is strong evidence that the opioids are influencing the palatability of sweet solutions. This hypothesis is strengthened by work done by Kirkham & Cooper (1988). Their study also examined the ability of naloxone to alter sham drinking of sucrose. By gradually

increasing the concentration of the sucrose, the authors were able to demonstrate an alteration in intake which resembled a halving of the sucrose concentration. That is, naloxone treated rats consumed 20% sucrose as if it were 10%, and 10% as if it were 5%. As postingestive consequences were minimized by using the sham feeding preparation, this result strongly suggests that opioid antagonists altered the palatability of the sucrose.

Utilizing an alternate way to assess palatability, Parker et al. (1992) have shown that morphine increases, while naltrexone reduces, positive responses to sweet stimuli, using intraoral infusions in a taste reactivity paradigm. Doyle et al. (1993) examined the influence of morphine on facial reactivity to infusion of a sucrose-quinine solution into the mouth. Morphine treatment resulted in an increase in positive hedonic responses to the oral infusions. No changes in aversive responses were observed, indicating that morphine selectively enhanced the positive hedonic component of the bitter-sweet infusate.

Most research on the effects of opioid antagonism on feeding behavior have employed naloxone and naltrexone, general opioid antagonists without specificity for any opioid subreceptor. In situations where general opioid antagonists have effects, then specific opioid subreceptor antagonists are sometimes utilized in order to further clarify the role of the opioid system in the behavioral process being studied. Three types of opioid receptor subtypes have been identified. The μ (mu), κ (kappa), and δ (delta) receptor subtypes have all been implicated in food intake and weight regulation (see Bodnar, 1996 for a review). Specifically, the μ and κ receptor subtypes have been implicated in the intake of sugar solutions under both real-feeding (Beczkowska et al, 1992) and sham-feeding (Leventhal et al., 1995) conditions.

Despite the large body of literature on food intake and the opioid system, relatively little work has been done on the role of the opioids in flavor conditioning. Dum et al. (1983) demonstrated that the ingestion of highly palatable stimuli causes the release of beta-endorphin in the hypothalamus of rats. Additionally, when a flavor is paired with the administration of low doses of opioid agonists, rats will come to prefer that flavor over a flavor paired with vehicle injections (Lett & Grant, 1989; Mucha & Herz, 1985). These results indicate that the endogenous opioid system may play an important role in flavor preference conditioning. It might be hypothesized that the consumption of palatable food causes an opioid release which reinforces the eating of the food that caused the release. A prediction which follows from this hypothesis is that opioid antagonism prior to exposure to palatable food would block the reinforcing effects of eating that food. In other words, opioid antagonism might block flavor preference learning. An undetermined piece of information is whether palatable foods such as sucrose cause hypothalamic opioid release when they are delivered directly to the stomach.

The role of the opioid system in flavor preference conditioning has been examined in only a few studies. Yu et al. (1999) examined the effects of naltrexone on flavor-flavor conditioning in a sham feeding paradigm. Their study separately examined the effects of the drug on the acquisition and the expression of a conditioned flavor preference. The first experiment was an expression study, in which food-deprived rats were trained to sham feed a CS+ flavored 16% sucrose (the US) and a CS– flavored 0.2% saccharin solution. The rats were trained with limited exposure (10 ml) to the flavored solutions on alternating days. When tested for preference the rats preferred the CS+, and

naltrexone treatment reduced total intake, but did not significantly reduce the magnitude of the preference. When the animals were retrained without an intake limit they also learned a strong preference. In this experiment naltrexone did not reduce intake or alter preference during two-bottle tests.

In a separate experiment Yu et al. (1999) investigated the influence of naltrexone administered during training, on flavor-flavor preference learning. Food-deprived rats were trained as in the first experiment, without a limit, and half of the rats (Drug group) received an injection of 0.1 mg/kg naltrexone prior to every training session. The remaining rats (Control group) were injected with saline prior to every training session. In preference testing, both groups of rats displayed a preference for the CS+ flavor. This preference was not attenuated by naltrexone treatment. These results indicate that naltrexone does not interfere with the acquisition or the expression of a flavor preference conditioned by the flavor of sucrose, in sham-feeding rats.

The role of the opioid system in flavor conditioning has also been investigated using both glucose and ethanol as conditioning agents. Mehiel (1996) presented food-deprived rats with CS+ flavored 10% glucose solution (the US) and CS– flavored water on alternating training days. When tested for preference when saline treated, the rats drank more of the CS+ flavor. When tested with naloxone treatment, however, their total intake was suppressed as compared to the vehicle preference test, and they did not significantly prefer either CS.

In a second experiment, Mehiel (1996) trained rats with a CS+ flavored 5.5% ethanol solution (the US) and a CS– flavored .25% saccharin solution. The author hypothesized that any preference learned for the ethanol paired flavor would be due to the

postingestive effects of ethanol, as opposed to the flavor of ethanol, which he assumed is unpalatable to rats. However, the author did not assess preference directly; rather, he compared the one bottle intakes of the training flavors with and without naloxone treatment. Generally speaking, preference is a measure of the relative amount of each solution an animal will consume, when given a choice between solutions. Preference is typically measured in two-bottle tests. When presenting a rat with a single bottle one is measuring acceptance; that is, how much a rat will consume of a given solution over the course of a test. Mehiel (1996) reported that, when saline treated, the rats took more of the CS+ flavor than of the CS– flavor. Naloxone treatment reduced CS+ intake, but not the intake of the CS-.

A third experiment involved rats drinking CS+ flavored glucose (the US) and CS– flavored saccharin in training. One group of rats was injected with naltrexone prior to every CS+ training session and saline prior to every CS– training session; a second group was trained with the reverse order of injections. When tested for preference, the group which received naltrexone on CS+ trials did not prefer either flavor, but the group which was injected with naltrexone on CS– days expressed a CS+ preference.

Mehiel's (1996) results seem to conflict with the Yu et al. (1999) finding that opioid blockade via naltrexone was ineffective in reducing the expression or acquisition of a flavor-flavor preference in sham-feeding rats. However, significant procedural differences exist between the studies. Particularly relevant is that Mehiel's rats, unlike the rats is the Yu et al.(1999) study, were real-feeding, and so experienced post-ingestive feedback from consumed nutrients. Additionally, Yu et al. gave naltrexone during both CS+ and CS– training days, not just on CS+ or CS– days, as in the Mehiel study.

Administering the antagonist on only CS+ or CS– days is problematic. Opioid antagonists can produce flavor aversions (Parker & Rennie, 1992), therefore, rats in the Mehiel (1996) study may have developed an aversion for whichever CS was paired with antagonist treatment. This possibility cannot be controlled for when administering the antagonist with only one of the two CS solutions.

Ramirez (1997) conducted a study using a flavor acceptance paradigm in which he paired IG carbohydrate infusions with saccharin intake. In this study Ramirez conditioned an increased acceptance of a saccharin solution by pairing with infusions of a relatively dilute (6%) maltodextrin solution. Another group of rats consumed a saccharin solution while being infused with water; this group did not show increased acceptance. After training the rats were treated with naloxone (0.3 and 0.1 mg/kg body weight) prior to one-bottle tests. Although Ramirez used unflavored saccharin solutions and a between group design, his saccharin solutions can be considered to be a "CS+" (in the maltodextrin infused group) and "CS-" (in the water infused group) for the purposes of this discussion.

During testing the rats which received saccharin as a CS+ drank significantly more than did the group which received saccharin as a CS-. Ramirez interprets the increased acceptance in the CS+ group as evidence for an increased hedonic response to saccharin after it had been paired with maltodextrin infusions. When treated with naloxone the CS+ group suppressed intake to a greater degree than did the CS– trained rats. At the 0.3 mg/kg dose, naloxone suppressed intake in both groups. The low dose of naloxone (0.1 mg/kg) reduced intake only in the group who received maltodextrin infusions. While the higher dose of naloxone did reduce intake in both groups, it had

larger and more reliable effects on the intake of the rats in the CS+ group. Because opioid antagonism has been reported to modulate the hedonics of palatable fluids, Ramirez interprets the naloxone results as further evidence that the hedonic value of the saccharin was increased in the CS+ group by the infusion of carbohydrate.

Taken together, these results seem to implicate the opioid system in the conditioning of flavor-nutrient, as opposed to flavor-flavor, preferences. Specifically, Yu et al. (1999) demonstrated that a functional opioid system is not necessary for the acquisition or expression of a flavor-flavor preference. Ramirez (1997), on the other hand, demonstrates a suppressive effect of naltrexone on the carbohydrate-conditioned increased acceptance of a saccharin solution.

Why the dopamine system?

Prior to a discussion of the dopamine system, it is important to clarify some terminology. Briefly, two families of dopamine receptors have been identified. The D_1 subfamily consists of the D_1 and D5-R receptors, while the D_2 subfamily consists of the D_2, D3 and D4-R receptors. A primary functional difference between the receptor subfamilies is that stimulation of receptors in the D_1 subfamilies results in increases in cyclic AMP, while D_2 subfamilies receptors inhibit cyclic AMP (Vallone et al., 2000). Many antagonist drugs, including raclopride and SCH23390, discriminate well between, but not within receptor subfamilies (Vallone et al., 2000). For the purposes of this paper, I will adopt the common convention of using the terms D_1 and D_2 receptors to refer to the two receptor subfamilies.

The dopamine system, like the opioid system, has been implicated in mediating food reward (Smith, 1995). Dopamine D_1 and D_2 receptor subtype antagonists reduce the intake of sucrose (Muscat & Wilner, 1989; Schneider et al., 1986). Xenakis and Sclafani (1981) demonstrated that pimozide (a D_2 antagonist) reduced the intake of a palatable glucose-saccharin mixture, relative to water intake. Additionally they demonstrated that this reduction was functionally equivalent to reducing the concentration of the sweet solution (Xenakis & Sclafani 1982). Radhakishun et al. (1988) demonstrated that, in food-deprived animals, eating caused an increase in nucleus accumbens dopamine levels that persisted until the termination of eating.

Muscat & Willner (1989) have compared the effects of sulpiride (a D_2 antagonist) and SCH23390 (a D_1 antagonist) on one and two bottle comparisons of sucrose and water intake in acutely (24h) food and water deprived rats. Both antagonists lowered the preference for 1% sucrose over water. In single bottle tests, both antagonists lowered the intake of low (<5%) sucrose concentrations. This pattern of suppression is opposite to that achieved when the authors reduced the deprivation period to 1 hr (Muscat & Wilner, 1989), indicating that these drugs reduce intake via mechanisms other than hunger reduction.

A large body of research characterizing the role of dopamine in the reward elicited by sweet tastes had been done by Smith and his coworkers, utilizing the sham feeding procedure. Using this procedure, which greatly reduces postingestive effects of nutrients, it has been demonstrated that pimozide reduces the intake of a range of sucrose concentrations (Geary and Smith, 1985). This experiment also demonstrates that the dopamine blockade produced intake effects similar to those produced by reducing the

sucrose concentration. This result was extended by an analysis of licking microstructure, which demonstrated that dopamine receptor antagonism that reduced intake of 10% sucrose down to the levels of 5% sucrose also produced patterns of licking that resembled those seen with 5% sucrose (Schneider et al., 1990). This result is important, because it provides evidence that the reductions in intake following dopamine antagonism are not due to motoric effects. Schneider et al.(1986) expanded these results to demonstrate that both SCH23390, a selective D_1 antagonist, and raclopride, a selective D_2 antagonist, reduced the intake of sham-fed 10% sucrose. While the sham feeding results suggest that dopamine antagonism may affect the perception of sweetness, Wilner et al. (1990) have demonstrated in a T-maze task that pimozide did not change the rats ability to discriminate between very dilute concentrations of sucrose, used as discriminatory cues. This provides evidence that dopamine antagonists do not affect the perceived intensity of sweet solutions.

As previously stated, much of the evidence on the action of dopamine antagonists seems to indicate that they reduce intake by reducing some hedonic component of the consumed substance; in particular, the sham feeding studies indicate a reduction in palatability. However, Treit & Berridge (1990) have demonstrated that the mixed D_1/D_2 (and sigma) receptor antagonist haloperidol neither increased nor decreased hedonic facial expressions to sucrose in a taste reactivity study. A similar pattern of results was obtained with the indirect agonist amphetamine in the same study. Using this and other results, Berridge (1996) has argued that the action of dopamine antagonism may be different than a simple reduction in the hedonic value of the ingested solution. Reward, according to Berridge, is comprised of the separable processes of "wanting" and "liking".

Wanting can be defined as "the subjective experience of needing or desiring something...produced by the psychological process of salience attribution". Liking is defined as "the subjective experience of a sensation as pleasurable...and the underlying evaluative and neural processes that directly produce this subjective experience" (Berridge, 1996). It is important to note that Berridge asserts that most behavioral methods (intake tests, preference, etc.) are measures in which both wanting and liking play a role and in which wanting and liking cannot be separated. The facial reactivity test, however, is a pure measure of liking, according to Berridge. Due to the influence of opiate blockade on palatability, as well as on intake measures, the opioid system seems to play a role in liking, and possibly wanting. While the classic anhedonia hypothesis of dopamine antagonism (Wise, 1978a, 1978b) would seem to implicate dopamine in liking, facial reactivity data does not support this hypothesis. Berridge (1996) speculates that dopamine is critical only to the wanting aspect of food reward.

Specific evidence for a role of dopamine in flavor conditioning comes from the work of Mark et al. (1991), which demonstrated an increase in nucleus accumbens dopamine release in naive rats in response to intraoral saccharin. When the saccharin solution was administered to rats with a previously conditioned aversion to that taste, neural dopamine release significantly decreased. Mark et al. (1994) broadened this finding by demonstrating that neural dopamine release is also modified by positive consequences of ingestion. In this study rats were trained with a CS+ flavor paired with IG Polycose infusions, and a CS– paired with water infusions. In one-bottle tests conducted in the absence of IG infusions, consumption of the CS+, but not the CS– was associated with an increase in accumbens dopamine release. Untrained animals

consuming the CS solutions showed no change in dopamine release. These studies demonstrate that learned preferences and aversions modify the intracellular dopamine response to a given flavor cue. Learning about other food-related cues may also influence dopamine release. Richardson & Gratton (1996) trained rats on a fixed ratio 1 schedule to lever press for the availability of milk. The presentation of the milk was paired with a cue light. In early training sessions nucleus accumbens dopamine release occurred in temporal conjunction with the presentation of the milk reward, but over sessions the release shifted forward in time to become associated with the cues signaling the start of the session.

The effects of dopamine antagonists on flavor preference conditioning have been examined in a limited number of studies. Hsiao and Smith (1995) trained rats to drink two differently flavored 10% sucrose solutions. The consumption of one flavor was preceded by the injection of the D_2 antagonist raclopride, the other flavor by saline. In a subsequent choice test with the two flavored solutions, the rats preferred the saline-paired flavor over the raclopride-paired flavor. This was taken as evidence that the reward potency of sucrose's sweet taste is reduced by D_2 antagonism. More recently, Yu et al. (2000) used a sham- feeding preparation to examine the effects of dopamine antagonism on the flavor preferences conditioned by the taste of sucrose. As mentioned above, rats trained to sham-feed a flavored 16% sucrose solution (CS+) and a differently flavored 0.2% saccharin solution (CS–), preferred the CS+ flavor when subsequently given the choice between the two flavors presented in mixed sucrose-saccharin solutions. Treating the rats with the D_2 antagonist raclopride or the D_1 antagonist SCH23390 prior to the choice test attenuated the expression of the preference for the CS+ flavor. In a

follow-up study, Yu et al. (Submitted) treated separate groups of rats with raclopride, SCH23390, or vehicle throughout sham-feeding training, and then conducted flavor preference tests in the presence or absence of the drugs. This study revealed that D_1 or D_2 antagonism throughout training attenuated preference conditioning compared to a control group, but not to a yoked control group that had its training intakes matched to that of the drug groups. These studies indicate a role for the dopamine system in flavor preferences conditioned by flavor-flavor associations.

Research Strategy

One of several methods of conditioning flavor-nutrient preferences is the intragastric infusion procedure. In this procedure, animals are trained to drink Kool-Aid flavored saccharin solutions, which are paired with intragastric infusions. More specifically, an animal might consume a cherry flavored saccharin, (CS+), paired with the intragastric infusion of a carbohydrate solution (US), and on alternate training days consume a grape flavored saccharin (CS-) paired with the intragastric infusion of water. After training, preference is assessed in a two-bottle choice test without nutrient infusions. This design has several advantages. In particular, the sweetness of the oral cues is equated, therefore any preference learned must be based upon the postingestive aspects of the US solutions. This factor is critical when examining the role of the opioid and dopamine systems in flavor preference conditioning, as antagonists may have differential effects on sweet taste and the postingestive effects of sweet carbohydrates. Some pharmacological studies of conditioned flavor preference (Mehiel, 1996) allowed the rats to consume the sugar by mouth so that sweet taste may have contributed to the preference conditioning effect. The goal of the present research was to evaluate the role of the opioid and dopamine systems in conditioning based on post-oral factors.

In the present research, some experiments examined the effects of dopamine and opioid antagonism on the *expression* of an established preference. This involved training the rats as described above, and then testing them for preference with saline injections, and with a range of antagonist doses. A second modification of our design was used to examine the effects of dopamine or opioid antagonism on the *acquisition*, or learning of, a flavor preference. In this design two (or three) groups of rats were trained as described

above. The control group(s) received vehicle injections prior to every training trial. The drug group received antagonist treatment prior to every training session. Preference was evaluated in both groups both with and without drug treatment. There are several advantages to this design. As previously mentioned, treating the animals with drug on only one of the two types of training days raises problems of interpretation. This design is also informative because it can account for state-dependent effects. If rats are trained under antagonist treatment, but only tested without the drug, a state dependent preference might not be revealed. Finally, testing the control rats both with and without drug treatment provides a built in test of the effect of the drug on the expression of an established preference.

General Methods

These general methods were employed in all of the studies described with the exception of Experiment 3.

Subjects. Male Sprague Dawley rats, obtained from Charles River Laboratories (Wilmington, MA) or bred in our colony from Charles River stock, served a subjects. The rats were individually housed in our vivarium, maintained at 21° C, in wire mesh cages, under a 12:12 hr light:dark cycle. Ad libitum food (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and water were available prior to surgery and during recovery. After recovery, animals were switched to a powdered version of the same diet. During some of the experiments rats were food-deprived to 85% of their free-feeding body weight. Animals were weighed daily and their food rations were adjusted to maintain the target weight.

Surgery. The rats were implanted with intragastric catheters by a method adapted from Davis and Campbell (1975). The animals were anesthetized with a ketamine:xylazine mixture (10:7.5; 1.1 mg/kg) and a silastic catheter (1.02 mm i.d., 2.16 mm o.d) was inserted into the fundus of the stomach and secured with sutures and polypropylene mesh. The catheter was routed subcutaneously to the head, where it connected to a Luer-Lok assembly which was secured to the skull with stainless steel screws and dental cement.

Apparatus. Testing was conducted in plastic cages (23 x 24 x 31.5 cm) with stainless steel mesh flooring. Above the cage a counterbalanced lever held an infusion swivel connected, by plastic tubing, at one end to a syringe pump and at the other end to the rat's Luer-Lok assembly. The rats drank from one or two stainless steel drinking spouts which were accessible via two holes at the front of the cage. The spouts were attached to bottles fixed in a motorized retractor unit which automatically inserted and removed the spouts at the beginning and the end of a session. Licking was monitored by an electronic drinkometer connected to a microcomputer which activated the syringe pump as the animal drank. Intragastric infusions were delivered at a rate of 1.3 ml/min and the computer matched the volume of the infusion to the volume which the rat orally consumed. This apparatus allowed for eight animals to be tested simultaneously.

Test Solutions. The CS solutions were 0.2% w/w sodium saccharin (Sigma, St. Louis, MO) solutions flavored with 0.05% Kool-Aid (General Foods, White Plains, NY). The flavor pairs were cherry and grape, or strawberry and orange. The nutrient infusions were 16% w/v sucrose. Half of the rats received one Kool-Aid flavor as the CS+ paired

with IG sucrose, and another flavor as the CS-, paired with IG water. The flavor-infusion pairs were reversed for the remaining animals.

Procedure. Prior to surgery the rats were familiarized with sweet solutions by giving them ad libitum access to a 0.2% saccharin + 2% sucrose solution (2 days), followed by a 0.2% saccharin + 1% sucrose (2 days) and then a 0.2% saccharin solution (2 days). Food and water were also available. The extended sucrose-saccharin exposure period was used because we have found that it speeds the acquisition of saccharin drinking when the rats are later trained in the test cages. After recovery from surgery, the rats were food-deprived to 85% of their post-surgical body weight. This level of deprivation facilitates drinking in short training sessions. Unless otherwise mentioned, rats were maintained at this level of deprivation throughout the experiments. Next, the rats were trained to drink unflavored saccharin in the test cages during 30 min/day sessions.

Formal training consisted of 10 one-bottle training sessions (30 min/day) with the CS+ and the CS-, paired with their appropriate infusions, presented on alternating days. In acquisition studies the experimental group was injected with the appropriate antagonist prior to every training session.

Following training, 30-min, two-bottle preference tests were conducted with the CS+ and CS– solutions, without IG infusions. Prior to test sessions the rats were injected with saline or the appropriate antagonist, in a volume of 1 ml/kg body weight. The timing and dosing of drug injections are described in the methods sections of the individual experiments.

Statistical Analysis. Intakes of the CS+ and CS– solutions were averaged over one-bottle training sessions for analysis via t tests, in one-group designs, and by analysis of variance (ANOVA) in multiple-group designs. Intakes in two-bottle tests were averaged within doses and analyzed using repeated measures ANOVA, followed by tests of simple main effects and Newman Keuls post hoc tests, where appropriate. The two-bottle data was also expressed and analyzed (by ANOVA) as percent CS+ intake (CS+ intake/total intake x 100). This transformation indicates what proportion of total two-bottle test intake was intake of the CS+ solution.

Part I: Effects of Opioid Receptor Antagonism on Nutrient-Conditioned Preference and Acceptance

The first three experiments examined the effects of opioid antagonism on nutrient- conditioned flavor preference and acceptance, using the general opioid antagonist naltrexone.

Experiment 1A: Effects of Naltrexone on the Expression of a Conditioned Flavor-Nutrient Preference in Food-Restricted Rats

Conditioned preferences for nonnutrative flavors previously paired with IG carbohydrate delivery have been demonstrated repeatedly in our laboratory (Sclafani, 1999). This first experiment determined the effects of opioid antagonism on the expression of a preference conditioned by intragastric sucrose. Rats were first trained to prefer a CS+ flavor by pairing it with intragastric sucrose infusions, and then were administered a range of naltrexone doses in order to determine if the expression of CS+ preference was dependent upon the endogenous opioid system.

Methods

Subjects. Twelve male Sprague Dawley rats (375-400 g) were surgically prepared and food-deprived as described above.

Test Solutions. The CS solutions consisted of 0.2% sodium saccharin solutions flavored with 0.05% cherry or grape Kool-Aid. The nutrient infusions were 16% w/v sucrose. Half of the rats received cherry as the CS+ paired with IG sucrose, and grape as the CS– paired with IG water; the flavor-infusion pairs were reversed for the remaining animals.

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

The rats were next adapted to drink unflavored saccharin in the test cages during 30 min/day sessions. For the first six sessions, they were not attached to the infusion system; subsequently they were attached but not infused (6 sessions) and finally infused with water as they drank the saccharin solution (6 sessions). During this adaptation period some rats with low intakes were given a palatable 2% maltodextrin + 0.2% saccharin solution to stimulate drinking. All rats were drinking the 0.2% saccharin prior to the start of formal training.

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

Following training, two-bottle preference tests were conducted with the CS+ and CS– solutions without IG infusions. Ten min prior to test sessions, the rats were injected subcutaneously with saline or naltrexone (Sigma Chemical Co.) at doses of 1.0, 2.5, and

5.0 mg/kg of body weight. All doses of naltrexone were administered in a volume of 1 ml/kg. The order of presentation for the 2.5 mg/kg and the 1 mg/kg doses were counterbalanced and the rats received each dose once; all rats received the 5 mg dose at the same time. This dose was tested twice, on two sequential days, after tests with the lower doses. At least one vehicle session preceded each dose level.

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

Results

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

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

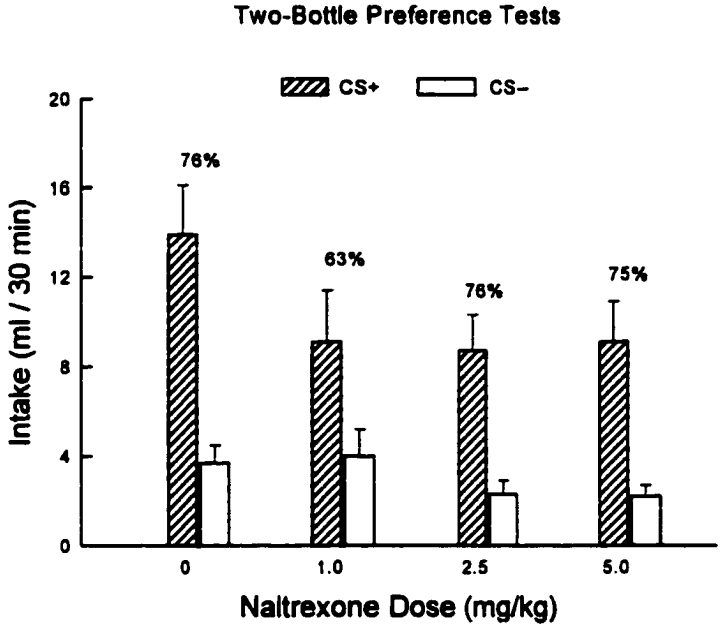

FIG. 1. Intakes (means + SEM) of the CS+ and the CS– solutions during 30 min, two-bottle preference tests with food deprived rats in Experiment 1A. Ten minutes prior to testing the rats were injected with 0 (vehicle) 1.0, 2.5, or 5.0 mg/kg of naltrexone. The CS solutions were grape or cherry flavored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

Discussion

This experiment confirms prior reports that rats learn to prefer a flavor paired with IG carbohydrate infusions over a flavor paired with IG water infusions (Sclafani, 1995; Sclafani, 1999). The new finding here is that the expression of this preference was not attenuated by naltrexone treatment. The drug suppressed total CS intake in this intragastric infusion paradigm, which both extends and is consistent with prior work demonstrating that opioid antagonists reduce saccharin intake (Beczkowska et al., 1993; Lynch, 1986; Lynch & Libby, 1983). However, this intake reduction did not attenuate the relative preference for CS+ over CS– solutions, as the % CS+ preferences did not change. These results indicate that a functioning opioid system is not necessary for the expression of a conditioned flavor-nutrient preference in food restricted rats.

Experiment 1B: Effects of Naltrexone on the Expression of a Conditioned Flavor-Nutrient Preference in Ad Libitum-fed Rats

Experiment 1A demonstrated that opioid antagonism decreased intake without reducing preference in food-deprived rats. There is evidence, however, suggesting that opioid antagonists are more effective in reducing intake in nondeprived rats than in deprived rats (Levine et al., 1995; Lynch & Libby, 1983). These results raise the possibility that naltrexone might have greater effects on preference expression on non-deprived rats. Therefore, the present experiment investigated the effects of naltrexone on preference expression in rats maintained on ad libitum food.

Methods

Ten of the rats from Experiment 1A served as subjects for this experiment. They were first given six one-bottle retraining sessions with the CS solutions from Experiment 1A, paired with their appropriate IG infusions as in Experiment 1A. Their food rations were gradually increased and by the 4th day of training food was available ad libitum except during the 30 min/day sessions. Next, the rats were given a series of two-bottle tests with the CS+ vs. CS– solutions. The rats were injected subcutaneously with vehicle (2 sessions), naltrexone (2.5 mg/kg, 2 sessions), vehicle (4 sessions), and naltrexone (5.0 mg/kg, 2 sessions), in that order, 10 min prior to the choice tests.

Results

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

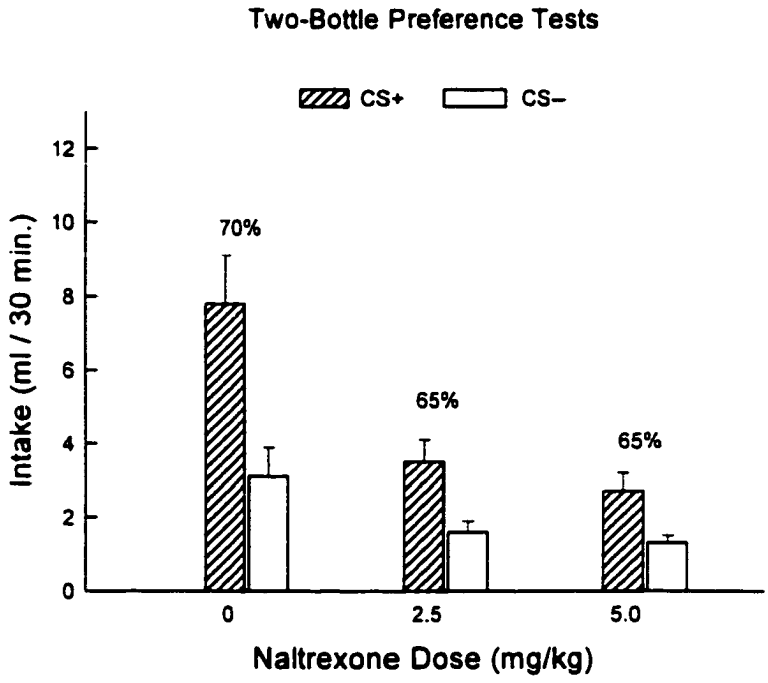

FIG. 2. Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests with food ad libitum animals in Experiment 1B. Ten minutes prior to testing the rats were injected with 0 (vehicle) 2.5, or 5.0 mg/kg of naltrexone. The CS solutions were grape or cherry flavored saccharin,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

Discussion

The intake suppression produced by naltrexone treatment was greater in the food ad libitum animals in the present experiment than it was in the nondeprived animals in Experiment 1A, confirming the findings that opioid antagonists are more effective in reducing intake in nondeprived rats (Levine et al., 1995; Lynch & Libby, 1983). The absolute intake data indicate that naltrexone may inhibit the expression of a conditioned flavor preference in nondeprived rats. That is, the rats drank significantly more CS+ than CS– when vehicle treated, but not when treated with naltrexone. The percent CS+ intakes, however, did not differ between vehicle or drug conditions. A "floor effect" may have contributed to the lack of a significant difference in the absolute intakes of the CS+ and CS– after naltrexone treatment. The rats consumed very little (3.1 ml/30 min) of the CS– in the vehicle test which did not allow for much reduction following naltrexone treatment. Any reduction in total intake would, therefore, be primarily reflected as a reduction in CS+ intake. It is also critical to note that, although the rats did not drink more CS+ than CS– when vehicle treated, the percent CS+ intakes were not affected by naltrexone treatment.

Experiment 2A: Effects of 0.1 mg/kg Naltrexone on the Acquisition and Expression of a Conditioned Flavor-Nutrient Preference in Food-Restricted Rats

The second experiment determined if opioid receptor antagonism during training impaired the acquisition of a flavor preference conditioned by IG sucrose. It also provided further information on the effects of naltrexone on the expression of the conditioned flavor preference, as the Control group in this experiment was only exposed

to the opioid antagonist during preference tests. The rats were initially trained with a low naltrexone dose (0.1 mg/kg) because pilot work revealed that rats treated with a 1.0 mg/kg dose at the start of training consumed very little of the CS solutions (~ 2 g/session) and thus had little opportunity to learn the flavor-nutrient association. The rats were subsequently trained with a 1.0 mg/kg dose in Experiment 2B.

Methods

Subjects. Twenty-eight male Sprague-Dawley rats (380-410 g) bred in our laboratory served as subjects. The rats were fitted with gastric catheters as described above. Due to problems with their gastric cannula, three rats were removed from the study.

Procedure. Prior to surgery the rats were familiarized with sweet solutions by giving them ad libitum access to a 0.2% saccharin + 2% sucrose solution (2 days), followed by a 0.2% saccharin + 1% sucrose (2 days) and then a 0.2% saccharin solution (2 days). Food and water were also available. The extended exposure period was used because of the reluctance some rats displayed in Experiment 1 to drink the 0.2% saccharin solution. After recovery from the surgery, the rats were food-deprived to 85% of their post-recovery body weight. The rats were next adapted to the test cages and training procedure. They were trained to drink unflavored 0.2% saccharin during 30 min/day sessions first without being attached to the infusion system (3 sessions), then while attached but not infused (3 sessions), and finally while infused with water as they drank saccharin (5 sessions). During the last three sessions, the rats were subcutaneously injected with 1.0 ml/kg saline.

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

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

Results

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

The lower panel of Figure 3 presents the data from the vehicle-treated preference tests. In order to compare the effects of the training conditions on the acquisition of a flavor preference, an ANOVA was conducted comparing the vehicle-treated preference tests for the two groups. This analysis revealed no group differences; overall the animals

consumed more CS+ than CS– ($F(1,23)=78.54$, $p<.0001$) and there was no significant interaction. An ANOVA on the percent CS+ intakes also revealed no group differences.

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

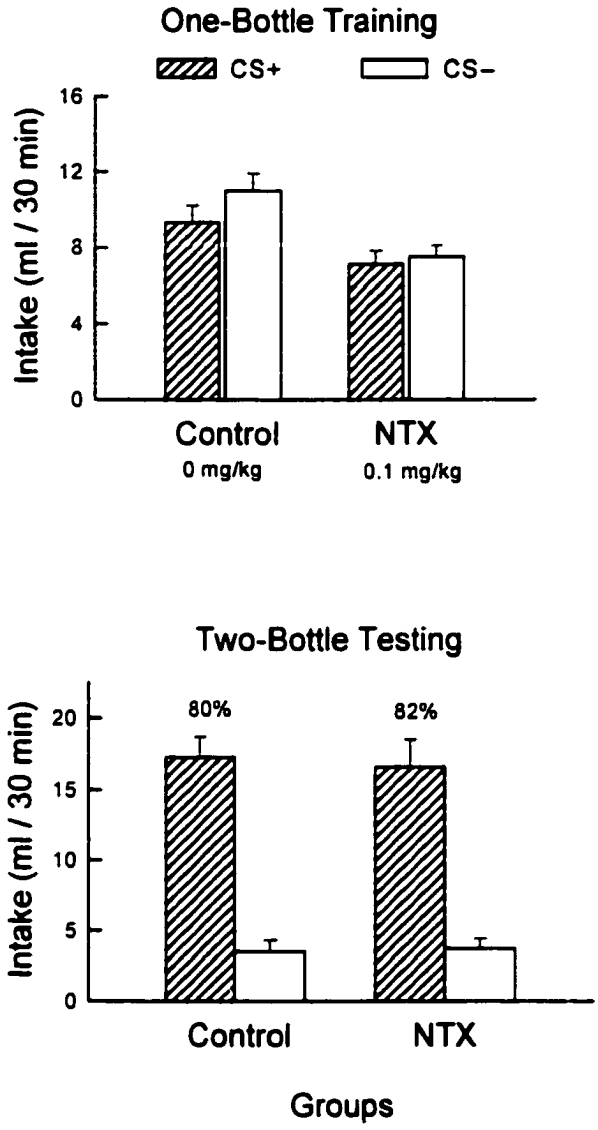

FIG. 3. Top:Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions with food deprived animals in Experiment 2A. The NTX group was injected with 0.1 mg/kg naltrexone prior to each training session and the Control group was injected with vehicle (0 mg/kg). The CS solutions were grape or cherry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests conducted following vehicle injections in Experiment 2A. The numbers atop the bars represent the percent CS+ intake.

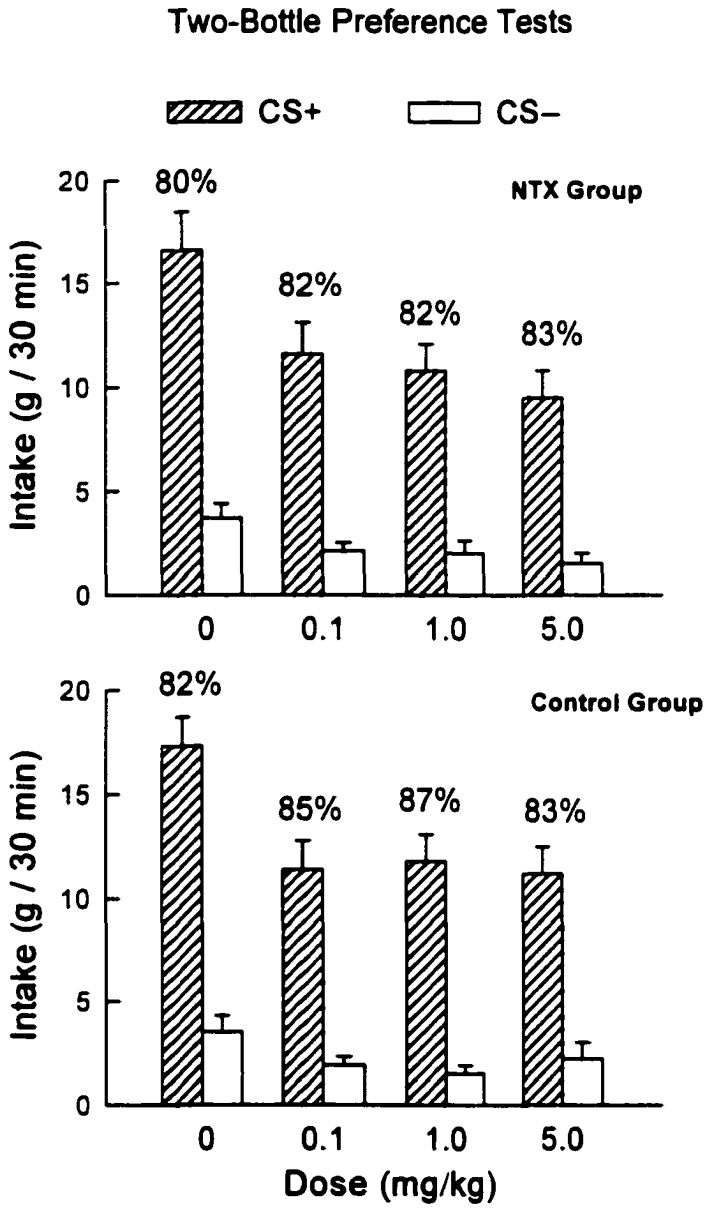

FIG. 4. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests with food deprived rats in Experiment 2A. Ten minutes prior to testing the rats were injected with 0 (vehicle) 0.1, 1.0, or 5.0 mg/kg of naltrexone. The CS solutions were grape or cherry flavored saccharin,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

Discussion

These data demonstrate that treating rats with naltrexone at 0.1 mg/kg during training did not attenuate the acquisition of a flavor preference conditioned by IG sucrose infusions. The NTX rats acquired a CS+ preference similar to that of the Controls rats, despite drinking less CS+ and being infused with less sucrose during training. The results obtained with the Control group provide a replication of Experiment 1A, in that these rats were treated with naltrexone only during preference testing. As in the first experiment, naltrexone treatment prior to the two-bottle tests failed to attenuate the expression of the CS+ preference.

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

Experiment 2B: Effects of 1.0 mg/kg Naltrexone on the Acquisition and Expression of a Conditioned Flavor-Nutrient Preference in Food-Restricted Rats

The rats from Experiment 2A were redistributed into two new NTX and Control groups. The new NTX group contained 6 rats from the former NTX group and 7 rats from the former Control group. The new Control group contained 6 rats from the former NTX and 6 rats from the former Control groups. The rats in these new groups were equated for their CS+ preferences and total intakes during the two-bottle tests of Experiment 2A.

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

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

Results

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

Figure 5 (lower panel) presents the data from the vehicle-treated preference tests. In order to compare the effects of the training conditions on the acquisition of a flavor preference, an ANOVA was conducted comparing the vehicle-treated preference tests for the two groups. Overall, the animals consumed more CS+ than CS– ($F(1,23)=139.48$, $p<.0001$), there were no differences between the groups, and no significant interactions. An ANOVA on the percent CS+ intakes also revealed no group differences.

Figure 6 presents the data from the drug-treated preference tests. The rats in both groups consumed more CS+ than CS– in the two-bottle tests ($F(1,23)=132.8$, $p<.0001$). There was also an overall effect of naltrexone dose ($F(1,23)=130.7$, $p<.0001$) indicating that, in both groups, total intakes during the drug-treated tests were less than during the

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

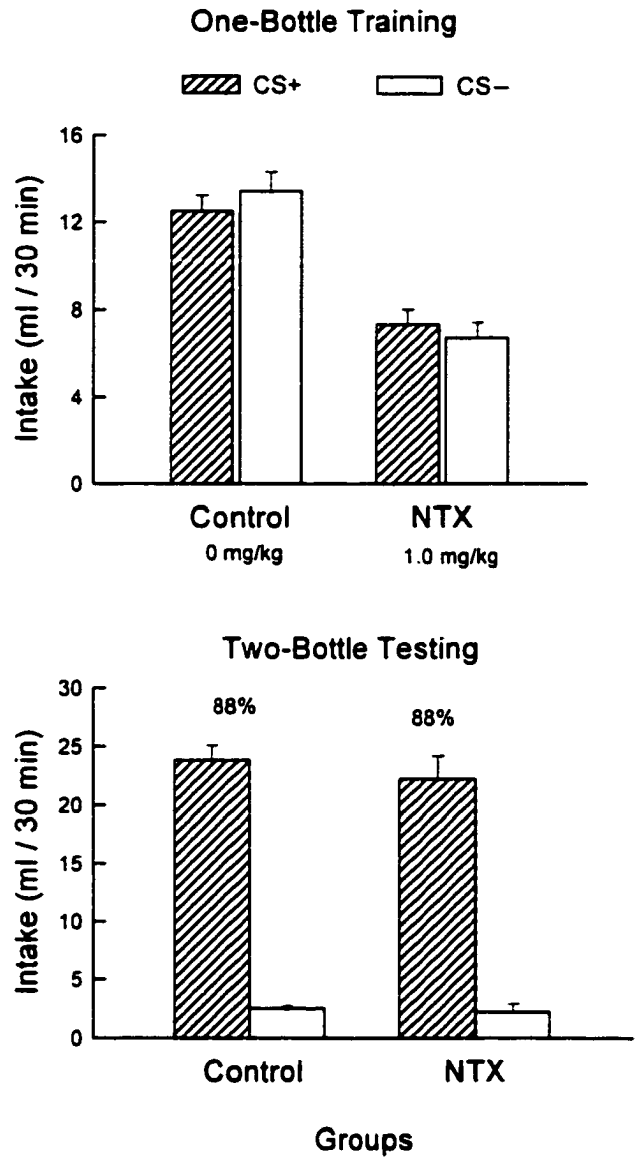

FIG. 5. Top: Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions with food deprived animals in Experiment 2B. The NTX group was injected with 1.0 mg/kg naltrexone prior to each training session and the Control group was injected with vehicle (0 mg/kg). The CS solutions were orange or strawberry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests conducted following vehicle injections in Experiment 2B. The numbers atop the bars represent the percent CS+ intake.

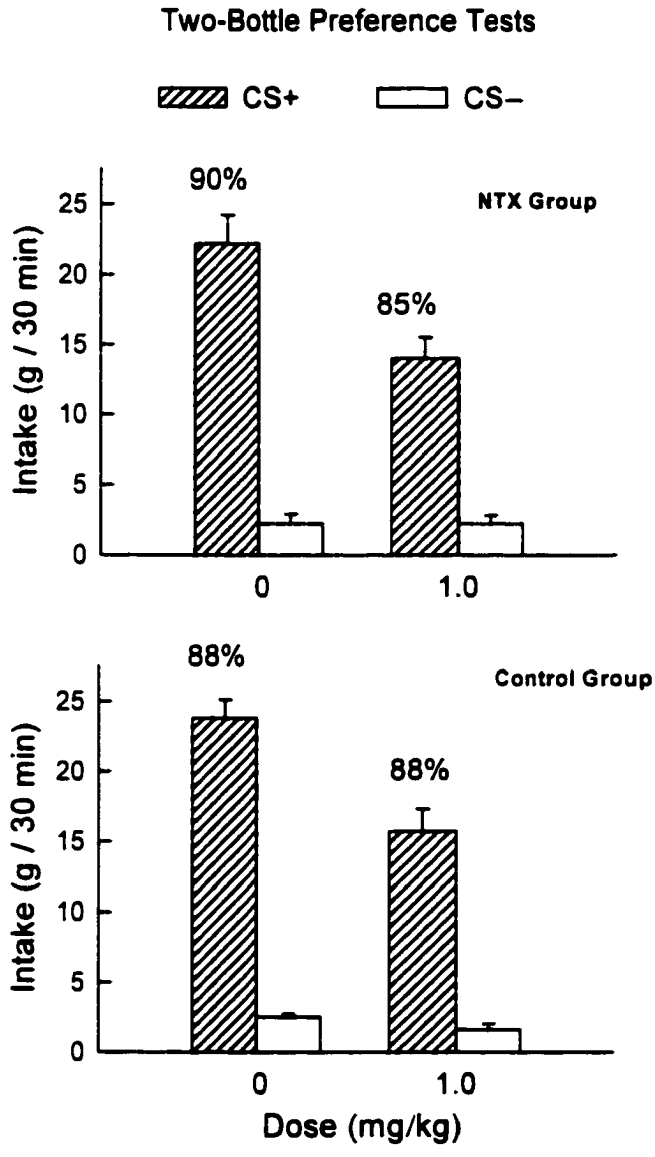

FIG. 6. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests with food deprived rats in Experiment 2B. Ten minutes prior to testing the rats were injected with 0 (vehicle) or 1.0 mg/kg of naltrexone. The CS solutions were orange or strawberry flavored saccharin, and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

Discussion

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

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

Experiment 2C: Effects of Naltrexone on the Expression of a Conditioned Flavor Nutrient Preference in Ad libitum Fed Rats

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

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

Results

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

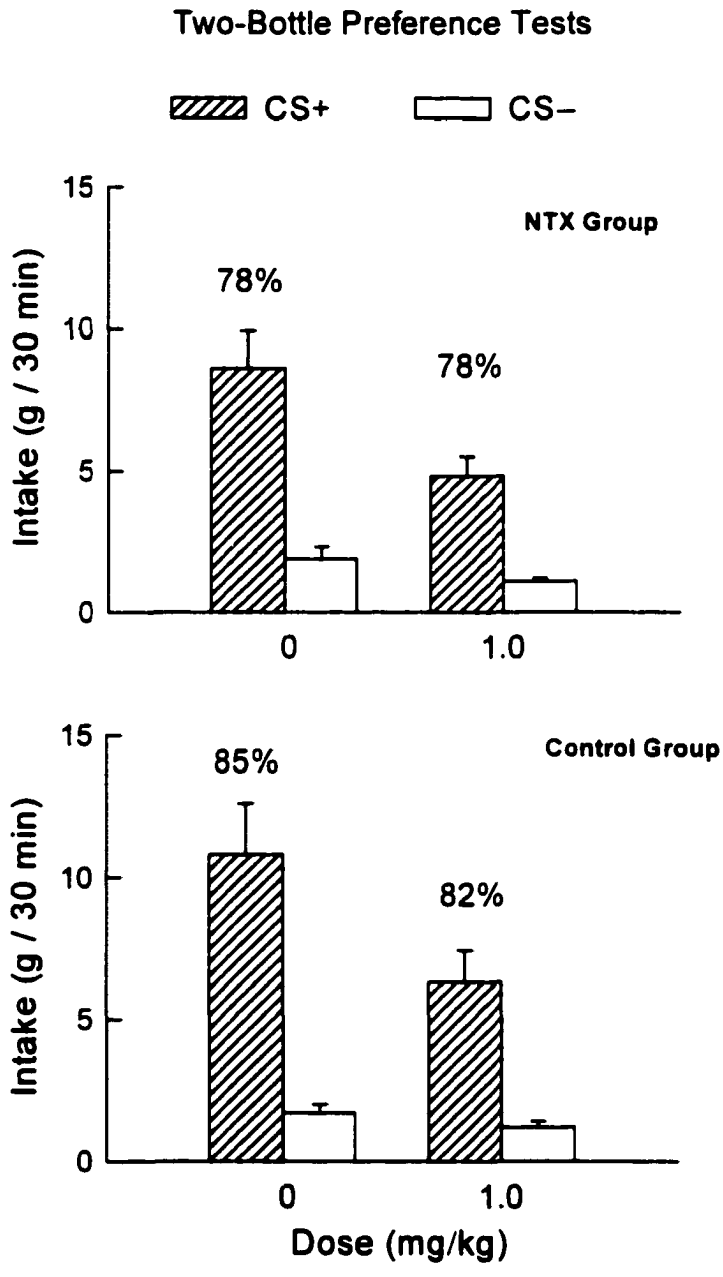

FIG. 7. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests with ad libitum fed rats in Experiment 2C. Ten minutes prior to testing the rats were injected with 0 (vehicle) or 1.0 mg/kg of naltrexone. The CS solutions were orange or strawberry flavored saccharin, and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

Discussion

These results are similar to those obtained in Experiment 1B and 2B in that naltrexone treatment produced a greater decrease in CS+ solution intake than CS– solution intake during two-bottle testing. However, unlike Experiment 1B, the ad libitum-fed rats in both groups drank significantly more CS+ than CS– even when drug treated. This may have occurred because the rats in the present experiment had a stronger initial CS+ preference than did the rats in Experiment 1B. It is also the case that higher drug doses were used in Experiment 1B, but this does not readily explain the different results, as no dose effects were observed in any of the experiments of this series. Note that in both experiments naltrexone did not significantly decrease the percent CS+ intakes. As in previous experiments, the selective reduction in CS+ intake produced by naltrexone is difficult to interpret because of a possible floor effect on CS– intakes.

Together, the results of Experiments 1 and 2 provide little evidence that the acquisition or expression of a conditioned flavor preference are dependent upon the endogenous opioid system. However, Ramirez (1997) reported an effect of opioid antagonism on the expression of an increased flavor acceptance conditioned by IG carbohydrate infusions. Experiment 3 was conducted in order to test the effects of opioid antagonism on an increased flavor acceptance conditioned using methodology more analogous to our preference experiments.

Experiment 3: Effects of Naltrexone on Conditioned Flavor Acceptance

In addition to conditioning an increase in the intake of a CS+ solution relative to a CS– solution, which is measured in two-bottle tests, IG nutrient infusions may also condition an increase in the absolute intake of the CS+ solution intake, which is measured in separate one-bottle tests with the CS+ and CS– solutions. Increased acceptance is more difficult to obtain, however, in part because the satiating action of nutrient infusions counteract their intake stimulating effect (Ramirez, 1997). Furthermore, conditioned flavor acceptance appears to extinguish more rapidly than conditioned flavor preference, suggesting that different neurobehavioral mechanisms may mediate these conditioned responses (Drucker et al., 1994; Perez et al., 1997). While Experiments 1 and 2 provide little evidence for opioid involvement in nutrient-conditioned flavor preferences, a recent report by Ramirez (1997) implicates the opioid system in the mediation of conditioned flavor acceptance. As previously mentioned, Ramirez reports that naloxone (0.1 or 0.3 mg/kg) decreased solution intake more in rats drinking saccharin paired with IG carbohydrate than in rats drinking saccharin paired with IG water. In view of these results, and the primarily negative results obtained in Experiments 1 and 2, the present experiment investigated whether naltrexone reduces the conditioned acceptance of a CS+ solution in one-bottle intake tests. To maintain comparability with Experiments 1 and 2, a within-group design was employed using the same CS flavors as in Experiments 2B and 2C. As in the Ramirez study (1997), the CS+ was paired with IG infusions of dilute carbohydrate (6% maltodextrin) and dilute saccharin solutions (0.05%) were used as CSs.

The rats were initially trained 20 hr/day with the CS flavors because it has been demonstrated this is a particularly effective way of conditioning increased flavor acceptance (Perez et al., 1998). For drug testing, 30 min/day sessions were conduced with the animals minimally (95%) food-deprived.

Methods

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

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

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

Procedure. At the start of the experiment the rats were housed in the training cages and adapted to a feeding schedule in which lab chow and water were available for 2 hr each day, followed by 2 hr of no food or fluid, and then 20 hr access to fluid only (which included the 12 hr dark period). Initially, water paired with IG water was available during the 20 hr access period. The rats were then given alternating one-bottle

access (20 hr/day) to the CS+ solution paired with IG maltodextrin infusions and the CS– solution paired with IG water for a total of 8 days. This was followed by a two-bottle test with the CS+ vs. CS– solutions for two 20 hr/day sessions. During this test, intake of the CS+ solution was paired with IG maltodextrin; CS– intake, which was expected to very low, was not paired with infusions because of apparatus limitations. The rats were next given one-bottle access to the CS solutions, each paired with their appropriate infusions, during alternating 30 min/day sessions. Infusions were continued during the testing phase as acceptance effects extinguish rapidly in the absence of reinforcement (Perez et al., 1998). One hr after the daily 30-min sessions, water was provided ad libitum and a food ration was given which maintained the rats at approximately 95% of their free-feeding body weight.

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

Results

Over the course of the 20-hr one-bottle training sessions, the rats substantially increased their intake of the CS+ solution relative to the CS– solution. During the last

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

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

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

The rats drank more CS+ than CS– solution in the vehicle and 2.5 mg/kg naltrexone tests ($F(1,10) =49.75$, $p<.001$; Figure 9). Naltrexone reduced overall CS

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

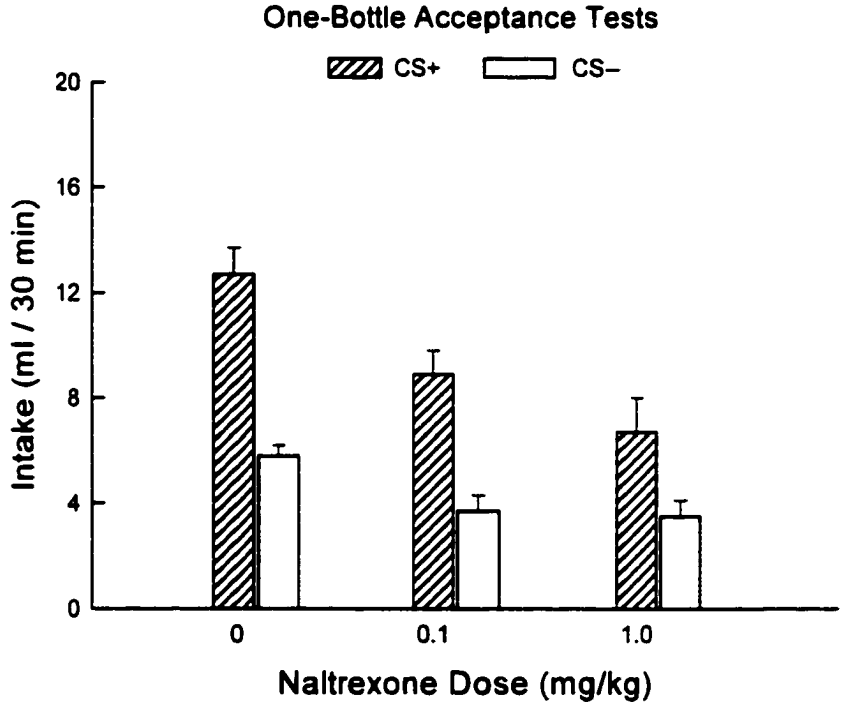

FIG. 8. Intakes (means +SEM) of the CS+ and the CS– during 30 minute, one-bottle acceptance tests with food-restricted rats in Experiment 3. Ten minutes prior to testing the rats were injected with 0 (vehicle), 0.1 or 1.0 mg/kg of naltrexone. The CS solutions were orange or strawberry flavored saccharin,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

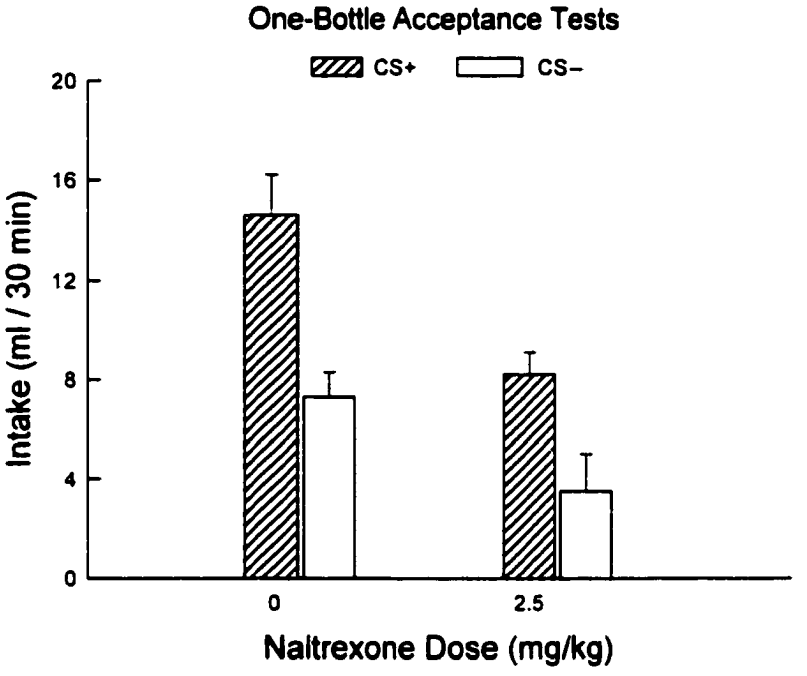

FIG. 9. Intakes (means +SEM) of the CS+ and the CS– during 30 minute, one-bottle acceptance tests with food-restricted rats in Experiment 3. Ten minutes prior to testing the rats were injected with 0 (vehicle) or 5.0 mg/kg of naltrexone. The CS solutions were orange or strawberry flavored saccharin, and the CS+ was paired with intragastric maltodxtrin and the CS– with intragastric water infusion throughout training and testing.

Discussion

Confirming previous results (Perez et al., 1998), the rats consumed substantially more of the CS+ solution paired with IG carbohydrate infusions than of the CS– flavor paired with IG water during one- and two-bottle 20 hr/day tests. They continued to overconsume the CS+, relative to the CS–, during the subsequent 30 min/day one-bottle tests. Evidence that this overconsumption represents a conditioned increase in the acceptability of the CS+ flavor, rather than a direct response to the nutrient infusions, is provided by prior data showing that CS+ intakes remain elevated during initial extinction tests when water rather than nutrient is infused (Perez et al.,1998; Ramirez, 1997).

Naltrexone treatment reduced the intakes of both CSs during the one-bottle tests, although CS+ intake was suppressed more than CS– intake as indicated by the significant dose by CS interaction. This partially replicates the finding of Ramirez (1997) that naloxone decreased solution intake more in rats drinking a saccharin solution paired with IG carbohydrate than in rats drinking a saccharin solution paired with IG water. As stated earlier, the unflavored saccharin solutions used in the Ramirez (1997) study can be considered to be a "CS+" and "CS–", comparable to the CS solutions in the present experiment. The findings of the two experiments differ in that Ramirez reported that the lowest drug dose (0.1 mg/kg) decreased only "CS+" intake, but in the present experiment the 0.1 mg/kg decreased the intake of both the CS+ and CS– solutions. Furthermore, naltrexone did not suppress CS+ intake more than CS– intake when the data are expressed as a percent of the vehicle baseline intakes.

There are many differences between the present experiment and the Ramirez study that may account for the discrepant results. Of particular note, the vehicle baseline

intakes of the "CS–" solution were lower in the Ramirez study than in the present study (~ 4 ml vs. ~ 6.5 ml/30 min) which could explain why he observed a more specific drug effect on "CS+" intake. Ramirez (1997) rejected a floor effect interpretation because he found that the dopamine antagonist pimozide suppressed "CS–" intake relative to baseline. However, opioid antagonists, unlike dopamine antagonists, typically do not suppress licking rates during the first several minutes of a drinking bout (Kirkham & Cooper, 1988; Xenakis & Sclafani, 1981, but see Higgs & Cooper, 1998). Therefore, if baseline bout size is low, rats may stop drinking before the drug's intake-reducing actions are expressed.

Part II: Effects of Dopamine receptor subtype antagonism on nutrient-conditioned flavor preference

Experiment 4: Effects of Raclopride on the Expression of a Conditioned Flavor-Nutrient Preference in Food-Restricted Rats

Experiments 1-3 indicate that the endogenous opioid system is not critically involved in the acquisition or expression of a sucrose conditioned place preference. Attention was therefore shifted to the dopamine system. As discussed in the introduction to this dissertation, the endogenous dopamine system has been implicated as a mediator of food reward (Smith, 1995).

Prior studies investigating the effects of dopamine antagonist on flavor preference have focused upon flavor-flavor conditioning in real-feeding (Hsiao & Smith, 1995) and sham-feeding rats (Yu et al., 2000). The present study focused on flavor-nutrient conditioning by training rats with the CS+ flavor paired with IG sugar infusions. Experiment 4 was conducted to determine if the expression a sucrose-conditioned preference is dependent upon the dopamine D_2 receptor system.

Methods

Subjects. Ten male Sprague Dawley rats (415-455 g) were housed individually in wire mesh cages maintained on a 12:12 hr light/dark cycle.

Surgery. The rats were surgically prepared as described in the general methods.

Test Solutions. The CS solutions consisted of 0.2% sodium saccharin solutions flavored with 0.05% cherry or grape Kool-Aid. The nutrient infusions were 16% w/v sucrose. Half of the rats received cherry as the CS+ paired with IG sucrose, and grape as

the CS– paired with IG water; the flavor-infusion pairs were reversed for the remaining animals.

Procedure. After recovery from surgery, the rats were familiarized with unflavored 0.2% saccharin solution by giving them 24 hr access to saccharin as well as water. All rats were then food restricted and maintained at 85% of their post-recovery body weight.

The rats were next adapted to drink unflavored saccharin in the test cages during 30 min/day sessions. For the first three sessions, they were not attached to the infusion system; subsequently they were attached but not infused (5 sessions) and finally infused with water as they drank the saccharin solution (5 sessions).

Formal training consisted of ten one-bottle training sessions (30 min/day) with the CS+ and the CS– solutions, paired with their appropriate infusions, presented on alternating days. The left-right position of the CS bottles was counterbalanced across days and animals. During the last four training sessions the rats received intraperitoneal (IP) injections of isotonic saline (vehicle; 1.0 ml/kg body weight) 15 min prior to the start of the session to familiarize them with the injection procedure.

Following training, two-bottle preference tests were conducted with the CS+ and CS– solutions without IG infusions. Fifteen min prior to test sessions, the rats were injected with saline or raclopride (Research Biochemical Intl., Natick, MA) at doses of 0 (vehicle), 200, 400, and 800 nmol/kg of body weight, in a volume of 1 ml/kg. The doses were given for 2 days each, in ascending order, with two days of vehicle treated preference separating each of the drug doses.

Results

The rats consumed more of the CS– than the CS+ (mean ±sem: 12.5 ±1.0 and 11.2 ±0.8 g, respectively) during training ($t(9)=2.26$, $p<.05$).

The results of the preference tests appear in Figure 10. Overall, the rats drank significantly more CS+ than CS– solution ($F(1,9)=116.67$, $p<.0001$). There was a significant effect of raclopride treatment ($F(3,27)=33.76$, $p<.0001$), and there was an interaction between CS and raclopride treatment ($F(3,27)=17.71$, $p<.0001$).

Tests of simple main effects revealed that raclopride treatment reduced the intake of the CS+ ($p<.0001$), but not of CS–. The rats drank significantly more CS+ than CS– at the vehicle ($p<.0001$), 200 nmol/kg ($p<.0001$), and 400 nmol/kg ($p<.0001$) doses, but not at the 800 nmol/kg dose. An ANOVA on the percent CS+ intakes revealed a significant effect of raclopride dose ($F(3,24)=4.7$, $p<.05$). A Newman-Keuls post-hoc analysis revealed that the percent CS+ preference at the 800 nmol/kg dose was significantly lower than all of the other doses ($p<.05$), while the percent CS+ preference at the other doses did not differ from each other.

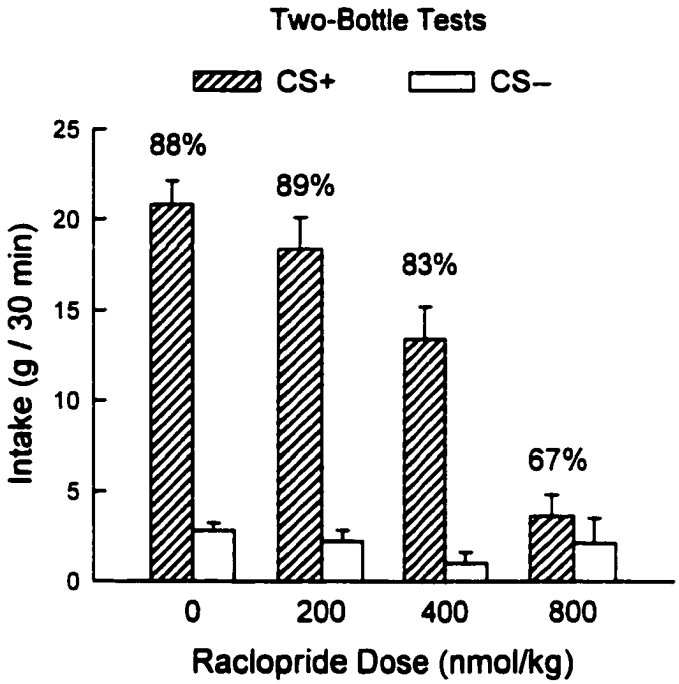

FIG. 10. Intakes (means + SEM) of the CS+ and the CS– solutions during 30 min, two-bottle preference tests with food deprived rats in Experiment 4. Fifteen minutes prior to testing the rats were injected with 0 (vehicle) 200, 400, or 800 nmol/kg of raclopride. The CS solutions were grape or cherry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. The numbers atop the bars represent the percent CS+ intake at that dose.

Discussion

As in previous experiments in this dissertation, rats learned to prefer a flavor paired with IG sucrose infusions over a flavor paired with IG water infusions. Raclopride treatment suppressed total intake, without suppressing preference, at all but the highest dose. The highest dose (800 nmol/kg) nearly eliminated intake, which resulted in nonsignificant differences in the intake of the CS solutions. The raclopride treatment did suppress the intake of the CS+, and not the CS– during preference testing. However, the baseline CS– intake was quite low in this experiment (2.8 ml in the vehicle tests), therefore there was very little room to see a suppression of the intake of this CS across doses. Therefore the seemingly specific effects of raclopride on CS+ intake might reflect a suppression of total intake obscured by a floor effect on CS– intakes. In conclusion, while raclopride reduces total intake, it has little effect on the expression of a conditioned flavor preference, except at doses which nearly eliminate intake.

Experiment 5: Effects of 200 nmol/kg Raclopride on the Acquisition and Expression of a Conditioned Flavor-Nutrient Preference in Food-Restricted Rats

Given that raclopride had little effect on the expression of a conditioned flavor preference, I sought to determine if the D_2 dopamine receptor system was critically involved in the acquisition of a preference. This experiment also provided further information on the effects of D_2 antagonism on the expression of the conditioned flavor preference, as the control group was trained and tested in a manner similar to the rats in Experiment 4. The rats were trained with a 200 nmol/kg dose of raclopride, as pilot data had indicated that this dose reduced, but did not eliminate intake of the CS solutions. .

Methods

Subjects. 18 male Sprague-Dawley rats (497-532 g) bred in our laboratory were used as subjects. The rats were fitted with gastric catheters as described in the general methods. Two rats were removed from the study due to problems with their gastric catheters.

Procedure. The rats were divided into two groups equated for their pre-training saccharin intakes. The RAC group (n=9) received 200 nmol/kg raclopride 15 min prior to the daily one-bottle training sessions, and the Control group (n=7) group received vehicle injections prior to training.

Formal training consisted of 10 one-bottle training sessions with the CS+ and the CS– paired with IG infusions of 16% sucrose and water, respectively. The CS solutions were grape- and cherry-flavored saccharin solutions. Following training, two-bottle preference tests were conducted with the CS+ vs. CS– solutions without IG infusions. During preference testing both groups were treated identically and were given injections of vehicle and, in ascending order, 200, 400, and 800 nmol/kg of raclopride 15 min prior to the two-bottle sessions. Each raclopride dose was presented for two consecutive sessions, and two vehicle sessions preceded each dose level.

Results

During one-bottle training the RAC group drank significantly less of the CS solutions than did the Control group ($F(1,14)=8.91$, $p<.01$) (Figure 11, upper panel). Compared to the Control group, the RAC group drank 47% and 29% less, respectively, of the CS+ and the CS– solutions. There was no effect of CS; there was, however, an interaction between group and CS ($F(1,14)=5.12$, $p<.05$). Tests of simple main effects

revealed that the RAC group consumed significantly more CS– than CS+ during training ($p<.05$), while the Control group drank more CS+ ($p<.01$), and more CS– ($p<.05$) than did the RAC group.

The lower panel of Figure 11 presents the results of the vehicle-treated preference tests. The ANOVA on the vehicle-treated two-bottle tests revealed no group differences. Overall, the animals consumed more CS+ than CS– ($F(1,14)=42.59$, $p<.0001$), and there was a significant interaction between Group and CS ($F(1,14)=14.33$, $p<.01$). Tests of simple main effects revealed that the Control group drank more CS+ than did the RAC group ($p<.001$), while the intake of CS– did not differ between groups. The Control group drank significantly more CS+ than CS– ($p<.0001$), while the RAC group did not. An ANOVA on the percent CS+ intakes revealed that the Control group had a significantly higher % CS+ preference than did the RAC group ($F(1,14)=21.87$, $p<.001$) (Fig 11).

As illustrated in Figure 12, the rats in both groups consumed more CS+ than CS– in the drug-treated two-bottle tests ($F(1,14)=30.57$, $p<.0001$). There was also an overall effect of raclopride dose ($F(3,42)=37.8$, $p<.0001$). There was an interaction between group and CS ($F(1,14)=10.95$, $p<.01$), as well as an interaction between raclopride dose and CS ($F(3,42)=7.26$, $p<.001$). There was a three-way interaction between group, CS, and raclopride treatment ($F(3,42)=3.39$, $p<.05$). Due to the three way interaction, separate ANOVAs were conducted on the data from each group.

The Control group drank significantly more CS+ than CS– ($F(1,6)=20.13$, $p<.01$). There was also an effect of raclopride treatment ($F(3,18)=17.65$, $p<.0001$), and an interaction between CS and raclopride dose ($F(3,18)=7.43$, $p<.01$). Tests of simple main

effects revealed that the rats drank more CS+ than CS– at the vehicle ($p<.01$), 200 ($p<.05$), and 400 ($p<.05$), but not the 800 nmol/kg dose. Raclopride treatment reduced the intake of the CS+ ($p<.0001$), but not the CS–.

The RAC rats drank significantly more CS+ than CS– ($F(1,8)=6.16$, $p<.05$). There was also an effect of raclopride treatment ($F(3,24)=20.87$, $p<.0001$); there was no interaction between raclopride treatment and CS.

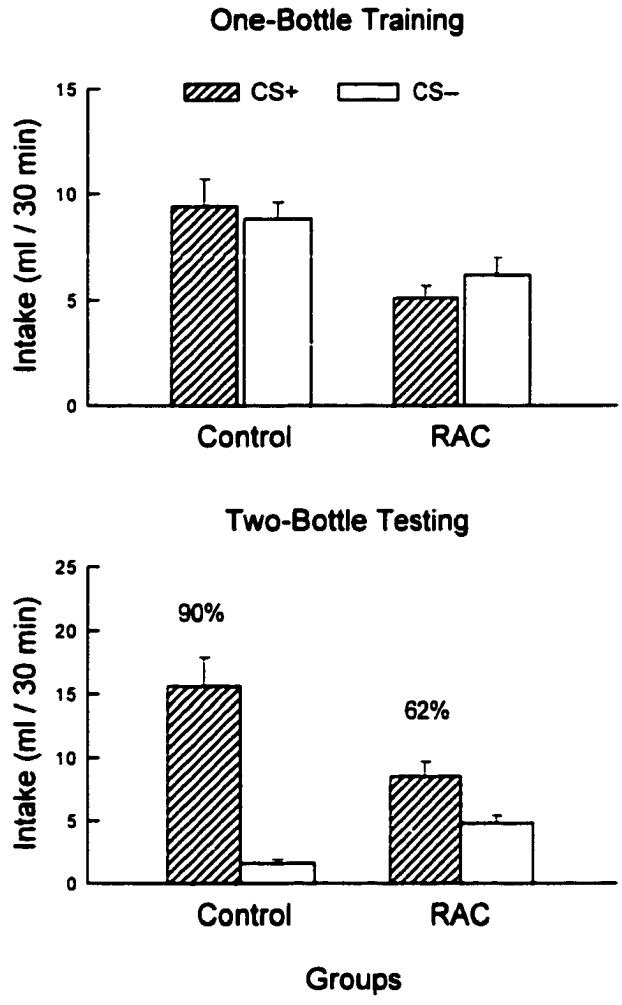

FIG. 11. Top: Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions in Experiment 5. The RAC group was injected with 200 nmol/kg raclopride prior to each training session, the Control group was injected with vehicle. The CS solutions were grape or cherry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests conducted following vehicle injections in Experiment 5. The numbers atop the bars represent the percent CS+ intake.

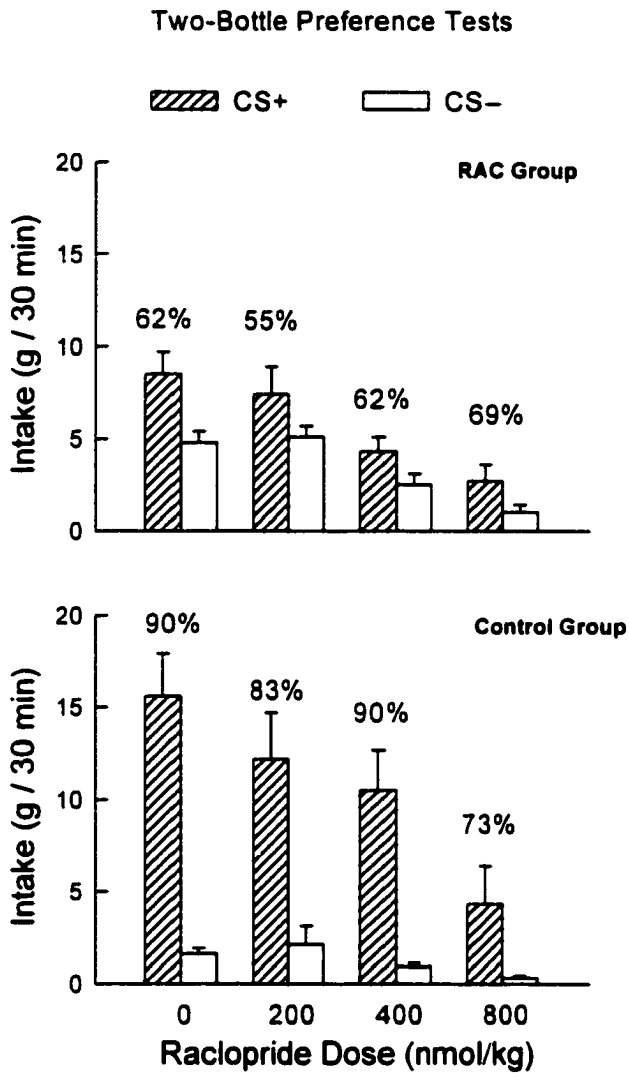

FIG. 12. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests in Experiment 5. Fifteen minutes prior to testing the rats were injected with 0 (vehicle) 200, 400, or 800 nmol/kg of raclopride. The CS solutions were grape or cherry flavored saccharin. The CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training, but no infusions were given during preference testing. The top panel represents the data for the RAC group which received 200 nmol/kg raclopride during training. The bottom panel represents the data for the Control group that was injected with vehicle during one-bottle training. The numbers atop the bars represent the percent CS+ intake at that dose.

Discussion

Raclopride treatment during training suppressed the acquisition of a CS+ preference. Analysis of the choice tests conducted following vehicle injections indicated that the RAC group did not have a significant preference for the CS+, although ANOVA of the vehicle and drug tests combined revealed that overall, the RAC group consumed more CS+ than CS–. Raclopride also suppressed CS+, as well as CS– intakes during one-bottle training which resulted in the RAC group being infused with less sucrose on CS+ training days than was the Control group. This may account for the drug's effect on reducing the CS+ preference. Note that while naltrexone suppressed CS+ intakes during training but did not attenuate CS+ preference in Experiment 2, the suppression was not as pronounced as that produced by raclopride in the present experiment. The impact of reduced one-bottle training intakes on raclopride's effect on CS+ preference learning was investigated in the next experiment.

The effects of raclopride treatment on the CS+ preference of the Control group replicates the findings of Experiment 4. That is, the drug suppressed the intake of the CS+ during the two-bottle tests, but at all doses except the highest (800 nmol/kg) the Control rats consumed more CS+ than CS–. As in Experiment 4, the failure of raclopride to suppress CS– intake may be due the low baseline intakes of this solution.

Experiment 6: The Effects of 200 nmol/kg Raclopride on the Acquisition and Expression of a Conditioned Flavor Preference, with a Yoked Control Group

The attenuation of CS+ preference conditioning produced by raclopride in Experiment 5 may have been secondary to the reduced CS+ intakes and paired sucrose

infusions during one-bottle training. This possibility was evaluated in the present experiment by replicating Experiment 5 with the addition of a yoked control group, which had its training intakes matched to that of the RAC group. This multiple group design allowed for an examination of the effects of raclopride on both the acquisition (RAC group) and expression (Control group) of a flavor preference, while controlling for the intake-reducing effects of raclopride during training (Yoked group).

Methods

Subjects. 28 male Sprague-Dawley rats (476-512 g) bred in our laboratory were used as subjects. The rats were surgically prepared, housed, and maintained as described in the general method.

Test Solutions. The CS solutions consisted of 0.2% sodium saccharin solutions flavored with 0.05% cherry or grape Kool-Aid. The nutrient infusions were 16% w/v sucrose. Half of the rats in each group received cherry as the CS+ paired with IG sucrose, and grape as the CS– paired with IG water; the flavor-infusion pairs were reversed for the remaining animals.

Procedure Prior to surgery the rats were familiarized with sweet solutions by giving them ad libitum access to a 0.2% saccharin + 2% sucrose solution (2 days), followed by a 0.2% saccharin + 1% sucrose (2 days) and then a 0.2% saccharin solution (2 days). Food and water were also available. The extended sucrose-saccharin exposure period was used because I have found that it speeds the acquisition of saccharin drinking when the rats are later trained in the test cages. After recovery from the surgery, the rats were food-deprived to 85% of their post-recovery body weight. The rats were next adapted to the test cages and training procedure. They were trained to drink unflavored

0.2% saccharin during 30 min/day sessions first without being attached to the infusion system (3 sessions), then while attached but not infused (3 sessions), and finally while infused with water as they drank saccharin (5 sessions). During the last three sessions, the rats were injected with 1.0 ml/kg saline. Based upon their training intakes and body weights the rats were divided into 3 groups: RAC (n=10), Control (n=8), and Yoked (n=10).

Formal training consisted of ten 30 min one-bottle training days with the CS+ and the CS–, and their appropriate infusions, presented on alternate days. The left-right position of the CS bottles was counterbalanced, following an ABBA pattern. The RAC group received an IP injection of raclopride, at a dose 200 nmol/kg body weight, 15 min prior to the start of the training sessions. The Control and the Yoked group received IP saline injections 15 min prior to each session. The RAC and Control groups were run on the same day, while the Yoked group was run 2 days behind. The oral intakes and IG infusions of the individual animals in the Yoked group were limited to the mean intake of the RAC group on the preceding corresponding CS day.

Following training two-bottle preference tests were conducted with the CS+ and CS– solutions, with no IG infusions. Fifteen min prior to testing rats were treated with IP vehicle injections or with injections of 200, 400, or 800 nmol/kg of raclopride. Each dose of raclopride was given on two sequential days, with 2 days of vehicle separating each pair of drug days; the doses were given in ascending order. The intake of the Yoked group was not limited during preference testing.

Statistical Analysis. Intakes of the CS+ and CS– solutions were averaged over one-bottle training sessions and analyzed with an ANOVA. Note that the data for the Yoked group was not included in this ANOVA, due to the imposed drinking limit.

The results of the two-bottle testing with vehicle treatment were compared between groups, to assess the effects of the training conditions on preference acquisition. Intakes in two-bottle tests were averaged within doses and analyzed using repeated measures ANOVA, followed by tests of simple main effects and Newman Keuls post hoc tests, where appropriate. In cases where the ANOVA indicated interactions between group and other variables, separate ANOVAs were conducted on the two-bottle data from each group. The two-bottle data were also expressed as percent CS+ intake (CS+ intake/total intake x 100).

Results

As illustrated in Figure 13 (upper panel), the intakes of the RAC and Yoked groups were well matched during one-bottle training, and the two groups consumed approximately 35% less of the CS+ and CS– solutions than did the Control group. The ANOVA confirmed that the RAC vs. Control group difference was significant ($F(1,16)=6.67$, $p<.05$) and revealed no CS effect or interaction between CS and Group. In the two-bottle choice tests conducted following vehicle-treatment, overall the rats consumed more CS+ than CS– ($F(1,25)=48.6$, $p<.0001$) and there no group differences or interactions. The percent CS+ intakes of the Yoked and RAC group were somewhat lower than that of the Control group, but these differences did not reach significance (Figure 13, lower panel).

The effect of raclopride treatment on the expression of the CS+ preference is summarized in Figure 14. Overall, the rats drank significantly more CS+ than CS– ($F(1,25)=42.6$, $p<.0001$) and raclopride reduced intake ($F(3,75)=87.12$, $p<.0001$). There was no significant group difference, but there was an interaction between raclopride treatment and CS ($F(3,75)=22.01$, $p<.0001$) as well as an interaction between Group, raclopride treatment, and CS ($F(6,75)=2.73$, $p<.05$). Because of the three- way interaction, individual ANOVAs for each group were performed. The Control group analysis revealed a significant CS by Drug interaction ($F(3,21)=20.67$) $p<.0001$). Simple main effects tests indicated that the Control group consumed more ($p<.01$) CS+ than CS– at the 0 (vehicle) and 200 nmol/kg doses but not at the 400 or 800 nmol/kg doses. The drug reduced ($p<.001$) the intake of the CS+ ($F(3,41)=41.84$) but not of the CS–.

The RAC group analysis also revealed an interaction between CS and raclopride treatment ($F(3,27)=6.61$, $p<.002$). Further tests indicated that the RAC animals consumed significantly more ($p<.05$) CS+ than CS– at the 0 and the 200 nmol/kg doses but not at the higher doses. Raclopride reduced ($p<.05$) the intake of both the CS+ and the CS–. The Yoked group drank significantly more CS+ than CS– ($F(1,9)=7.88$, $p<.03$) and decreased their intake with raclopride treatment ($F(3,27)=38.04$, $p<.0001$); there was no interaction between CS and drug dose.

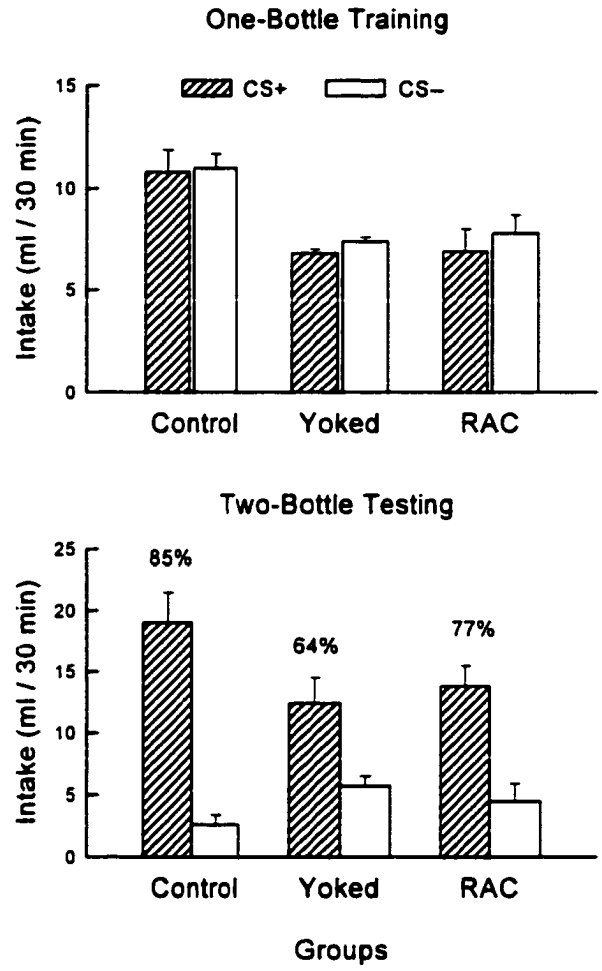

FIG. 13. Top: Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions in Experiment 6A. The RAC group was injected with 200 nmol/kg raclopride prior to each training session, the Control and the Yoked groups were injected with vehicle. The Yoked group had its CS intake limited to that of the RAC group. The CS solutions were grape or cherry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests conducted following vehicle injections in Experiment 6A. The numbers atop the bars represent the percent CS+ intake.

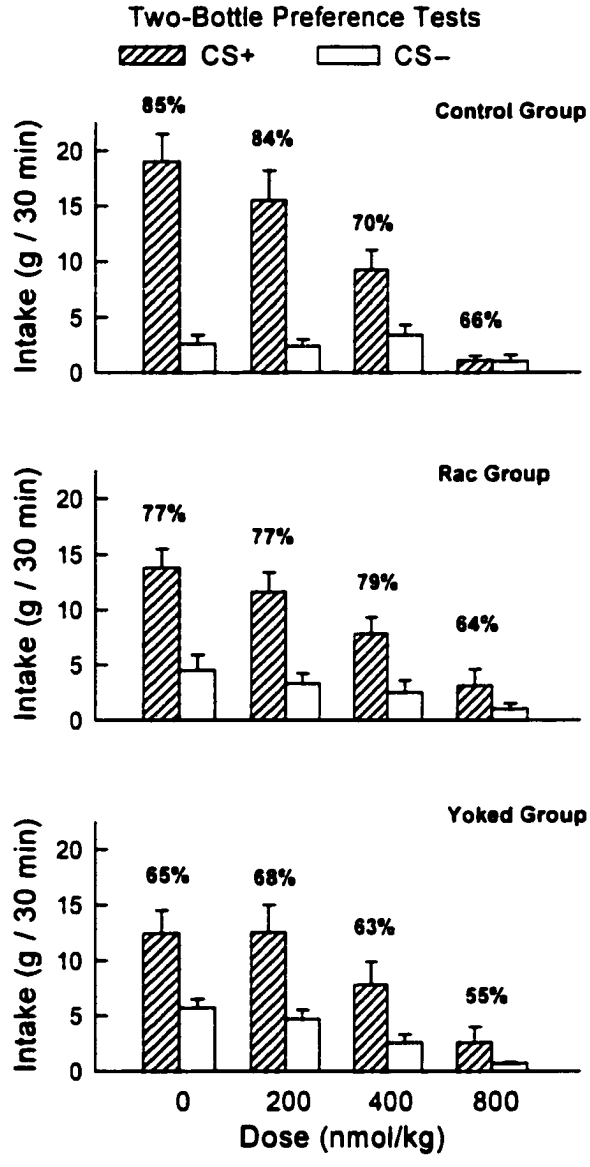

FIG. 14. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests in Experiment 6A. Fifteen minutes prior to testing the rats were injected with 0 (vehicle) 200, 400, or 800 nmol/kg of raclopride. The CS solutions were grape or cherry flavored saccharin. The CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training, but no infusions were given during preference testing. The top panel represents the data for the Control group that was injected with vehicle during one-bottle training; the center panel represents the data for the RAC group which received 200 nmol/kg raclopride during training; the bottom panel represents the data from the Yoked group, which was injected with vehicle during training and had its CS intake matched to that of the RAC group. The numbers atop the bars represent the percent CS+ intake at that dose.

Discussion

In the present experiment pretreatment with 200 nmol/kg body weight raclopride did not impair rats' ability to acquire a conditioned flavor preference. The rats in the RAC group learned a CS+ preference similar to that of the Control group, as expressed when vehicle treated, despite drinking less during training. This conclusion is also supported by the similar percent CS+ intakes across the 3 groups. Both the Yoked and the RAC groups acquired a preference for the CS+ over the CS–, despite drinking less than the Control group during training. The percent CS+ preference of the Yoked and RAC groups were also similar. The failure to see a difference in the magnitude of the preference between these two groups strongly suggests that raclopride did not affect the acquisition of a preference in this experiment. It is of interest that the RAC group in the present experiment expressed a preference when vehicle treated, while the RAC group in Experiment 5 did not. This difference may have occurred because of differences in training intakes. The RAC group from Experiment 5 drank a mean of 5.1 and 6.2 g of the CS+ and the CS–, respectively, while the RAC group in Experiment 6 drank 6.9 g of the CS+ and 7.8 g of the CS–. This greater CS exposure during training may have been responsible for the stronger preference expressed by the RAC group in Experiment 6.

Data from the Control group demonstrates that these rats learned a strong preference for the CS+ over the CS–, as expressed under vehicle treatment. The Control group continued to consume more CS+ than CS– when treated with the 200 nmol/kg dose of raclopride, but did not consume significantly more CS+ than CS– at either the 400 or 800 nmol/kg dose. In the Control group, raclopride treatment suppressed the intake of

the CS+, but not the CS– during preference testing. A floor effect may have contributed to the seemingly specific effect on intake. The Control rats consumed very little of the CS– (2.6 g/30 min, as opposed to 4.5 and 5.7 g/30 min for the RAC and Yoked groups, respectively) in the vehicle test, which did not allow for much reduction following raclopride treatment. Therefore any reduction in total intake would necessarily be expressed as a reduction in CS+ intake. Floor effects also explain the lack of preference at the 800 nmol/kg dose; this dose greatly suppressed intake, resulting in small and similar intakes of the CS solutions.

Although the Control rats did not consume significantly more CS+ than CS– at the 400 nmol/kg raclopride dose, indicating an effect of raclopride on preference expression, they displayed a 70% CS+ preference at this dose. The lack of statistical significance was due to one of the eight Control rats which failed to prefer the CS+. These points are raised because in Experiment 5, using nearly identical methodology, a 400 nmol/kg dose of raclopride dose did not block the expression of a CS+ preference in the Control group. Experiment 6B provides further information on the effects of the 400 nmol/kg dose on the expression of the CS+ preference in Control rats and also determined if this dose blocks the acquisition of the preference when administered to the RAC group throughout training.

Experiment 6B: The Effects of 400 nmol/kg raclopride on the Acquisition and Expression of a conditioned flavor preference

The rats were redistributed into three new RAC, Control, and Yoked groups. The new RAC group (n=10) was made up of 3 rats from the former Control group, 3 from the

former RAC group, and 4 from the former Yoked group. The new Control group (n=8) was made up of 2 rats from the former Control group, 3 from the former RAC group, and 3 from the former Yoked group. The new Yoked group (n=10) was made up of 3 rats from the former Control group, 4 from the former RAC group, and 3 from the former Yoked group. The rats in these new groups were equated for their CS+ preferences and total intakes during the two-bottle tests of Experiment 6A.

The rats were trained as in Experiment 1A except that the CS solutions contained 0.2% saccharin flavored with orange and strawberry (Kool-Aid flavors), and the RAC group was treated with 400 nmol/kg raclopride throughout one-bottle training.

Following training, two-bottle preference tests were conducted with the CS+ and CS– solutions. The three groups were treated identically. They were injected with vehicle prior to the first two sessions, 400 nmol/kg raclopride prior to the next two sessions, and vehicle prior to the last two sessions. The 800 nmol/kg dose was not tested because it nearly eliminated intake in the two-bottle tests in Experiment 6A.

Results

The intakes of the newly constituted RAC and Yoked groups were matched during one-bottle training and the two groups consumed approximately 50% less of the CS+ and CS– solutions than did the Control group (Figure 15, top panel). The ANOVA confirmed that a significant difference existed between the training intakes of the RAC and Control groups ($F(1,16)=13.79$, $p<.01$) and there was no interaction between CS intake and Group. Overall, the Control and RAC groups consumed slightly, but significantly more CS+ than CS– during one-bottle training ($F(1,16)=5.15$, $p<.05$) (Fig 15). In the two-bottle choice tests conducted following vehicle treatment the rats

consumed more CS+ than CS– ($F(1,25)=64.2$, $p<.0001$) and there were no significant group differences or interactions. The percent CS+ intakes of the Yoked and RAC group were somewhat lower than that of the Control group, but these differences were not significant (Figure 15, lower panel).

The effect of raclopride treatment on two-bottle CS preference is summarized in Figure 16. Overall, the rats drank significantly more CS+ than CS– ($F(1,25)=57.8$, $p<.0001$) and the 400 nmol/kg raclopride dose reduced CS intakes ($F(1,25)=95.78$, $p<.0001$). There were no significant group differences or interactions with group and CS or dose. There was, however, an interaction between raclopride treatment and CS ($F(1,25)=13.76$, $p<.01$). Given this nteraction the drug effect on CS intake was explored further by analyzing the data from the three groups combined. Tests of simple main effects revealed that the rats drank more ($p<.0001$) CS+ than CS– following both vehicle and 400 nmol/kg raclopride treatment. While the interaction between drug and CS indicated that raclopride suppressed CS+ intake to a greater degree than CS– intake, simple main effects tests revealed that drug treatment reduced the intake of both the CS+ ($p<.0001$) and the CS– ($p<.0001$).

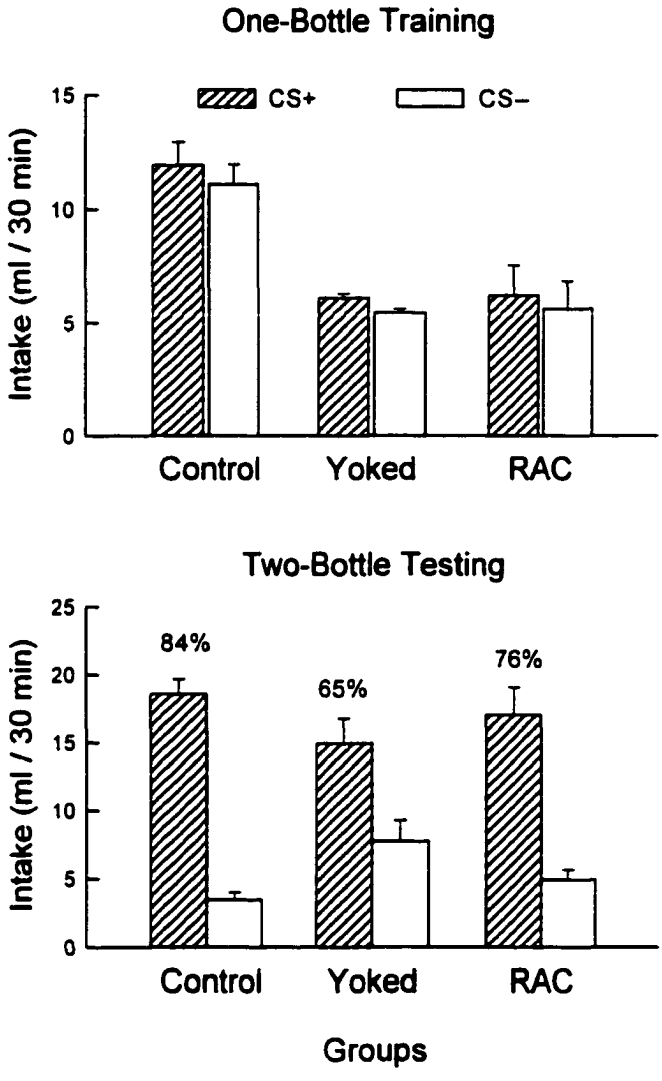

FIG. 15. Top: Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions in Experiment 6B. The RAC group was injected with 400 nmol/kg raclopride prior to each training session, the Control and the Yoked groups were injected with vehicle. The Yoked group had its CS intake limited to that of the RAC group. The CS solutions were orange or strawberry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests following vehicle injections in Experiment 1B. The numbers atop the bars represent the percent CS+ intake.

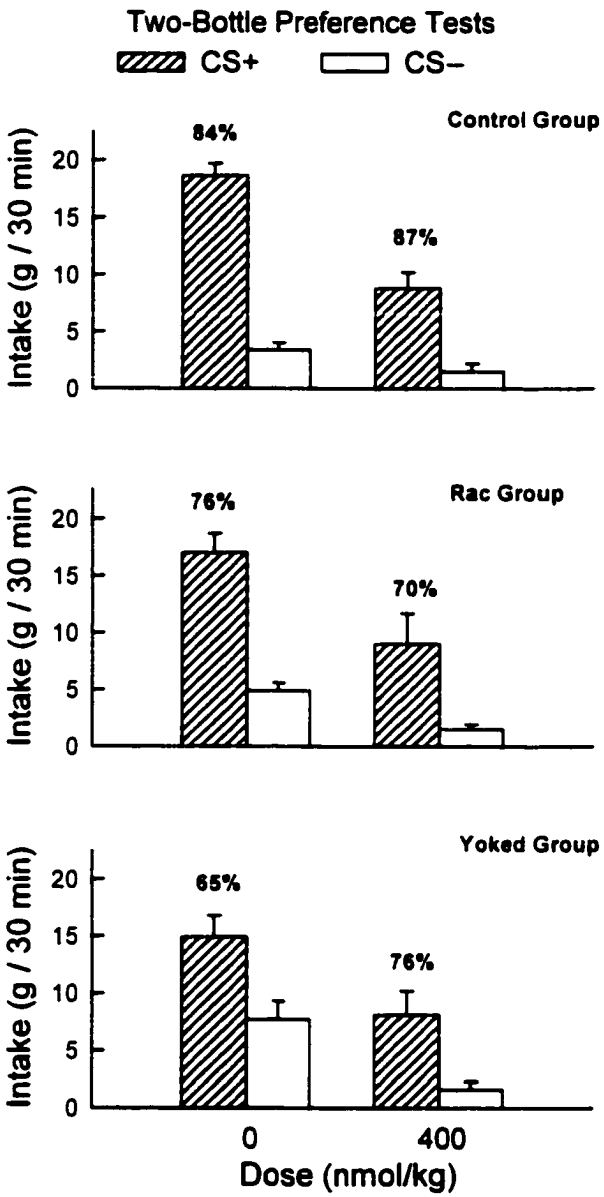

FIG. 16. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests in Experiment 6B. Fifteen minutes prior to testing the rats were injected with 0 (vehicle) or 400 nmol/kg of raclopride. The CS solutions were orange or strawberry flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training but no infusions were given during preference testing. The top panel represents the data for the Control group that was injected with vehicle during one-bottle training; the center panel represents the data for the RAC group which received 400 nmol/kg raclopride during training; the bottom panel represents the data from the Yoked group, which was injected with vehicle during training and had its CS intake matched to that of the RAC group. The numbers atop the bars represent the percent CS+ intake at that dose.

Discussion

These results demonstrate that treating rats with raclopride at 400 nmol/kg during one-bottle CS training, despite reducing training intakes by half, did not attenuate the acquisition of flavor preference conditioned by IG sucrose infusions. The rats in the RAC group did not differ from the Control and Yoked rats in their CS intakes during the two-bottle tests with vehicle treatment.

Raclopride (400 nmol/kg) treatment during the two-bottle tests did not attenuate the preference for the CS+. In fact, the Control and Yoked rats displayed slightly greater percent CS+ intakes in the drug tests than in the vehicle tests. Raclopride did, however, significantly reduce total CS intake and did so by reducing the intakes of both the CS+ and the CS–. The robust preference displayed by the Control groups following the 400 nmol/kg raclopride dose contrasts with the findings obtained in Experiment 6A, but replicates Experiment 5. Taken together, the results of Experiments 6A and 6B indicate that D_2 dopamine receptors are not critically involved in the acquisition or the expression of a nutrient conditioned flavor preference.

Experiment 7: The Effects of SCH23390 on the Acquisition and Expression of a conditioned flavor preference

Both D_1 and D_2 dopamine receptors have been implicated in mediating the rewarding actions of food (Smith, 1995). Experiment 7 investigated whether blocking D_1 receptors with SCH23390 alters the acquisition and/or expression of carbohydrate-conditioned flavor preferences.

Subjects 29 male rats (481-513 g) were used as subjects; these rats were surgically prepared and maintained as described above.

Procedure After recovery from the surgery, the rats were food-deprived to 85% of their post- recovery body weight. The rats were trained to drink unflavored 0.2% saccharin in 30 min sessions, in the test chamber, first without being attached to the infusion apparatus (6 days), then while attached but not infused (6 days), and finally while infused with water (6 days). During this adaptation period some rats with low intakes were given 2% Polycose + 0.2% saccharin to stimulate drinking. All rats were drinking the 0.2% saccharin at the start of formal training. Based upon their training intakes and body weights the rats were divided into 3 groups: SCH (n=9), Control (n=10), and Yoked (n=10).

Formal training consisted of ten 30-min one-bottle training sessions with the CS+ and the CS-, and their appropriate infusions, presented on alternate days. The left-right position of the CS bottles was counterbalanced, following an ABBA pattern. The SCH group received 200 nmol/kg body weight SCH23390 (Research Biochemical Intl., Natick, MA) IP, 15 min prior to the start of the training sessions. The Control and the Yoked group received IP saline injections prior to each session. The SCH and Control groups were run on the same day, while the Yoked group was run 2 days behind. The oral intakes and IG infusions of the Yoked group were yoked to the average of the SCH group on the preceding corresponding CS day.

Following training two-bottle preference tests were conducted with the CS+ and CS– solutions. IG infusions were not administered during preference testing. Fifteen min prior to testing rats were treated with vehicle injections or with injections of 200 or 400 nmol/kg of SCH23390. The SCH23390 doses were administered in ascending order and each dose was given on two sequential days, with at least 2 days of vehicle

separating each pair of drug days. The intake of the Yoked group was not limited during preference testing.

Results

The CS+ and CS– intakes of the SCH and Yoked groups were well matched during one-bottle training and the two groups consumed approximately 60% less of the CS solutions than did the Control group (Figure 17, upper panel). The ANOVA confirmed that the SCH group drank less than did the Control group ($F(1,17)=25.2$, $p<.0001$) and revealed that overall CS+ intakes were less than CS– intakes in both groups ($F(1,17)=4.49$, $p<.05$). In the two-bottle choice tests conducted under vehicle treatment, the total intakes of the three groups did not differ, but there was a significant interaction between Group and CS ($F(2,26)=20.45$, $p<.0001$)(Figure 17, lower panel). Further analysis revealed that the both the Control and Yoked rats consumed more ($p<.001$) CS+ than CS– in the choice tests, whereas the SCH groups consumed similar amounts of the two CS solutions. The groups also differed in their percent CS+ intakes ($F(2,26)=21.56$, $p<.0001$). In particular, the percent CS+ intake for the SCH group was less than that of the Control and the Yoked groups, which did not differ from each other.

Figure 18 summarizes the effects of SCH23390 treatment on the two-bottle intakes of the CS+ and CS–. Overall, the three groups did not differ in their total CS intakes and they all reduced their intakes when treated with SCH23390 ($F(2,52)=87.37$, $p<.0001$). There were significant interactions between drug treatment and CS ($F(2,52)=11.63$, $p<.0001$), Group and CS ($F(2,26)=15.12$, $p<.0001$), and Group, Drug and CS ($F(4,52)=3.73$, $p<.01$). Because of the three-way interaction, individual ANOVAs for each group were performed. The Control group analysis revealed a significant CS by

Drug interaction ($F(2,18)=18.7$) $p<.0001$). Simple main effects tests revealed that the Control group consumed more ($p<.01$) CS+ than CS– at the vehicle and 200 nmol/kg doses but not at the 400 nmol/kg dose. The drug reduced the intake of the CS+ ($F(2,35)=52.8$), $p<.0001$), but not of the CS–. The Yoked group analysis also yielded a CS by Drug interaction ($F(2,18)=5.28$) $p<.02$). Like the Controls, the Yoked rats consumed more ($p<.001$) CS+ than CS– at the vehicle and 200 nmol/kg dose but not at the 400 nmol/kg dose. The drug reduced ($p<.0001$) their intake of the CS+ but not of the CS–. In contrast to the Control and Yoked rats, the SCH group did not drink more CS+ than CS– at any dose and the drug reduced their intakes of both CS solutions.

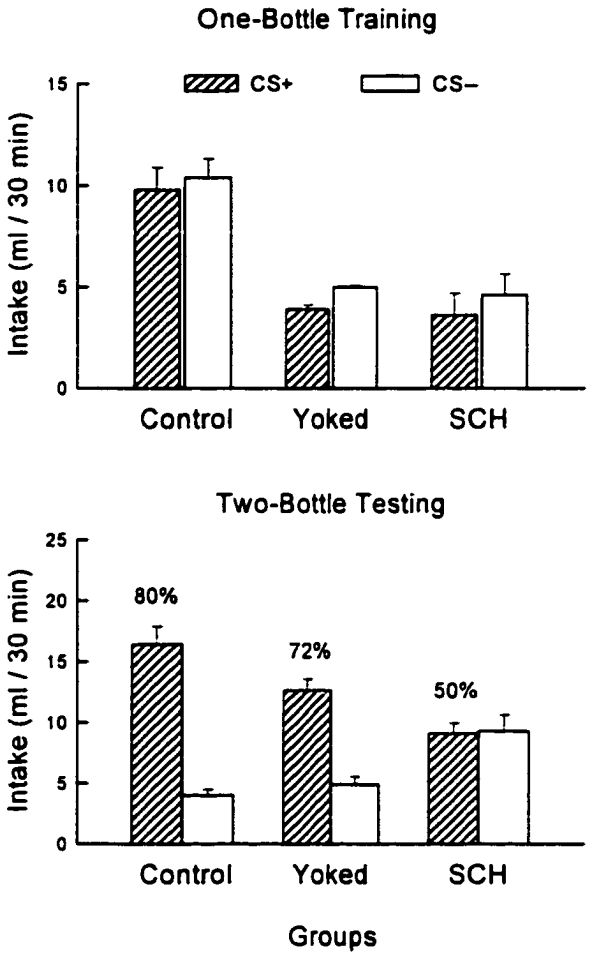

FIG. 17. Top: Intakes (means +SEM) of the CS+ and the CS– during 30 min, one-bottle training sessions in Experiment 7. The SCH group was injected with 200 nmol/kg SCH23390 prior to each training session, the Control and the Yoked groups were injected with vehicle (0 nmol/kg); the Yoked group had its CS intakes limited to that of the SCH group. The CS solutions were cherry or grape flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training. Bottom: Intakes (means + SEM) of the CS+ and the CS– during 30 minute, two-bottle preference tests following vehicle injections in Experiment 7. The numbers atop the bars represent the percent CS+ intake.

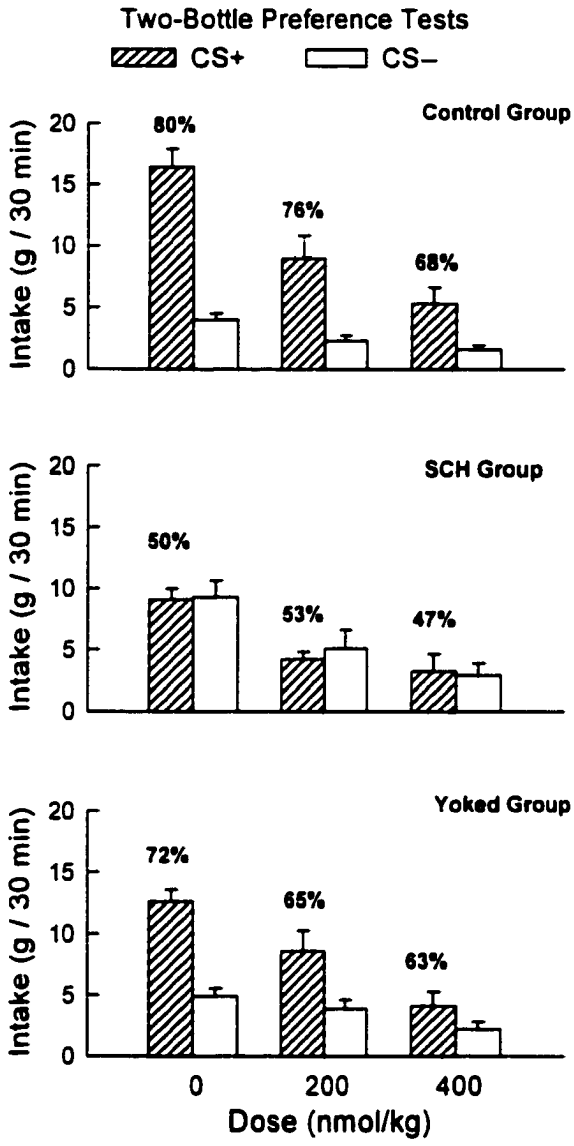

FIG. 18. Intakes (means + SEM) of the CS+ and the CS– during 30 min, two-bottle preference tests in Experiment 7. Fifteen minutes prior to testing the rats were injected with 0 (vehicle), 200 or 400 nmol/kg of SCH23390. The CS solutions were cherry or grape flavored saccharin, and the CS+ was paired with intragastric sucrose and the CS– with intragastric water infusions during training but no infusions were given during preference testing. The top panel represents the data for the Control group that was injected with vehicle during one-bottle training; the center panel represents the data for the SCH group which received 200 nmol/kg SCH23390 during training; the bottom panel represents the data from the Yoked group, which was injected with vehicle and had it's CS intake matched to that of the SCH group during training. The numbers atop the bars represent the percent CS+ intake at that dose.

Discussion

There are two important findings of this experiment. The first is that treatment with SCH23390 before every training trial blocked the acquisition of a conditioned flavor preference. The SCH group displayed no preference for either CS solution when vehicle treated. Additionally, the percent CS+ intake for the SCH group was significantly lower than it was for the other groups. When treated with SCH23390 during preference the SCH group continued to show no preference, demonstrating that they had not learned a preference which was dependent upon the drug-state under which they were trained. This contrasts with the Control and Yoked groups, which both demonstrated significant preferences (80% and 72%, respectively) when vehicle treated. It is critical to note that the lack of preference in the SCH group cannot be attributed to reduced intake during training, as the Yoked group, which had its CS and US exposure matched to that of the SCH group, did learn a preference. The second important finding is that the 200 nmol/kg dose of SCH23390, which blocked the acquisition of the CS+ preference in the SCH group, did not block the expression of the CS+ preference in the Control group. The Control rats reduced their CS+ intake following the 200 nmol/kg dose, but their preference was only slightly reduced relative to the vehicle tests (80% to 76%). The 400 nmol/kg dose produced a greater intake suppression and the Control group no longer significantly preferred the CS+, although the mean CS+ intake was still twice that of the CS–. An examination of the data from the individual animals in the Control group revealed that only the three rats that consumed very little during the 400 nmol/kg tests (total CS intakes less than 4 g) failed to prefer the CS+ to the CS–. Thus, a "floor" effect may account for the lack of a significant CS+ preference at the 400 nmol/kg dose,

however the present data cannot completely rule out a role of the D_1 receptor in preference expression.

General Discussion

The present data confirms previous reports that rats learn to prefer flavors paired with the postingestive effects of carbohydrates (Sclafani, 1990; Sclafani, 1995; Sclafani, 1999), and that opioid and dopamine receptor antagonism suppresses the intake of sweet solutions (Levine et al, 1995; Smith, 1995). The new findings in this dissertation are that opioid antagonism does not affect the acquisition of a conditioned flavor preference (Experiment 2), and has only minimal effects on the expression of a conditioned preference (Experiments 1 and 2) or a conditioned flavor acceptance (Experiment 3). Antagonism of the D_2 dopamine receptor system with raclopride did not affect the acquisition (Experiments 6A and 6B) of a sucrose conditioned flavor preference, and only affected the expression of such a preference at doses which nearly eliminated intake (Experiments 4-6). Finally, the D_1 antagonist SCH23390 blocked the acquisition of a flavor preference conditioned by intragastric sucrose (Experiment 7), and only affected the expression of such a preference only at doses which greatly reduced total intake.

Opioid Receptor Antagonism

In the five different two-bottle tests conducted in Experiments 1 and 2, naltrexone consistently suppressed total CS intakes but did not reduce percent CS+ intakes. In three of these tests, the drug suppressed CS+ intake more than CS– intake, but in only one case did the rats fail to consume more CS+ than CS– following drug treatment (Experiment 2B). As discussed above, this may have been due to a floor effect, as CS intakes in this test were lower than in the remaining four tests. Overall, these data indicate that a fully functioning opioid system is not critical for the expression of a flavor preference conditioned by IG carbohydrate infusions. Nevertheless, the drug by CS interaction

observed in several of the experiments indicates that a role for the opioid system in conditioned flavor preferences cannot be fully ruled out. An inherent difficulty in evaluating this issue is that low CS– intakes during two-bottle tests make it difficult to observe nonselective decreases in CS intakes. As discussed below, theoretical considerations also preclude eliminating opioid involvement in the expression of learned flavor preferences.

In contrast to the present findings, several studies have reported that opioid antagonists suppress the preference for saccharin and sugar solutions, which might suggest that different neurochemical systems mediate learned and unlearned flavor preferences. However, there are important methodological differences between these studies which limit comparisons. Note in particular, that some of the data cited as evidence that naloxone reduces saccharin preference actually show decreased saccharin acceptance rather than decreased preference per se (Gosnell & Majchrzak, 1989; Lynch, 1986; Lynch & Burns, 1990; Lynch & Libby, 1983). That is, although the nondeprived rats in these studies were offered the choice between saccharin and water, their water intakes were virtually nil and were not reported. More compelling evidence for naloxone-induced reduction in saccharin preference comes from studies of water-deprived rats given saccharin vs. water tests in which water consumption was measurable. In these experiments, naloxone reduced saccharin intake and water intake remained unchanged or even increased (Cooper, 1983; LeMagnen et al., 1980; Siviy & Reid, 1983). This outcome may be related to the fact that the rats were motivated by thirst to drink water and by taste to drink saccharin, and opioid antagonists are most effective in suppressing taste-motivated drinking (Sclafani et al., 1982). Note that water

restriction reduces the expression of a learned preference for a carbohydrate-paired CS+ flavor over a water-paired CS– flavor (Drucker et al., 1994). Thus, it may be inappropriate to use water-restricted rats to evaluate drug effects on nutrient-conditioned flavor preferences.

Opioid antagonists have also been found to alter preferences for solid foods in food-restricted rats. In particular, two studies observed that naloxone (0.3 - 3 mg/kg) or naltrexone (0.1 - 5 mg/kg) reduced the intake of a preferred food while intake of the less preferred food remained the same or even increased (Cooper & Turkish, 1989; Glass et al., 1996). The intake and preference reductions observed in these experiments were more pronounced than those observed in the present study. This may be due to differences in test substances (solid foods versus flavored saccharin solutions) and/or deprivation conditions (overnight food deprivation versus chronic food restriction). In addition, the choice foods used in the prior experiments, high-fat and high-carbohydrate semisynthetic diets (Glass et al., 1996) or chocolate cookie and lab chow (Cooper & Turkish, 1989), differed in flavor, nutrient composition, and caloric density, whereas the CS solutions used in the choice tests of the present study differed only in their cue flavor and training history. Another potentially important difference is that only nutritive choice items were used in the prior experiments whereas the CS solutions used in the present study had been paired with nutritive and nonnutritive infusions. It may be that the all-or none nature of the nutrient reinforcement used in the present study, and the strong preferences it produced, obscured more subtle effects of opioid antagonism on flavor preferences. This possibility can be addressed by training rats with two CS+ solutions paired with different nutrient concentrations (e.g., 8% maltodextrin vs. 16% maltodextrin) which condition

more moderate flavor preferences (i.e., CS+16% preferred to CS+8%)(Lucas et al., 1998). Another approach is to pair the CS+ solutions with different nutrients (e.g., isocaloric carbohydrate and fat infusions) which also condition moderate flavor preferences (i.e., CS+carbohydrate preferred to CS+fat)(Lucas et al., 1998). The use of different nutrients is also of interest in view of reports of nutrient-specific effects obtained with opioid antagonists and agonists (Glass, 1998).

In Experiment 3, drug effects on carbohydrate-conditioned flavor acceptance were investigated using one-bottle tests and the data were similar to the conditioned preference results of the first two experiments. Naltrexone decreased the absolute but not percent intake of the CS+ relative to the CS–, and the rats continued to consume more CS+ than CS– in the one-bottle tests. As noted above, these results differ somewhat from those reported by Ramirez (1997), but different vehicle baseline intakes may account for the discrepancy.

The minimal effects of naltrexone on the expression of a CS+ preference and acceptance in this study does not necessarily argue against the hypothesis that flavor-nutrient learning involves an opioid-mediated shift in hedonic evaluation (Mehiel, 1996; Ramirez, 1997). It is conceivable, for example, that nutrient conditioning enhances the CS+ preference and acceptance in a way analogous to increasing the sweetness of the CS+ solution (recall that both the CS+ and CS– solutions contained saccharin). Naltrexone may attenuate the hedonic response to both CS solutions such that the relative difference between the CS+ and CS– remain about the same and the rat therefore continues to drink more CS+ than CS–. As a simple test of this idea, we determined the effects of naltrexone on the preference rats display for a 0.2% saccharin solution over a

slightly less sweet 0.15% solution (Azzara and Sclafani, unpublished findings). Naltrexone (1.0, 2.5, 5.0 mg/kg) significantly reduced 0.2% saccharin intake without reducing the already low intake of 0.15% saccharin, but the rats continued to consume more 0.2% saccharin than 0.15% saccharin in the two-bottle tests. Furthermore, the percentage of total intake consumed as 0.2% saccharin was not significantly reduced by the drug; percent intakes were 86% in the vehicle test, and 74% to 85% in the drug tests. These findings mirror the present results obtained with the CS+ and CS– solutions.

While the naltrexone expression results are not incompatible with an opioid mediation hypothesis, the acquisition data challenge the idea that the opioid system is critically involved in flavor preference learning. In Experiments 2A and 2B treating rats with naltrexone prior to the one-bottle training sessions had no effect on the magnitude of the CS+ preference they displayed in subsequent two-bottle tests. Furthermore, the NTX group responded like the Control group to naltrexone injections during the two-bottle tests. The failure of naltrexone treatment during training to reduce subsequent CS+ preference is particularly noteworthy because the drug reduced the rats' exposure to the CS and US during training. Interestingly, reduced CS exposure did result in a reduced preference in a subsequent experiment (Experiment 5). I will return to this issue later in this discussion.

The results of Experiments 1-3 indicate that the ability of IG carbohydrate infusions to condition a CS+ flavor preference is not mediated by opioid receptor activity. Although Mehiel (1996) hypothesized that opioid activity is involved in carbohydrate conditioned flavor preferences, his results are difficult to interpret because the animals were treated with naloxone only on CS+ or CS– training sessions. Opioid antagonists

can produce flavor aversions (Parker & Rennie, 1992), therefore, rats in the Mehiel (1996) study may have developed an aversion for whichever CS was paired with antagonist treatment. This possibility cannot be controlled for when administering the antagonist with only one of the two CS solutions. Mehiel (1996) also proposed an opioid mediation of ethanol-conditioned flavor preferences. The present data are based on carbohydrate conditioning only and thus it remains possible that the opioid system has an important role in the conditioning effects of other nutrients including ethanol.

In apparent contrast with our results with opioid antagonism, Lynch (1986) reported that naloxone blocks the normal acquisition of a saccharin preference in rats. Lynch (1986) observed that daily naloxone injections prevented the gradual increase in saccharin intake displayed by saline treated rats. Although his rats had access to both saccharin and water, water intakes were not reported because the nondeprived rats drank virtually no water. Furthermore, saccharin vs. water preference was not measured following the end of drug treatment. In a subsequent experiment, Lynch and Burns (1990) observed that daily naloxone injections almost completely inhibited sucrose and saccharin intake over 10 training sessions, but when subsequently tested without the drug sucrose and saccharin intake rapidly increased. In fact, the naloxone treatment appeared to stimulate subsequent sucrose intake. Water was available during these tests but intakes were not reported because they were so low. Lynch's data show that naltrexone suppressed the acceptability of the saccharin and sucrose solutions during drug treatment, but did not block the preference for these solutions in subsequent drug-free solution vs. water tests. These results are not much different from the present findings: naltrexone

treatment during one-bottle training limited the intake of flavored saccharin solutions, but did not suppress CS+ intake or preference in the two-bottle vehicle tests (Experiment 2).

The present results complement the recent finding that naltrexone has minimal effects on flavor-flavor preference conditioning by the sweet taste of sucrose (Yu et al., 1999). Naltrexone treatment during training or prior to choice testing did not block the acquisition or expression of a preference for the sucrose-paired flavor.

In summary, Experiments 1-3 demonstrated that the opioid antagonist naltrexone did not suppress the acquisition of flavor preferences conditioned by intragastric carbohydrate infusions, and had minimal effects on the expression of carbohydrate-conditioned flavor preference and acceptance. Nevertheless, naltrexone reduced the total intakes of the saccharin-sweetened CS solutions which confirms prior findings obtained with unflavored saccharin and sugar solutions. These findings indicate that opioid activity modulates the consumption of palatable flavors but does not specifically mediate sucrose-based flavor preference learning, in an intragastric infusion paradigm. As the general opioid antagonist naltrexone had little effect on nutrient-conditioned flavor preference, specific receptor subtype antagonists were not tested.

Dopamine Receptor Antagonism

In Experiments 4-7, the role of dopamine receptors in the acquisition and expression of nutrient-conditioned preference was investigated by treating rats with selective D_1 or D_2 antagonists during training and/or testing. The new findings in these experiments are that antagonism of the D_2 dopamine receptor system with raclopride did not block the acquisition (Experiment 6) of a sucrose conditioned flavor preference,

while having effects of the expression of such a preference only at doses which greatly reduced total intake (Experiments 4-6). However, the D_1 antagonist SCH23390 blocked the acquisition, but not the expression, of a flavor preference conditioned by intragastric sucrose (Experiment 7).

Experiment 4 demonstrated that D_2 antagonism only suppressed flavor preference expression at the 800 nmol/kg dose, which dramatically suppressed total intake. Although the antagonist treatment significantly reduced the intake of the CS+, but not the CS–, it is reasonable to believe that a floor effect prevented the intake of the CS– from being significantly suppressed.

In Experiment 5 raclopride treatment during training suppressed the acquisition of a conditioned flavor preference. The mechanisms by which this occurred, however, were not readily discernable. Raclopride treatment during training greatly suppressed intake, and the reduced CS exposure may have caused the reduced preference learning in the RAC group. Experiment 6 examined this possibility by adding a yoked control group that had its training intakes matched to that of the RAC group. In this experiment the RAC as well as the Yoked group developed a significant CS+ preference, although their percent CS+ intakes (77% and 64%, respectively) were somewhat lover than that of the Control group (85%). Since the RAC group in Experiment 5 drank less during training than did the RAC group in Experiment 6, their reduced exposure to the CS solutions may account for their lack of preference. It is of interest to note that, although the differences were not significant, the RAC group in Experiment 6 had a higher percent CS+ intake than did the Yoked control.

In Experiment 6 rats treated with raclopride at 200 or 400 nmol/kg during training learned a significant preference for the CS+ over the CS–, as expressed when vehicle treated, despite the fact that the drug reduced their training intakes by 30-50% relative to Controls. The RAC, Control, and Yoked groups continued to drink more CS+ than CS– when treated with raclopride at 200 or 400 nmol/kg, although at the 400 nmol/kg dose the CS intake differences were not significant in Experiment 6A. As stated earlier, the attenuation of preference at 400 nmol/kg in this experiment may be an anomaly, as in Experiments 4, 5, and 6B 400 nmol/kg raclopride did not block preference expression. The highest dose of raclopride (800 nmol/kg) reduced total intakes to very low levels and eliminated the CS+ preference in Experiments 4, 5 and 6. The fact that raclopride suppressed CS+ intakes more than CS– intakes in these two-bottle tests may be taken as evidence for a role of D_2 receptors in conditioned flavor preference. As with the opioid experiments, an inherent difficulty in evaluating the two-bottle preference data is that the low CS– intakes during the vehicle tests limits the magnitude of the drug's suppressive effect on CS–. Relevant to this point is the previously mentioned study by Ramirez (1997) in which IG maltodextrin infusion conditioned increased saccharin intake in one group more than did IG water infusion in another group. In a subsequent one-bottle test, the D_2 antagonist pimozide suppressed saccharin intake more in the water-infused group than in the maltodextrin-infused group. As mentioned in the preceding section, other training techniques which produce greater intake of the CS+ and the CS– during two bottle tests might be useful in determining if raclopride specifically attenuates CS+ intake, or simply suppresses total intake. Taken together, these results suggest that D_2

receptors are not critically involved in flavor preferences conditioned by intragastric nutrient infusions.

In contrast to the results obtained with the D_2 antagonist, the D_1 antagonist SCH23390 blocked the flavor preference conditioning by IG sucrose infusions. In Experiment 7 the rats treated with 200 nmol/kg of SCH23390 throughout training had a percent CS+ intake of 50% in the two-bottle vehicle tests which contrasts with the 80% and 72% CS+ intakes of the Control and Yoked groups. The Control and Yoked groups continued to prefer the CS+ to the CS– when treated with the 200 nmol/kg dose, although their CS+ intake was reduced by the drug. Intake was further suppressed by the 400 nmol/kg dose of SCH23390 and the Controls no longer consumed significantly more CS+ than CS–. However, given low CS– intakes, this loss of preference may have been due a floor effect. Thus, the present results revealed two types of selective drug effects: the D_1 but not the D_2 antagonist blocked preference learning, and the D_1 antagonist, at the dose that prevented learning in the SCH group, did not block the expression of the learned preference in the Control rats.

The present results with dopamine D_1 and D_2 antagonists are interesting in light of recent work by Yu et al. (2000, submitted). These authors observed that both raclopride and SCH23390 attenuated the expression of flavor preferences conditioned by the taste of sucrose, relative to the taste of saccharin, but did not block the acquisition of the preference, relative to a Yoked control group. In these studies the sweet taste of sucrose was the US, as the rats sham-fed the solutions during training and testing (flavor-flavor conditioning). The rats in the present study, on the other hand, had the CS+ flavor paired with intragastric sucrose infusions and their flavor preference was conditioned by the

sugar's postingestive effects (flavor-nutrient conditioning). Together these results from Yu et al.(2000) and the present study implicate the D_1 receptor as critical in the acquisition of a flavor-nutrient preference, but not a flavor-flavor preference. The D_1 receptor is also critical for the expression of a flavor-flavor preference, while D_1 antagonism only blocks the expression of a flavor-nutrient preference at doses which nearly eliminate intake. D_2 receptor antagonism did not block the acquisition of a flavor-nutrient preference or a flavor-flavor preference, relative to yoked control groups. D_2 antagonism did not block the expression of a flavor-nutrient preference although it did attenuate the expression of a flavor-flavor preference. Together, these findings are consistent with the idea that flavor-flavor and flavor-nutrient learning involve different behavioral and neural processes. This conclusion remains tentative, however, given that the flavor-flavor and flavor-nutrient preference conditioning training paradigms differed in a number of respects.

The finding that D_2 receptor antagonism with raclopride did not block flavor preference learning reinforced by the taste (Yu et al., 2000; sub.) or postingestive actions (present study) of sucrose might appear to conflict with the sucrose conditioning data reported by Hsiao and Smith (1995). However, fundamental differences in the training procedures of these studies can account for the contrasting results. First, unlike the present study, but like the Yu et al. studies, Hsiao and Smith (1995) emphasized the reinforcing action of the sweet taste of sucrose. They did this by having rats "real-feed" flavored sucrose solutions and limited postingestive effects by using short training sessions (5 min). Second, whereas the present study and Yu et al. (2000; submitted) paired both the CS+ and CS– flavors with raclopride treatment, Hsiao and Smith paired

one flavored sucrose solution with the drug and a second flavored sucrose solution with saline. The decreased preference they observed for the raclopride-paired flavor was attributed to the drug reducing the reinforcing potency of the sucrose solution's sweet taste. This conclusion is not inconsistent with acquisition data reported by Yu et al. because their rats were trained with the drug paired with both sucrose and saccharin, so that the differential reinforcing effects of the two sweet solutions may have been maintained. Finally, the dose of raclopride used in the Hsiao and Smith (1995) study (800 nmol/kg) was considerably higher than the training dose employed by Yu et al. (submitted) and in the present study. It is possible that flavor preference conditioning by IG sucrose infusions would be prevented if rats were trained with the 800 nmol/kg dose, but this dose might reduce training intakes to very low levels. Note that in the present experiments the 800 nmol/kg dose of raclopride nearly eliminated intake in the well-trained animals.

The differential effects of the D_1 and D_2 antagonists in blocking flavor-nutrient conditioning are consistent with prior findings suggesting that D_1 receptors have a more fundamental role in learning produced by food and other rewards than do D_2 receptors (Benninger & Miller, 1998). Of particular relevance to the present study is the report of Caulliez et al. (1996) that D_1 but not D_2 antagonism blocked taste aversion learning in rats. In their experiment, water-restricted rats were trained to drink a saccharin solution which was followed by lithium chloride poisoning. Microinjections of the D_1 antagonist SCH23390 into the lateral hypothalamus blocked the acquisition of a conditioned taste aversion to the saccharin whereas microinjections of the D_2 antagonist, sulpiride, did not attenuate taste aversion learning relative to vehicle injections. These results along with

the present findings indicate that the endogenous dopamine system, and the D_1 receptor in particular, is involved in learning about both the positive and negative consequences of food. The Caulliez et al. (1996) study further suggests the lateral hypothalamic area as one possible site where D_1 receptors act to modulate flavor-nutrient learning.

Psychobiological Issues

In view of the extensive evidence linking the opioid system to affective aspects of reward, the failure of naltrexone to influence the acquisition of sucrose-conditioned flavor preferences in the present study suggests that this type of conditioning may not involve a shift in the hedonic evaluation of the flavor. Berridge (1996) has proposed that food reward can be subdivided into "wanting," which is related to incentive motivation, and "liking," which corresponds with hedonic evaluation and palatability. He further argues that the opioid system is primarily involved in the liking component of reward, while the dopamine system is the primary mediator of the wanting component of reward. The minimal effects obtained in the present study with opioid antagonists suggests that flavor-nutrient learning might involve a change in dopamine-mediated incentive motivation ("wanting"). The role of the endogenous dopamine system in the acquisition and expression of conditioned flavor preferences was examined in Experiments 4-7, and, as stated above, it was demonstrated that the acquisition of a carbohydrate-conditioned flavor preference was blocked by the administration of a dopamine D_1 antagonist.

A more recent review by Berridge and Robinson (1998), has put forth the theory that dopamine, which is linked to the "wanting" aspect of reward, is not critical for hedonic reward learning. This conclusion is based in part on their finding that 6-OHDA

lesions, which dramatically reduced brain dopamine levels, did not block taste aversion learning in rats. The conditioned aversion was measured using the taste reactivity test which, according to Berridge and Robinson, is the only way to distinguish food "liking" from "wanting." It is possible, therefore, that the SCH rats treated with the D_1 antagonist in Experiment 7 learned to "like" the CS+ flavor paired with IG sucrose, but this hedonic learning was not observed because the two-bottle intake test used primarily measures "wanting" and the drug treatment blocked conditioned wanting. Recall, however, that our negative results with opioid antagonism led us to hypothesize that a hedonic shift was not involved in the acquisition of a flavor preference. Our finding that the opioid antagonist naltrexone did not prevent flavor-nutrient preference conditioning would seem to argue that hedonic changes are not involved in this type of learning, however preference learning was evaluated using two-bottle intakes only. Whether flavor-nutrient learning actually results in an increased hedonic response to the CS+ flavor, as measured by the taste reactivity test, remains to be established.

Another recent theory of the role of dopamine in reward comes from Ikemoto and Panksepp (1999), who hypothesized that the meso-accumbens dopamine system allows animal to adapt to novel situations by focusing approach and investigation towards salient stimuli. If those stimuli are related to biologically relevant rewards, then the dopamine system enables those stimuli to acquire incentive properties. Ikemoto and Panksepp (1999) further hypothesize that while the dopamine system is critical to incentive learning, once responses to incentive stimuli are well established their behavioral expression is only minimally dependant upon dopamine release. An explicit prediction of this theory is that meso-accumbens dopamine blockade would interfere with the

acquisition, but not the expression of a conditioned flavor preference. The results obtained with the D_1 antagonist fit this prediction quite well, however, it is critical to note that Ikemoto and Panksepp (1999) have speculated that dopamine outside of the meso-accumbens system may control well established behaviors. Note that the present experiments were not designed to dissociate between various theories on the role of dopamine in reward. Further research is needed to characterize the nature of the learning processing involved in flavor-nutrient conditioning and the involvement of different receptor subtypes in this learning.

The present results are also interesting in light of the Mark et al. (1994) study showing that rats trained with a CS+ flavor paired with IG Polycose infusions subsequently show an increase in nucleus accumbens dopamine when drinking the CS+ but not the CS–. This finding suggests that dopamine antagonists should block the expression of a CS+ preference which was not observed in the present study except at highest drug doses. As noted above, Ikemoto & Panksepp (1999) hypothesized that well trained behaviors are not dependent upon the meso-accumbens dopamine system. Therefore, although dopamine release may occur in conjunction with the consumption of a CS+, the CS+ preference may not be dependent upon this dopamine release after extensive training. It would be of interest to compare accumbens dopamine release in response to CS consumption in animals treated with SCH23390 or vehicle during training.

In summary, the present experiments revealed that the opioid antagonism does not block the acquisition of a flavor preference conditioned by intragastric sucrose. Opioid antagonism has minimal effects on the expression of such a preference. Like the opioid

antagonist, the dopamine D_2 antagonist raclopride did not suppress the acquisition of a flavor preference conditioned by intragastric sucrose infusions. Dopamine D_2 antagonism had minimal effects on the expression of this preference except at high doses that substantially suppressed total intake. In contrast, the dopamine D_1 antagonist SCH23390 blocked the acquisition of a preference for a flavor paired with intragastric sucrose infusions but had minimal effects on the expression of this preference. These findings indicate that the D_1 .but not the D_2 receptor subtype is critically involved in flavor conditioning by the postingestive actions of sucrose.

Together, the results of this series of experiments suggests that flavor preference learning based upon intragastric sucrose infusions is mediated by the D_1 dopamine receptor system. Together with other research (Beninger & Miller, 1998; Caulliez et al. 1996) the D_1 receptor is emerging as a potential mediator in learning about flavors, and in reward processes in general. However, the psychobiological mechanisms by which D_1 antagonism produces its effects are not understood. For example, D_1 antagonism may reduce reward, block associative processes, or block incentive salience attribution. These questions remain to be investigated. It should be noted, however, that the neuropharmacology of food reward is not fully understood, and so behavioral and psychobiological characterizations of flavor preference conditioning based on the effects of pharmacological manipulations remain tentative.

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