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D_1 but not D_2 dopamine receptor antagonism blocks the acquisition of a flavor preference conditioned by intragastric carbohydrate infusions

Anthony V. Azzara[a], Richard J. Bodnar[b], Andrew R. Delamater[a], Anthony Sclafani[a,*]

[a]*Experimental Doctoral Subprogram, Department of Psychology, Brooklyn College of City University of New York, Brooklyn, NY, USA*
[b]*Neuropsychology Doctoral Subprogram, Department of Psychology, Queens College of City University of New York, Queens, NY, USA*

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Abstract

The effects of dopamine D_1 (SCH23390) and D_2 (raclopride) receptor antagonists on the acquisition and expressions of flavor preferences conditioned by the postingestive actions of sucrose were investigated. Food-restricted rats were trained in one-bottle sessions to associate one flavored saccharin solution (CS+) with intragastric (ig) infusions of 16% sucrose, and another flavored saccharin solution (CS−) with water infusions. Flavor preferences were then measured in two-bottle tests. In Experiment 1A, rats that received the D_2 antagonist (raclopride, 200 nmol/kg; RAC group) throughout training consumed less CS+ and CS− than did saline-treated Control rats; a saline-treated Yoked group had its intake limited to that of the RAC group. All three groups displayed CS+ preferences during two-bottle tests when treated with saline or raclopride, except at doses that greatly suppressed intake. Experiment 1B obtained similar results with rats treated with 400 nmol/kg raclopride throughout training. In Experiment 2, rats that received the D_1 antagonist (SCH23390, 200 nmol/kg; SCH group) throughout training consumed less CS+ and CS− than did saline-treated Control rats; a saline-treated Yoked group had its intake limited to that of the SCH group. Unlike the Control and Yoked groups, the SCH group failed to prefer the CS+ to the CS− in two bottle tests. SCH23390 treatment during two-bottle testing did not block CS+ preference in the Control or Yoked groups, except at doses that greatly suppressed intake. We conclude that D_1, but not D_2, dopamine receptors are critically involved in the acquisition of a sucrose-conditioned flavor preference, and both receptor subtypes have a more limited role in the expression of this preference.

Keywords: Conditioned flavor preference; Acquisition; Expression; Intragastric sucrose; SCH23390; Raclopride

1. Introduction

In recent studies, our laboratories have investigated the pharmacology of flavor preference learning in rats using flavor–flavor and flavor–nutrient conditioning paradigms (Azzara et al., 2000; Yu et al., 1999, 2000a,b). In flavor–nutrient conditioning, rats learn to associate a cue flavor, the conditioned stimulus (the CS+), with the postingestive actions of a nutrient (Sclafani, 1995). For example, Azzara et al. (2000) trained rats to drink a novel flavored solution (the CS+), which was paired with an intragastric (ig) infusion of sucrose, the unconditioned stimulus (the US). On other trials, a different flavored solution (the CS−) was paired with an intragastric water infusion. After several training sessions, the rats displayed a strong preference for the CS+ flavor over the CS− flavor in a two-bottle choice test. In flavor–flavor (or flavor–taste) conditioning, rats learn to associate the cue flavor (the CS+) with an already preferred flavor, e.g., sweet taste (the US). Typically, the US is the flavor of a nonnutritive substance, e.g., saccharin, to eliminate the involvement of flavor-postingestive nutrient conditioning (Holman, 1975). In our flavor–taste conditioning studies, rats were trained to associate the CS+ flavor with a highly preferred sucrose solution and the CS− flavor with a less preferred saccharin solution (Yu et al., 1999, 2000a). The US was considered to be the sweet taste of the sucrose because postingestive actions were minimized by training and testing the rats with an open gastric fistula (sham-feeding procedure) (Yu et al., 1999, 2000a). While flavor–taste and flavor–nutrient learning

* Corresponding author. Tel.: +1-718-951-5606; fax: +1-718-951-4824.
E-mail address: asclafani@gc.cuny.edu (A. Sclafani).

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are clearly related, there is some evidence that they are mediated by different neural mechanisms. For example, flavor–nutrient learning is possible with much longer CS to US delays than can support flavor–flavor conditioning (Sclafani, 1995).

Initial drug studies of flavor preference learning focused on the opioid system because of its long-recognized role in feeding behavior. Although one report indicated that the opioid receptor antagonist naloxone attenuated conditioned flavor preferences (Mehiel, 1996), our studies failed to confirm this observation. In particular, Yu et al. (1999) found that the general opioid antagonist, naltrexone, did not prevent the acquisition or the expression of a flavor preference conditioned by the sweet taste of sucrose in sham-fed rats. In a parallel study, Azzara et al. (2000) observed that naltrexone treatment also did not prevent rats from learning or expressing a flavor preference conditioned by the postingestive actions of sucrose. In view of these negative results with an opioid antagonist, subsequent studies have focused on the role of the dopamine system in flavor preference learning.

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 and Willner, 1989; Schneider et al., 1986). Radhakishun et al. (1988) demonstrated that in food-deprived animals, eating caused an increase in nucleus accumbens dopamine release that persisted until the termination of eating. 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 an intraoral saccharin infusion. When the saccharin solution was administered to rats with a conditioned aversion to that taste, accumbens 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 intragastric Polycose infusions and a CS− flavor paired with water infusions. In one-bottle tests conducted in the absence of intragastric infusions, consumption of the CS+ solution but not the CS− solution was associated with an increase in accumbens dopamine. 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 and Gratton (1996) trained rats to lever press for milk rewards. 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 cue signaling the start of the session. All of these findings are presented against a larger background of research implicating the dopamine system in reward processes (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999).

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 to 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. (2000a) 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. (2000b) 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 the expression but not acquisition of flavor preferences conditioned by flavor–taste associations.

In the present study, we examined dopamine involvement in flavor preferences conditioned by the postingestive nutritive actions of sucrose. This was accomplished by pairing a CS+ flavor with the intragastric infusion of 16% sucrose and a CS− flavor with the intragastric infusion of water. Both flavors were presented in 0.2% saccharin solutions so that the CS+ and CS− solutions were equally sweet and differed only in their flavors and postingestive consequences. Drug groups were treated with a dopamine antagonist (raclopride or SCH23390) before every training trial to assess the drug effects on the acquisition of the CS+ preference. A control group was trained with vehicle injections, and tested for preference under vehicle and a range of antagonist doses, in order to assess drug effects on the expression of the CS+ preference. Finally, a Yoked control group had its intakes matched to that of the drug group to determine if any drug effects on preference conditioning were secondary to reduced CS intakes during training.

2. Experiment 1A: the effects of 200 nmol/kg raclopride on the acquisition and expression of a conditioned flavor preference

Prior work investigating the role of the D_2 receptor antagonist raclopride in flavor preference conditioning trained rats to drink flavored sucrose solutions (Hsiao and Smith, 1995; Yu et al., 2000a,b). The present experiment examined the effects of raclopride on the preference conditioning produced by intragastric sucrose infusions. The multiple group design allowed us to examine the drug effects on both the acquisition and expression of a flavor preference while controlling for the intake-reducing effects of raclopride during training. Other important design features are that the drug group was treated with raclopride on both CS+ and CS − training days to control for any aversive effects of the drug and that preference testing was conducted under both vehicle and drug states to identify any state dependent effects.

2.1. Methods

2.1.1. Subjects

Twenty-eight male Sprague–Dawley rats, obtained from Charles River Laboratories (Willmington, MA), weighing a mean of 539 g were used. The rats were housed individually in wire mesh cages, in a temperature-controlled room, with a 12:12 h light/dark cycle. All testing occurred during the light cycle. Rats had chow (Laboratory Rodent Diet, PMI Nutrition International, Brentwood, MO) and water available ad libitum prior to surgery and during recovery.

2.1.2. 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; 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.

2.1.3. Apparatus

Testing was conducted in plastic cages ($23 \times 24 \times 31.5$ 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 spouts attached to bottles containing saccharin solutions. The drinking spouts were accessible via two holes at the front of the cage; a motorized bottle holder automatically inserted and removed the spouts at the beginning and the end of a session. Licking was monitored by an electronic drinkometer connected to a microcomputer that activated the syringe pump as the animal drank. The intragastric infusion rate was 1.3 ml/min. The oral intake/infusion volume was maintained at approximately 1:1 by the microcomputer, which turned the infusion pump on for 3 s for every $20 \pm X$ licks emitted by the rat; the value X was adjusted for each rat depending upon the rat's lick efficiency (licks/oral intake in g). This system allowed the animals to control both the timing and amount of the infusion.

2.1.4. Test solutions

The CS solutions consisted of 0.2% sodium saccharin solutions (Sigma, St. Louis, MO) flavored with 0.05% cherry or grape Kool-Aid (General Foods, White Plains, NY). The nutrient infusions were 16% w/v sucrose (Pathmark Brand, Carteret, NJ). Half of the rats in each group received cherry as the CS+ paired with intragastric sucrose and grape as the CS − paired with intragastric water; the flavor-infusion pairs were reversed for the remaining animals.

2.1.5. 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 sucrose–saccharin exposure period was used because it facilitates the acquisition of saccharin drinking in the test cages. After recovery from surgery, the rats were food deprived to 85% of their postrecovery body weight and were adapted to the test cages and training procedure; all training and testing occurred during 30 min/day sessions, 6 days/week throughout the experiment. They were trained to drink unflavored 0.2% saccharin during sessions first without being attached to the infusion system (three sessions), then while attached but not infused (three sessions), and finally while infused with water as they drank saccharin (five sessions). During the last three sessions, the rats were injected intraperitoneally (ip) with 1.0 ml/kg saline. Based upon their training intakes, the rats were divided into three groups: RAC ($n = 10$), Control ($n = 8$), and Yoked ($n = 10$).

Formal training consisted of 10 one-bottle training sessions with the CS+ and the CS − presented five times each in alternating order. Oral intakes of the CS+ and CS − were paired with matched volume infusions of 16% sucrose and water, respectively, for the RAC and Control groups. The left–right position of the CS solutions was counterbalanced, following an ABBA pattern. The RAC group received an intraperitoneal injection of raclopride (Research Biochemical International, Natick, MA), at a dose 200 nmol/kg body weight, 15 min prior to the start of the daily training sessions, while the Control and the Yoked group received intraperitoneal saline vehicle injections. The RAC and Control groups were run on the same day, while the Yoked group was run 2 days behind. The oral intakes and intragastric 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 one-bottle training, a series of two-bottle preference tests was conducted with the CS+ and CS − solutions without intragastric infusions. Fifteen minutes prior to testing, the rats were injected intraperitoneally with vehicle or raclopride at 200, 400, or 800 nmol/kg. Each dose of raclopride was given on two successive sessions in an ascending dose order, and two vehicle sessions preceded each pair of drug tests; the left–right positions of the CS solutions were alternated over sessions. The intake of the Yoked group was not limited during preference testing.

2.1.6. Statistical analysis

CS intakes were corrected for spillage and measured to the nearest 0.1 g. The one-bottle training data were averaged over sessions and analyzed with an analysis of variance (ANOVA); the Yoked group was not included in this analysis because of its imposed CS intake limit. The two-bottle intakes under vehicle treatment were averaged over sessions and were analyzed to assess the effects of training conditions on CS preference acquisition in the three groups. The effect of raclopride on the two-bottle CS intakes of the three groups was 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. A significant difference between the two-bottle intakes of the CS+ and CS − was taken as primary evidence for a CS+ preference. The two-bottle intakes of the individual rats were also expressed as percent CS+ intakes (CS+ intake/total intake × 100) and analyzed by ANOVA. The percent intake data were used to compare CS+ preferences between groups and across drug conditions, which are particularly important when there are drug-induced changes in absolute intakes.

2.2. Results

As illustrated in Fig. 1 (top), 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.

The effect of drug treatment during training on flavor preference learning is indicated by the results of the two-bottle choice tests conducted following vehicle injection (Fig. 1, bottom). 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 were not significant (Fig. 1).

The effect of raclopride treatment on the expression of the CS+ preference is summarized in Fig. 2. 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

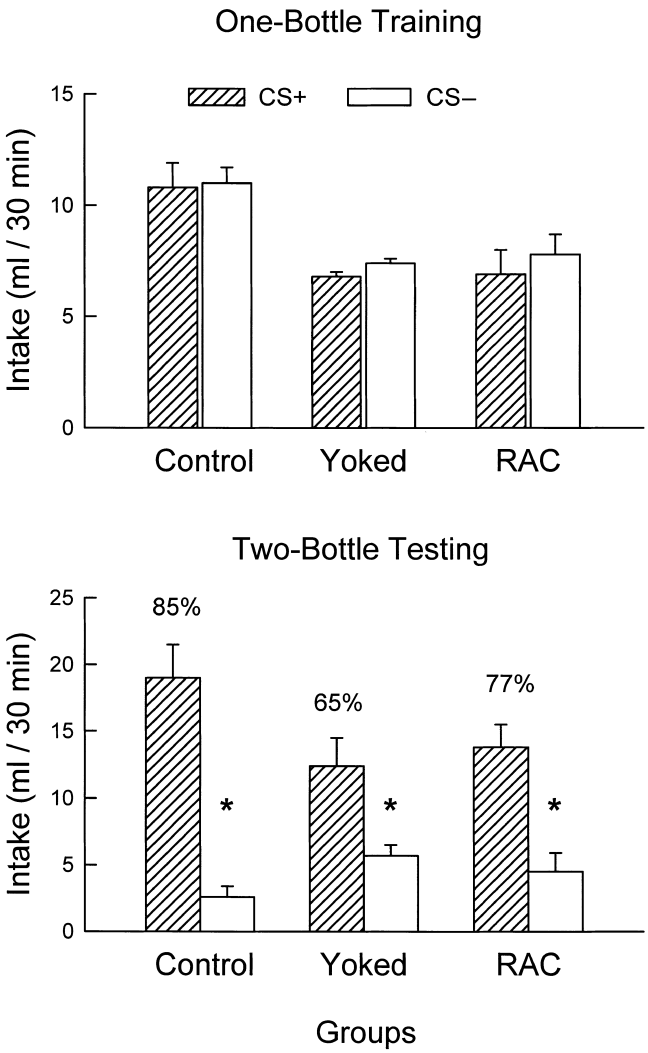

Fig. 1. Top: Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, one-bottle training sessions in Experiment 1A. The RAC group was injected with 200 nmol/kg raclopride prior to each training session; the Control and the Yoked groups were injected with vehicle. The Yoked group had its CS intake 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 (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests conducted following vehicle injections in Experiment 1A. The asterisk denotes a significant ($P < .05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

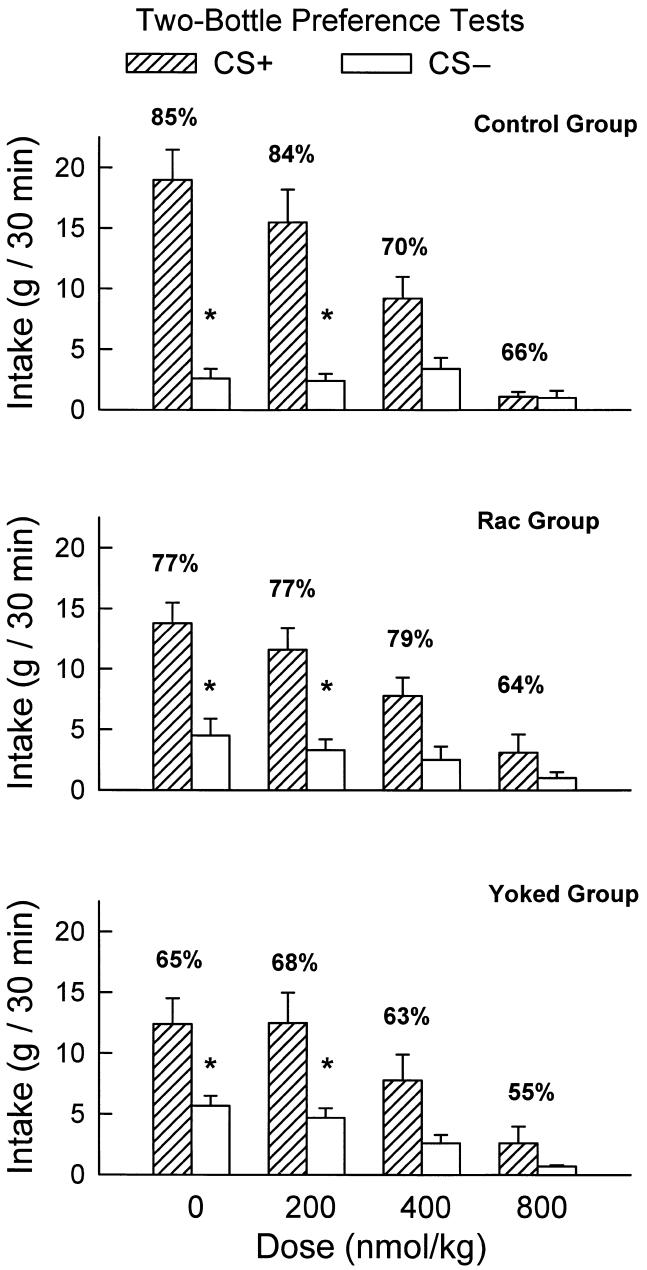

Fig. 2. Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests in Experiment 1A. Fifteen minutes prior to testing, the rats were injected with 0 (vehicle), 200, 400, or 800 nmol/kg of raclopride. The CS solutions were grape- or cherry-flavored saccharin. The CS+ was 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 asterisk denotes a significant ($P < .05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

analysis revealed a significant CS × Drug dose 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. Simple main effects tests also indicated that 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. Furthermore, 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 [$F(3,27) = 38.04$, $P < .0001$]. Although there was no interaction between CS and drug dose, individual tests indicated that the Yoked rats consumed more ($P < .05$) CS+ than CS − at the 0 and the 200 nmol/kg doses but not at the higher doses.

Analysis of the percent CS+ intakes during the two-bottle tests following vehicle or raclopride treatment revealed a drug effect [$F(3,75) = 5.42$, $P < .01$] but no Group or Group × Drug dose effect. Individual tests indicated that percent CS+ intakes were reduced ($P < .05$) at the 800 nmol/kg dose relative to the lower doses; no other dose differences were significant.

2.3. Discussion

In confirmation of prior studies, the rats learned to prefer the CS+ flavor paired with intragastric sugar infusions (Sclafani, 1995), and raclopride treatment reduced the intake of the sweet CS solutions (Smith, 1995). The new finding here is that raclopride treatment at a dose of 200 nmol/kg did not impair the acquisition or expression of the CS+ preference conditioned by intragastric sucrose infusions. While the RAC group consumed less of the CS solutions during one-bottle training than did the Control group, the percent CS+ intake of the RAC group did not differ from that of the Control group. The CS+ preference of the RAC group also did not differ from that of the Yoked group, which was given limited access to the CS solutions during training. 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 the preference.

The two-bottle test data from the Control and Yoked groups provide information on the effects of raclopride on the expression of a conditioned CS+ preference in rats not previously exposed to the drug. The Control rats consumed significantly more CS+ than CS − when treated with the vehicle and the 200 nmol/kg dose of raclopride but not when treated with the 400 and 800 nmol/kg doses. The results obtained with the two higher doses and the fact that raclopride treatment suppressed CS+ intake but not CS − intake in the Control rats suggest that the CS+ preferences are blocked by raclopride at higher doses. However, the seemingly specific action of the drug on CS+ intake may have been due to "floor effect" on CS − intake. That is, the

Control rats consumed very little of the CS − (2.6 g/30 min) in the two-bottle tests following vehicle treatment, which did not allow for much reduction in CS − intake following raclopride treatment. In the Yoked rats, which drank more CS − in the vehicle tests (5.7 g/30 min), raclopride treatment reduced the two-bottle intakes of both the CS+ and CS − . A floor effect may also explain the lack of CS+ preference at the 800 nmol/kg dose since this dose greatly suppressed intake in all groups, resulting in small and similar intakes of the CS solutions.

The Control rats did not consume significantly more CS+ than CS − at the 400 nmol/kg raclopride dose but this was due to one of the eight rats, which failed to prefer the CS+. Overall, the Control group showed a 70% CS+ preference at the 400 nmol/kg dose, which was not significantly less than the percent CS+ intakes at the lower drug doses. These points are raised because in two unpublished experiments using nearly identical methodology, a 400 nmol/kg dose raclopride dose did not block the expression of a CS+ preference but rather reduced it only slightly from about 87% (vehicle test) to 83% (Azzara and Sclafani, unpublished observations). Experiment 1B 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.

3. Experiment 1B: the effects of 400 nmol/kg raclopride on the acquisition and expression of a conditioned flavor preference

The rats from Experiment 1A were redistributed into three new RAC ($n=10$), Control ($n=8$), and Yoked groups ($n=10$). The new groups were equated for their prior group membership as well as for their CS+ preferences and total intakes during the two-bottle tests of Experiment 1A.

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 rats in the three groups 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 1A.

3.1. 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 (Fig. 3, top). 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. 3, top].

The effect of drug treatment during training on flavor preference learning is indicated by the results of the two-bottle choice tests conducted following vehicle injection

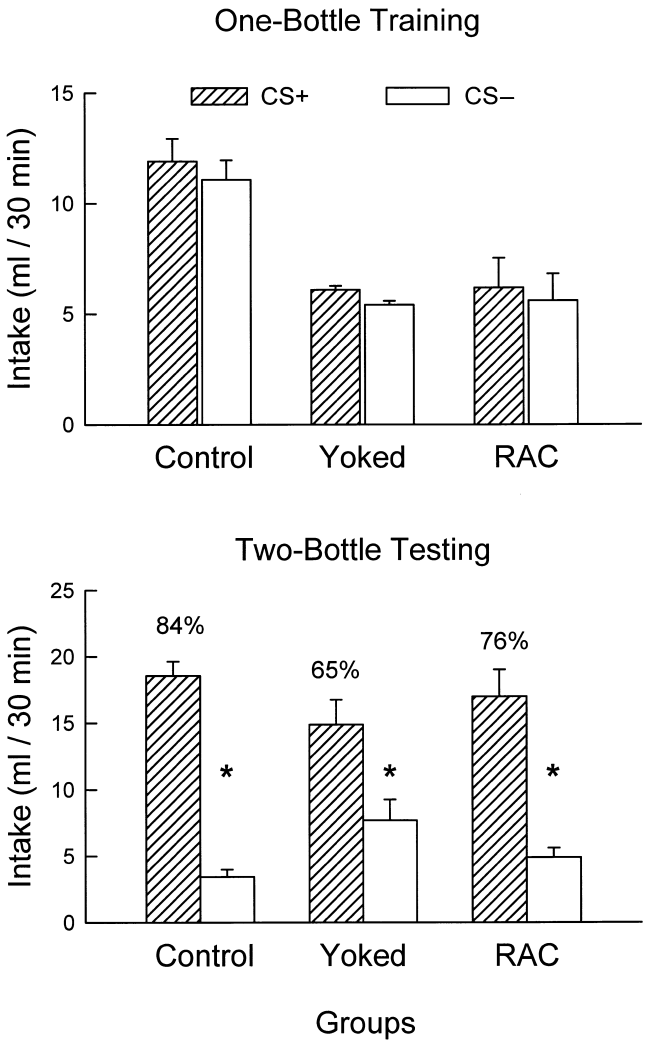

Fig. 3. Top: Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, one-bottle training sessions in Experiment 1B. The RAC group was injected with 400 nmol/kg raclopride prior to each training session; the Control and the Yoked groups were injected with vehicle. The Yoked group had its CS intake 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 (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests following vehicle injections in Experiment 1B. The asterisk denotes a significant ($P<.05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

(Fig. 3, bottom). Overall, 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 (Fig. 3).

The effect of raclopride treatment on the expression of the CS+ preference is summarized in Fig. 4. 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 dose and CS [$F(1,25) = 13.76$, $P < .01$] and the drug effect on CS intake was explored further by analyzing the data from the three groups combined. Despite the interaction, 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, and drug treatment reduced ($P < .0001$) the intake of both the CS+ and the CS −. Analysis of the percent CS+ intakes revealed no significant group or drug effect or interaction.

3.2. Discussion

These results demonstrate that treating rats with raclopride at 400 nmol/kg during one-bottle CS training, although it reduced training intakes by half, did not attenuate the acquisition of flavor preference conditioned by intragastric 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 expression of the CS+ preference. 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 1A but replicates two earlier experiments (Azzara and Sclafani, unpublished observations). Taken together, the results of Experiments 1A and 1B indicate that D_2 dopamine receptors are not critically involved in the acquisition or the expression of a nutrient-conditioned flavor preference.

4. Experiment 2: 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). The second experiment investigated whether blocking D_1 receptors with SCH23390 alters

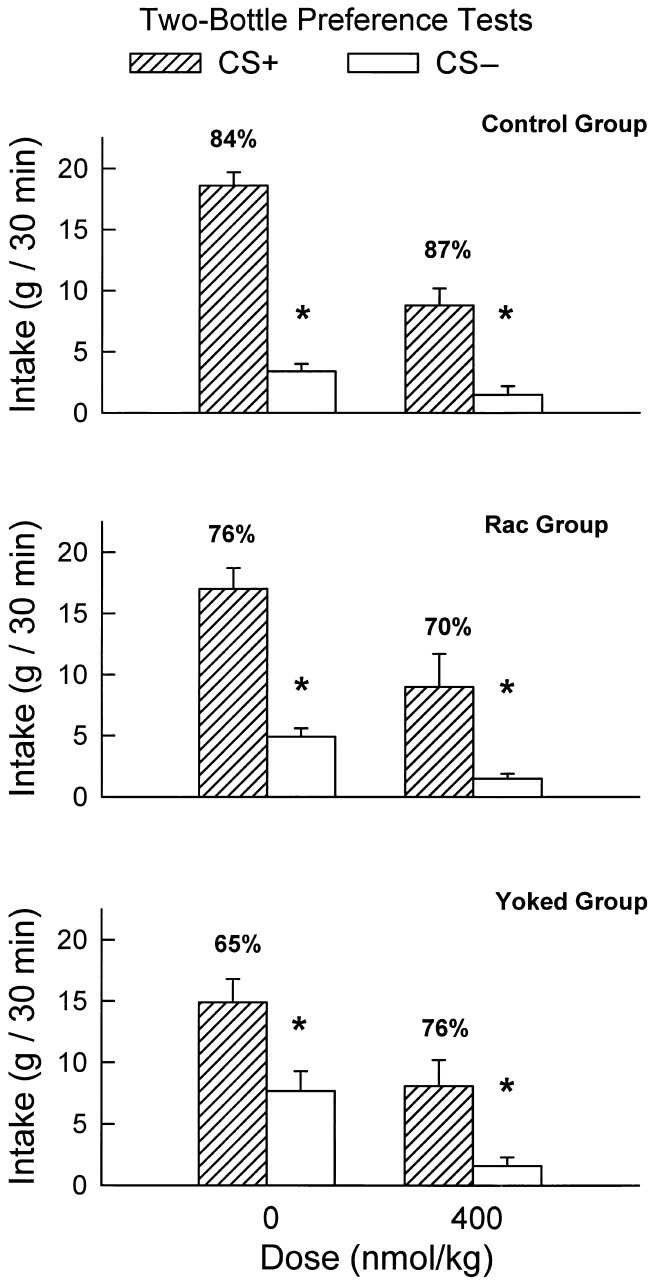

Fig. 4. Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests in Experiment 1B. Fifteen minutes prior to testing, the rats were injected with 0 (vehicle) or 400 nmol/kg of raclopride. The CS solutions were orange- or strawberry-flavored saccharin, and the CS+ was 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 asterisk denotes a significant ($P < .05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

the acquisition and/or expression of carbohydrate-conditioned flavor preferences.

4.1. Subjects

Twenty-nine experimentally naive, male Sprague–Dawley rats, obtained from Charles River Laboratories, weighing a mean of 502 g, were used; these rats were surgically prepared and maintained as in Experiment 1.

4.2. Procedure

The rats were exposed to saccharin + sucrose and saccharin solutions in their home cages, as in Experiment 1A. After recovery from the surgery, the rats were food deprived to 85% of their postrecovery body weight. The rats were trained 30 min/day to drink unflavored 0.2% saccharin 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 0.2% 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, the rats were divided into three groups: SCH ($n = 9$), Control ($n = 10$), and Yoked ($n = 10$).

Ten one-bottle training sessions with the CS+ and CS − were conducted as in Experiment 1A. The SCH group was injected with SCH23390 (200 nmol/kg ip) 15 min prior to the one-bottle sessions, whereas the Control and the Yoked groups were injected with the saline vehicle prior to each session. The rats were next given a series of two-bottle preference tests as in the first experiment. Fifteen minutes prior to testing, the rats were injected intraperitoneally with vehicle or SCH23390 at 200 or 400 nmol/kg. Each dose of SCH23390 was given on two successive sessions in an ascending order, and two vehicle sessions preceded each pair of drug sessions.

4.3. 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 (Fig. 5, top). 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 [$F(1,17) = 4.49$, $P < .05$].

The effect of drug treatment during training on flavor preference learning is indicated by the results of the two-bottle choice tests conducted following vehicle injection (Fig. 5, bottom). Overall, the total intakes of the three groups did not differ, but there was a significant interaction between Group and CS [$F(2,26) = 20.45$, $P < .0001$]. Further analysis revealed that both the Control and Yoked

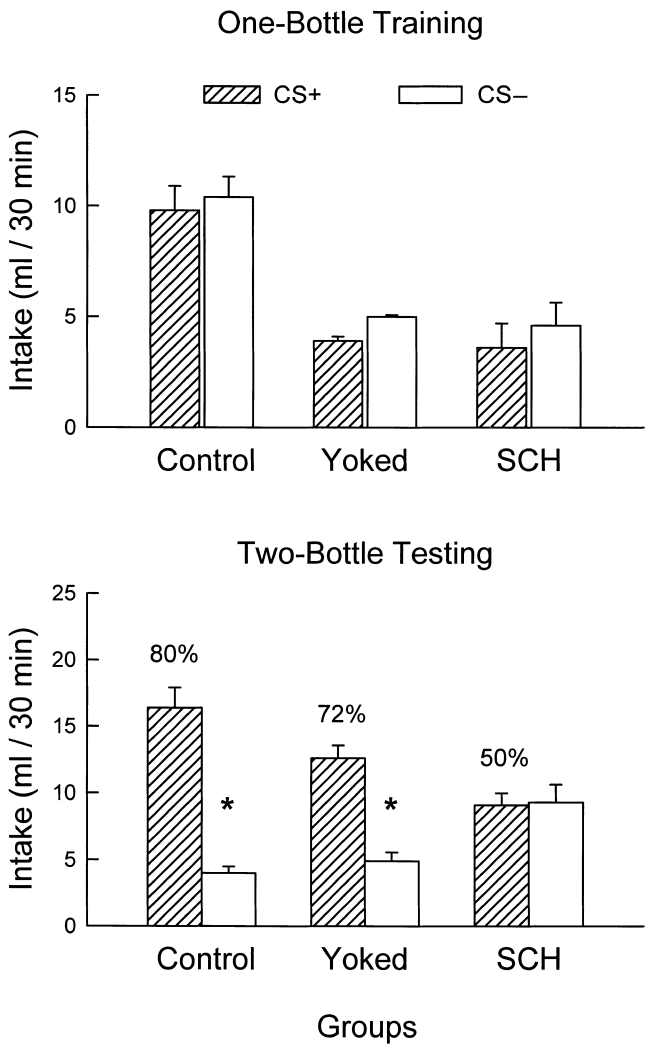

Fig. 5. Top: Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, one-bottle training sessions. in Experiment 2. The SCH group was injected with 200 nmol/kg SCH23390 prior to each training session; the Control and the Yoked groups were injected with vehicle (0 nmol/kg); the Yoked group had 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 (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests following vehicle injections in Experiment 2. The asterisk denotes a significant ($P < .05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

rats consumed more ($P < .001$) CS+ than CS − in the choice tests, whereas the SCH group 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 ($P < .01$) than that of the Control and the Yoked groups, which did not differ from each other.

Fig. 6 summarizes the effects of SCH23390 treatment on the expression of the CS+ preference. Overall, the three groups did not differ in their total CS intakes and they all reduced their intakes when treated with SCH23390

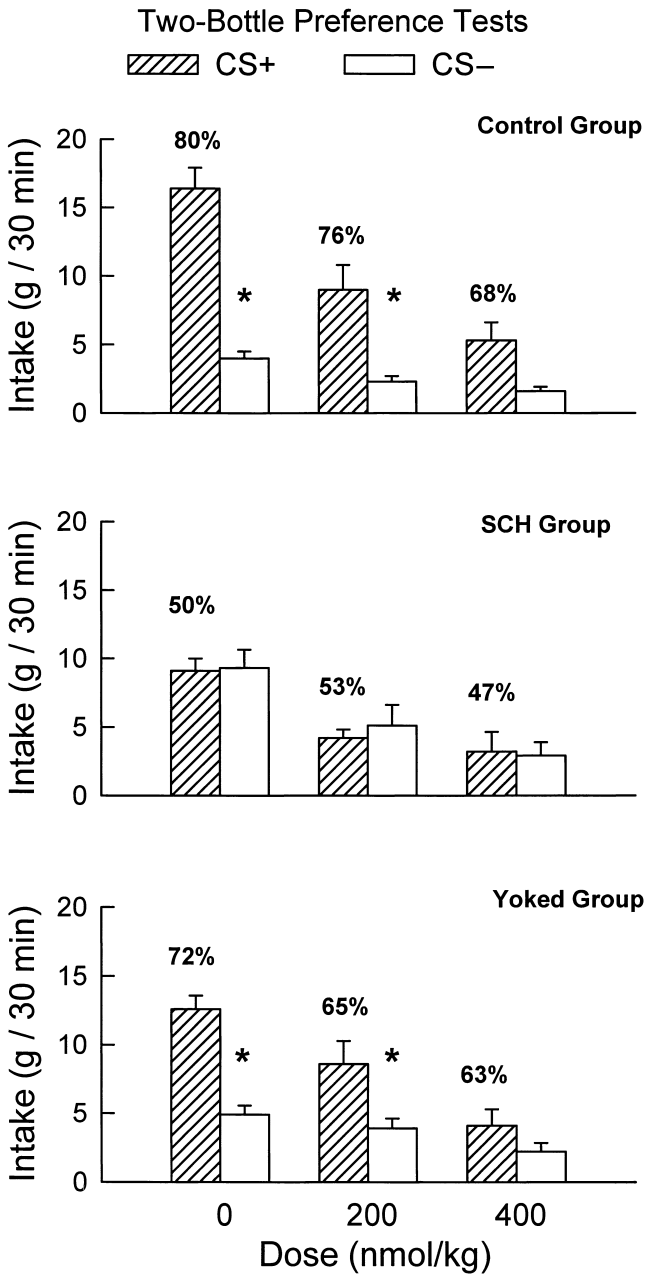

Fig. 6. Intakes (mean + S.E.M.) of the CS+ and the CS − during 30 min, two-bottle preference tests in Experiment 2. Fifteen minutes prior to testing, the rats were injected with 0 (vehicle), 200, or 400 nmol/kg of SCH23390. The CS solutions were cherry- or grape-flavored saccharin, and the CS+ was paired 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 its CS intake matched to that of the SCH group during training. The asterisk denotes a significant ($P<.05$) difference between CS+ and CS − intakes. The numbers atop the bars represent the mean of the individual rats' percent CS+ intakes.

[$F(2,52)=87.37$, $P<.0001$]. There were significant interactions between Drug dose 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 × 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 × 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 rats did not drink more CS+ than CS − at any dose and the drug reduced their intakes of both the CS+ ($P<.001$) and CS − ($P<.001$) solutions. Consistent with these findings, the percent CS+ intakes of the SCH rats were significantly less than those of the Control and Yoked rats [$F(2,26)=12.18$, $P<.001$]. There was no overall Drug effect on percent CS+ intakes or Drug × Group interaction.

4.4. Discussion

In addition to confirming prior studies showing that the D_1 antagonist SCH23390 suppresses the intake of sweet solutions (Smith, 1995), this experiment revealed two new findings. The first is that SCH23390 treatment throughout one-bottle training blocked the acquisition of a conditioned flavor preference. The SCH group displayed no preference for either the CS+ or CS − solution during the two-bottle tests under vehicle treatment. They continued to show no CS+ preference when treated with SCH23390 prior to the two-bottle tests, demonstrating that they had not learned a preference, which was dependent upon the training drug state. This contrasts with the Control and Yoked groups, which both demonstrated significant CS+ preferences (80% and 72%, respectively) when treated with vehicle. The failure of the SCH group to learn a CS+ preference cannot be attributed to their reduced intake during training in view of the preference displayed by the Yoked group, which had its CS and US exposure matched to that of the SCH group. It is also unlikely that daily SCH23390 treatment during training had an aversive effect that blocked preference learning. When first tested with the CS+ and CS − solutions in the absence of the drug (i.e., initial two-bottle vehicle tests), the SCH rats drank as much total solution as did the Control and Yoked groups.

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 (80–76%). The 400 nmol/kg dose produced a greater intake suppression and the Control group no longer significantly preferred the CS+ although their percent CS+ intake was 68%. This lack of preference is difficult to interpret given the overall reduction in total intake produced by the 400 nmol/kg dose.

5. General discussion

In the three experiments of this study, the Control rats acquired strong (80–85%) preferences for the cue flavor that was paired with intragastric infusions of sucrose, which extends prior findings on carbohydrate-conditioned flavor preferences (Sclafani, 1995). The role of dopamine receptors in the acquisition and expression of this sucrose-conditioned preference was investigated by treating rats with selective D_1 or D_2 antagonists during training and/or testing. The D_2 antagonist raclopride did not attenuate the acquisition of the sucrose-conditioned flavor preference, whereas the D_1 antagonist SCH23390 completely blocked the learning of the preference. Both antagonists had minimal effects on the expression of the conditioned preference at doses that did not drastically reduce solution intakes. At higher doses, the attenuated preferences we observed with both raclopride and SCH23390 can be understood in terms of a general intake-suppressive effect of the antagonists.

In Experiment 1, rats treated with raclopride at 200 or 400 nmol/kg during training learned a significant preference for the CS+ over the CS−, as expressed in the two-bottle tests following vehicle treatment, 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 significant only in Experiment 1B. At the 800 nmol/kg dose, raclopride reduced total intakes to very low levels and eliminated the CS+ preference. The fact that raclopride suppressed CS+ intakes more than CS− intakes in the two-bottle tests may be taken as evidence for a role of D_2 receptors in conditioned flavor preference. A 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 a conditioning study by Ramirez (1997) in which separate groups of rats were trained to drink a saccharin solution paired with intragastric maltodextrin infusion or a saccharin solution paired with intragastric water. Nutrient conditioning was evidenced by the elevated saccharin intake observed in the rats infused with maltodextrin relative to the water-infused rats. 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. 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 flavor preference conditioning by intragastric sucrose infusions. In Experiment 2, 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 their 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, failed to significantly block the expression of the learned preference in the Control rats.

A third type of selective drug effect is suggested by comparing the present results with those obtained in parallel studies performed in our laboratories. Yu et al. (2000a,b) observed that both raclopride and SCH23390 attenuated the expression of flavor preferences conditioned by the taste of sucrose, relative to the taste of saccharin. Additionally, the antagonists attenuated the acquisition of the preference compared to a control group but not compared to a Yoked control group. In these studies, the rats drank a CS+ flavored sucrose solution, which drained out an open gastric fistula so that the resulting CS+ preference was conditioned by the sweet taste of sucrose rather than by the sugar's postingestive actions (flavor–taste 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). The results obtained with these two training paradigms indicate that whereas the *acquisition* of flavor–nutrient preference learning is dependent upon D_1 receptors, the *expression* of a flavor–taste preference involves both D_1 and D_2 receptor activity. This conclusion remains tentative given that the two training paradigms differed in a number of respects, but the findings are consistent with the idea that flavor–taste and flavor–nutrient learning involve different behavioral and neural processes.

D_2 receptor antagonism with raclopride did not block flavor preference learning reinforced by taste (Yu et al., 2000a,b) and did not block preference learning based upon the postingestive actions (present study) of sucrose. These results would appear to conflict with the sucrose conditioning data reported by Hsiao and Smith (1995). However, fundamental differences in the training procedures of these studies may account for the contrasting results. First, unlike

the present study, but like the Yu et al.'s (2000a,b) 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. (2000a,b) paired both the CS+ and CS − flavors with raclopride treatment, Hsiao and Smith (1995) 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's (1995) study (800 nmol/kg) was considerably higher than the training dose employed by Yu et al. (2000b) and in the present study. It is possible that flavor preference conditioning by intragastric 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 Experiment 1A, 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 (Beninger and 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 LiCl-induced toxicosis. 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.

Various theories have been proposed to explain the behavioral functions of brain dopamine systems. According to Berridge and Robinson (1998), food reward can be subdivided into a 'wanting' component, which is related to incentive motivation, and a 'liking' component, which corresponds with hedonic evaluation. In their model, the dopamine system is the primary mediator of the 'wanting' component, whereas the opioid system is thought to mediate the 'liking' component. Berridge and Robinson (1998) further hypothesize that dopamine 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 2 learned to "like" the CS+ flavor paired with intragastric 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. Whether flavor–nutrient learning actually results in an increased hedonic response to the CS+ flavor, as measured by the taste reactivity test, is currently under investigation. 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, but again preference learning was evaluated using two-bottle intakes only (Azzara et al., 2000).

Another recent theory of the role of dopamine in reward comes from Ikemoto and Panksepp (1999), who hypothesized that the mesoaccumbens 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 dependent upon dopamine release. An explicit prediction of this theory is that mesoaccumbens 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, Ikemoto and Panksepp (1999) have also speculated that dopamine outside of the mesoaccumbens 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.'s (1994) study showing that rats trained with a CS+ flavor paired with intragastric Polycose infusions subsequently show an increase in nucleus accumbens dopamine release 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 the highest drug doses. As noted above, Ikemoto and Panksepp (1999) hypothesized that well-trained behaviors are not dependent upon the mesoac-

cumbens 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 dopamine D_2 antagonist raclopride did not suppress the acquisition of a flavor preference conditioned by intragastric sucrose infusions and 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. The results of parallel studies reported elsewhere (Yu et al., 2000a,b) implicate both receptor subtypes in the expression of flavor preferences conditioned by the sweet taste of sucrose.

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References

Azzara AV, Bodnar RJ, Delamater AR, Sclafani A. Naltrexone fails to block the acquisition or expression of a flavor preference conditioned by intragastric carbohydrate infusions. Pharmacol Biochem Behav 2000;67:545–57.

Beninger RJ, Miller R. Dopamine D_1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev 1998;22:335–45.

Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 1998;28:309–69.

Caulliez R, Meile M-J, Nicolaidis S. A lateral hypothalamic D_1 dopaminergic mechanism in conditioned taste aversion. Brain Res 1996;729:234–45.

Davis JD, Campbell CS. Chronic intrajugular, intraportal, gastric, and duodenal cannulae for the rat. In: Singh D, Avery DD, editors. Physiological techniques in behavioral research. Monterey: Brooks Cole, 1975. pp. 163–77.

Holman EW. Immediate and delayed reinforcers for flavor preferences in rats. Learn Motiv 1975;6:91–100.

Hsiao S, Smith GP. Raclopride reduces sucrose preference in rats. Pharmacol Biochem Behav 1995;50:121–5.

Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 1999;31:6–41.

Mark GP, Blander DS, Hoebel BG. A conditioned stimulus decreases extracellular dopamine in the nucleus accumbens after the development of a learned taste aversion. Brain Res 1991;551:308–10.

Mark GP, Smith SE, Rada PV, Hoebel BG. An appetitively conditioned taste elicits a preferential increase in mesolimbic dopamine release. Pharmacol Biochem Behav 1994;48:651–60.

Mehiel R. The effects of naloxone on flavor–calorie preference learning indicate involvement of opioid reward systems. Psychol Rec 1996;46:435–50.

Muscat R, Willner P. Effects of selective dopamine receptor antagonists on sucrose consumption and preference. Psychopharmacology (Berlin) 1989;99:98–102.

Radhakishun FS, VanRee JM, Westerink BHC. Scheduled eating increases dopamine release in the nucleus accumbens of food-deprived rats as assessed with on-line brain dialysis. Neurosci Lett 1988;85:351–6.

Ramirez I. Intragastric carbohydrate exerts both intake-stimulating and intake-suppressing effects. Behav Neurosci 1997;111:612–22.

Richardson NR, Gratton A. Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in rat. J Neurosci 1996;16:8160–9.

Schneider LH, Gibbs J, Smith GP. D-2 selective receptor antagonists suppress sucrose sham feeding in the rat. Brain Res Bull 1986;17:605–11.

Sclafani A. How food preferences are learned — laboratory animal models. Proc Nutr Soc 1995;54:419–27.

Smith GP. Dopamine and food reward. In: Fluharty S, Morrison AM, editors. Progress in psychobiology and physiological psychology vol. 16. New York: Academic Press, 1995. pp. 83–144.

Yu W-Z, Sclafani A, Delamater AR, Bodnar RJ. Pharmacology of flavor preference conditioning in sham-feeding rats: effects of naltrexone. Pharmacol Biochem Behav 1999;64:573–84.

Yu W-Z, Silva RM, Sclafani A, Delamater AR, Bodnar RJ. Pharmacology of flavor preference conditioning in sham-feeding rats: effects of dopamine receptor antagonists. Pharmacol Biochem Behav 2000a;65:573–84.

Yu W-Z, Silva RM, Sclafani A, Delamater AR, Bodnar RJ. Role of D_1 and D_2 dopamine receptors in the acquisition and expression of flavor-preference conditioning in sham-feeding rats. Pharmacol Biochem Behav 2000b;67:537–544.