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# Pharmacology of Flavor Preference Conditioning in Sham-Feeding Rats: Effects of Dopamine Receptor Antagonists

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YU, W.-Z., R. M. SILVA, A. SCLAFANI, A. R. DELAMATER AND R. J. BODNAR. *Pharmacology of flavor preference conditioning in sham-feeding rats: Effects of dopamine receptor antagonists.* PHARMACOL BIOCHEM BEHAV **65**(4) 635–647, 2000.—Opioid and dopamine systems are both implicated in the response to sweet solutions. Our laboratory previously reported that the opioid antagonist, naltrexone, reduced the intake of sweet solutions, yet had little or no effect on sucrose-conditioned flavor preferences in sham-feeding rats. The present study examined the role of dopamine $D_1$ and $D_2$ receptors in the expression of flavor preferences conditioned by the sweet taste of sucrose. All sessions were conducted under sham-feeding conditions to minimize postingestive influences. Training was accomplished by adding a novel flavor (CS+) to a 16% sucrose solution, a different flavor (CS−) to a less-preferred 0.2% saccharin solution in alternating, one-bottle sessions. Preferences were assessed in two-bottle tests with the CS+ and CS− flavors presented in mixed sucrose (8%)–saccharin (0.1%) solutions following systemic doses of 0, 50, 200, 400, or 800 nmol/kg of the $D_2$ antagonist, raclopride (Experiment 1) or the $D_1$ antagonist, SCH23390 (Experiment 2) under either food-restricted or unrestricted conditions. Rats significantly preferred the CS+ solutions in vehicle tests, and displayed equipotent and dose-dependent reductions in total intake and CS+ preference following either $D_1$ or $D_2$ receptor antagonism. Similar results were obtained with SCH23390 and raclopride in Experiment 3 conducted with water-restricted rats. These data indicate that dopaminergic $D_1$ and $D_2$ receptors play pivotal and functionally equivalent roles in the expression of flavor preferences conditioned by the sweet taste of sucrose. © 2000 Elsevier Science Inc.

Conditioned flavor preference  Sham-feeding preparation  Dopamine  $D_1$ receptor  $D_2$ receptor  SCH23390  Raclopride  Expression studies

LEARNING, together with innate taste biases, play a major role in food preferences [see review: (48)]. The conditioned flavor preference paradigm has been a useful procedure to study acquisition and expression of acquired food preferences in animals. In one version of the paradigm, an arbitrary flavor (the conditioned stimulus or CS+) is paired with a nutritive source (the unconditioned stimulus or US; e.g., sucrose solution), and a second flavor (the CS−) is paired with a nonnutritive source (e.g., saccharin solution) during one-bottle training sessions. Preference learning is then assessed in a two-choice test with the two flavors presented in a common base (e.g., sucrose–saccharin mixture) to ensure that any differential intake can be attributed to a learned response to the two cue flavors. Both the flavor and the postingestive consequences of the nutrient can function as an unconditioned stimulus in producing the preference for the cue flavor. That a palatable flavor alone is sufficient to condition flavor preferences (flavor–flavor conditioning) is demonstrated by studies in which a CS+ flavor is mixed into a preferred saccharin solution, and a CS− flavor is mixed in a less preferred saccharin solution or plain water (24,25). Other studies show that the postingestive actions of nutrients condition flavor preferences (flavor–nutrient conditioning) by pairing the CS+ flavor with intragastric (IG) nutrient infusions (48). Different neural processes may mediate these two types of flavor learning because flavor–nutrient conditioning is possible with delays between the CS and US of several minutes or more,

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whereas the US flavor must be closely associated with the CS flavor for flavor–flavor conditioning to occur [(18,24); see also: (33)].

Relatively little is known about the neurochemical and pharmacological mechanisms involved in flavor preference conditioning. Recent studies in our laboratories revealed that the general opioid antagonist, naltrexone, significantly reduced the intake of sweet solutions, yet had little or no effect on the acquisition or expression of flavor preferences conditioned by the sweet taste or postingestive actions of sucrose (49,65). The dopamine system has been implicated in reinforcement mechanisms related to food and water intake (2,3,8,34). Specifically, both $D_1$ and $D_2$ receptors are involved in the ingestive response to sweet solutions because $D_1$ and $D_2$ antagonists each reduce the intake of sugar and saccharin solutions (21,32,43–47,56,62). Further, Hsiao and Smith (26) found that $D_2$ receptor blockade with raclopride reduces sucrose-conditioned flavor preferences. In their paradigm, rats were trained with 10% sucrose solutions paired with two distinct flavors in which raclopride was paired with one flavor, and vehicle was paired with the second flavor.

The present study evaluated whether selective $D_2$ (raclopride) and $D_1$ (SCH23390) receptor antagonists altered the flavor preference conditioned by the taste of sucrose in rats trained under food-restricted or nonrestricted feeding conditions (Experiments 1 and 2) or under water restriction (Experiment 3). Parallel studies (Azzara et al., in preparation) to be reported elsewhere investigated whether these selective antagonists altered the flavor preference conditioned by the postingestive actions of sucrose. These parallel experiments provide information on the involvement of $D_1$ and $D_2$ receptors in both flavor–flavor and flavor–nutrient learning.

## EXPERIMENT 1A

Flavor–flavor learning has typically been studied by training rats with cue flavors added to palatable, nonnutritive fluids [saccharin solution, mineral oil emulsion; (18,24)]. Recently, our laboratory has adopted the sham-feeding procedure (58) to study flavor–flavor learning (65). In this preparation, ingested fluid drains out of an open gastric fistula, and thus the postingestive nutritive effects are minimized, although not completely eliminated (50). An advantage of this procedure is that nutritive as well as nonnutritive solutions can be used as unconditioned stimuli to produce flavor–flavor learning in the absence of postingestive nutritive conditioning. Also, rats consume substantial amounts of sapid solutions during sham-feeding sessions so that their exposure to the conditioning stimuli during one-bottle training sessions is maximized. In addition, their elevated intakes during two-bottle sham-feeding tests provide a high baseline to evaluate drug effects on flavor preferences.

To assess flavor conditioning by sweet tastes, our laboratory (65) trained rats to drink distinctively flavored (e.g., grape and cherry) 16% sucrose and 0.2% saccharin solutions during one-bottle sham-feeding trials. In subsequent two-bottle tests, the rats preferred the sucrose-paired flavor over the saccharin-paired flavor when both were presented in mixed sucrose–saccharin solutions. This flavor preference was attributed to the reinforcing effect of the sucrose taste because the sugar's postingestive actions were minimized by the open gastric fistula. Note that although saccharin and sucrose are both sweet, rats prefer concentrated sucrose solutions to saccharin solutions in two-bottle choice tests (14,31), and sham feed substantially more sucrose than saccharin in one-bottle tests (51). Thus, the taste of sucrose is a more potent unconditioned stimulus than the taste of saccharin in flavor–flavor learning.

Our prior study revealed that the general opioid antagonist, naltrexone, did not block the expression of the sucrose-conditioned flavor preference (65). The present experiment used the same sham-feeding testing procedure to determine if the selective $D_2$ receptor antagonist, raclopride (28,39), alters the flavor preference conditioned by the taste of sucrose.

### *Method*

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

*Test solutions.* The training solutions consisted of either 16% sucrose (Domino Sugar) or 0.2% sodium saccharin (Sigma Chemical Co., St. Louis, MO) flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY). Half of the rats had a cherry flavor added to the sucrose solution and a grape flavor added to the saccharin solution; the flavors were reversed for the remaining rats. In the two-choice preference tests the cherry and grape flavors were each presented in a mixed solution containing 8% sucrose + 0.1% saccharin. The taste of sucrose was considered the US, because it is strongly preferred to the taste of saccharin, and stimulates more sham feeding than saccharin at the concentrations employed in the present study (14,51,64). The flavor added to the sucrose solution is referred to as the CS+, and the flavor added to the saccharin solution is referred to as the CS−. For initial sham-feeding training, an 8% maltodextrin solution was used (BioServ, Frenchtown, NJ), which has a distinctive taste to rats.

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

*One-bottle training.* The rats were given 10 one-bottle, sham-feeding training sessions (30 min/day) with unlimited access to the CS training solutions. The CS− was presented on odd-numbered days, and the CS+ was presented on even-numbered days. Food was unavailable during all test times. On days 7–10, the rats received vehicle treatment (1 ml nor-

mal saline/kg body weight, SC) 30 min prior to the training session, during which they had access to two sipper tubes, one containing the CS− or CS+ solution, and the other containing water. This acclimated the rats both to the injection procedure and the presence of two sipper tubes during the choice tests. Water intake was negligible in these training trials. The position of the CS and water sipper tubes varied across days using a left–right–right–left pattern.

*Two bottle testing.* Following training, the rats were given eight two-bottle sham-feeding test sessions (30 min/day) with unlimited access to the CS+ and CS− flavors presented in mixed sucrose (8%)–saccharin (0.1%) solutions. The positions of the two sipper tubes were counterbalanced as described above. On day 1, subgroups of rats received vehicle (1 ml/kg, SC) or raclopride (Research Biochemicals Intl., Natick, MA) at doses of either 50 or 800 nmol/kg 30 min prior to the test sessions. This pattern of treatments was systematically altered over the ensuing 3 days such that all nine rats received two vehicle injections, and raclopride at doses of 50 and 800 nmol/kg. The pattern was then repeated on days 5–8 so that all nine rats received two more vehicle injections, and raclopride at doses of 200 and 400 nmol/kg.

*Statistics.* CS intakes were recorded to the nearest milliliter. Intakes during training were evaluated by a repeated-measures factorial analysis of variance with the CS− and CS+ conditions as one variable, and the 5 days of exposure as the second variable. Tukey corrected comparisons ($p < 0.05$) detected significant effects. The test data were evaluated with separate randomized-block analyses of variance performed on CS+ and CS− intake as a function of pooled vehicle and raclopride dose treatments, total intake as a function of vehicle and raclopride treatment, and CS+ preference scores as a function of vehicle and raclopride treatment. CS+ preference was defined as the percentage of CS+ intake/total intake.

### *Results*

*CS+ and CS− intake during training.* Significant differences in sham intakes were observed across training days, $F(4, 32) = 3.37$, $p < 0.021$, between the CS+ and CS− conditions, $F(1, 8) = 13.97$, $p < 0.006$, and for the interaction between days and conditions, [F(4),32) = 3.25], $p < 0.024$. Overall, the rats drank nearly four and one-half times more of the CS+ solution (25.7 ml) than the CS-solution (5.7 ml) during training (Fig. 1A). Whereas intake of the CS− solution remained stable over the 5 days of training, intake of the CS+

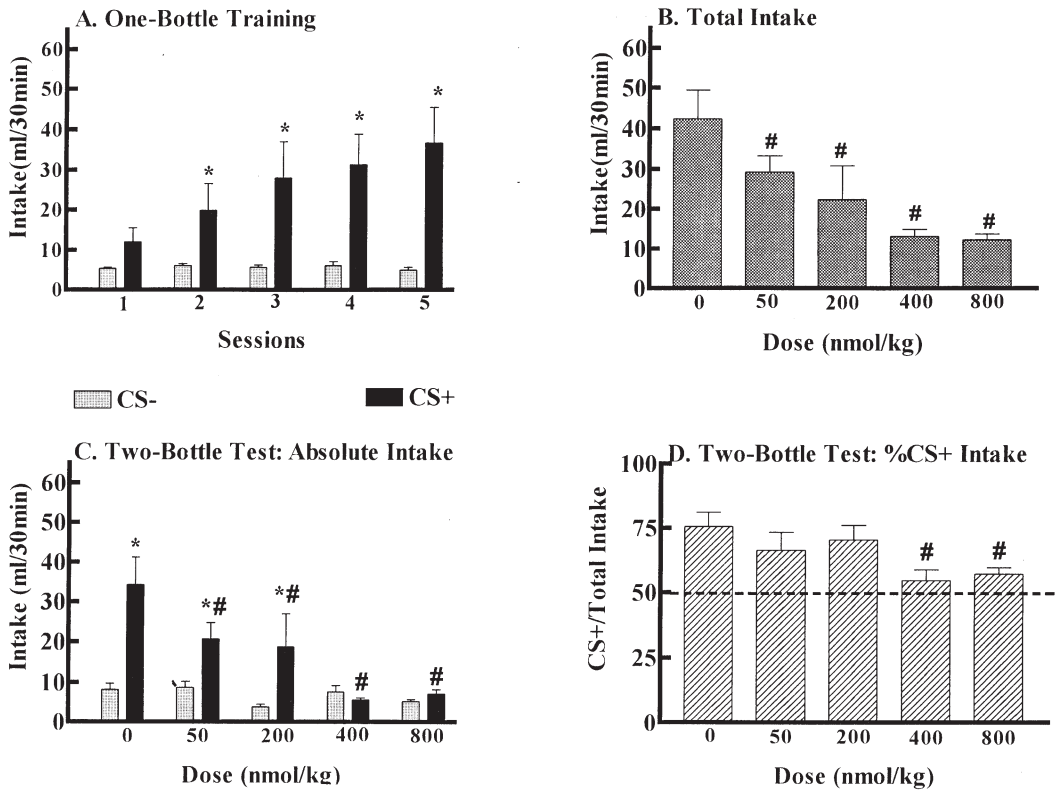


FIG. 1. Experiment 1A, Raclopride: Conditioned flavor preferences in food-restricted, sham-feeding rats. (A) Sham-feeding intakes (mean ± SEM) in one-bottle training tests (30 min) of either a saccharin (0.2%) solution paired with a novel grape or cherry (0.05%) flavor (CS−), or a sucrose (16%) solution paired with a novel cherry or grape flavor (CS+) in rats food-restricted to 85–90% of their normal body weight. (B) Sham-feeding total intakes (mean ± SEM, 30 min) of a combined saccharin (0.1%) and sucrose (8%) solution in two-bottle tests with the CS+ and CS− flavors, respectively, following pretreatment (30 min) with either vehicle (mean of four tests) or raclopride at doses of 50, 200, 400, or 800 nmol/kg. (C) Sham-feeding intakes of the CS+ flavored and CS− flavored solutions following raclopride relative to vehicle treatment. (D) Conditioned flavor preferences, defined as the percentage of CS+ intake over total intake following raclopride relative to vehicle treatment. In this and all subsequent figures, the * denotes significant differences between CS+ and CS− intakes for each corresponding treatment. In this and all subsequent figures, the # denotes significant differences between the particular antagonist treatment relative to its corresponding vehicle treatment.

solution significantly increased over the last 3 days of training (Fig. 1A).

*Raclopride and conditioned flavor preferences.* All doses of raclopride significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 32) = 8.03$, $p < 0.0001$; the two higher (400 and 800 nmol/kg) raclopride doses produced significantly greater reductions than the two lower (50 and 200 nmol/kg) doses (Fig. 1B). Reductions in total intake were noted following all doses of raclopride. Overall, the rats consumed more of the CS+ (17.6 ml) than of the CS− (6.3 ml) solution during these tests, $F(1, 8) = 10.56$, $p < 0.012$. However, there was a significant interaction between raclopride doses and CS solutions, $F(4, 32) = 4.51$, $p < 0.005$. Raclopride did not alter CS− intake, but significantly reduced intake of the CS+ solutions following all raclopride doses relative to vehicle treatment (Fig. 1C). Whereas CS+ intake was significantly higher than corresponding CS− intake following the vehicle, 50 and 200 nmol/kg doses of raclopride, CS+, and CS− intakes failed to differ from each other following the 400 and 800 nmol/kg doses of raclopride. Raclopride also significantly reduced the percent CS+ intake, $F(4, 32) = 3.76$, $p < 0.013$. The percent CS+ intake following vehicle (75.6%) was significantly reduced by the two higher 400 (54.5%) and 800 (57.1%) nmol/kg doses of raclopride (Fig. 1D).

### *Discussion*

This experiment confirmed that rats develop a reliable preference for a flavor paired with sucrose over a flavor paired with saccharin during one-bottle sham-feeding sessions as described previously (65). Because the same-feeding procedure minimized the postingestive actions of the sucrose solution, the CS+ preference is attributed to flavor–flavor conditioning. Consistent with previous reports observed with dopamine antagonists (21,32,43–47,56,62), raclopride significantly reduced intake of the sucrose + saccharin solutions during the two-bottle tests.

The novel finding is that raclopride significantly and dose-dependently reduced the preference for the CS+ flavored solution without altering the intake of the CS− flavored solution in the two-bottle tests. Such a selective effect by raclopride upon conditioned flavor preferences could be observed using two distinct statistical approaches: comparison of the absolute intakes of the CS+ and CS− solutions, and alterations in the percent CS+ intake. Note that the failure of raclopride to reduce CS− intake was not due to a floor effect, because the animals consumed measurable amounts (4–10 ml) of CS− during the two-bottle tests. However, there is a possibility that the adipsic effects of raclopride might be greater upon higher levels of intake than lower levels of intake, independent of the conditioning effect. These data support the observation by Hsiao and Smith (26) indicating that raclopride reduced sucrose-conditioned preferences. In their experiment, rats were trained with flavored 10% sucrose solutions with raclopride paired with one flavor, and vehicle paired with the other flavor during one-bottle training. In the choice test, the raclopride-paired flavored sucrose solution was less preferred than the vehicle-paired solution.

The rats in this experiment were food-restricted to maximize sampling of the CS+ and CS− solutions during training. In Experiment 1B, they were retested under ad lib feeding conditions. Because brain dopamine levels, particularly in the nucleus accumbens, are increased during food deprivation (9,19,30,40,61), it is important to determine whether raclopride's inhibition of conditioned flavor preferences is observed under normal feeding conditions.

## EXPERIMENT 1B

### *Method*

At the end of Experiment 1A, the nine rats were given ad lib access to food and water for 2 weeks. They were then given four 30-min retraining sessions with unlimited access to the CS+/sucrose and the CS−/saccharin solutions; water bottles were also available during these sessions. Following retraining, the rats were given two-bottle preference tests with the CS+ and CS− flavors presented in the sucrose-saccharin mixture. They received four vehicle injections, and one injection each of the 50, 200, 400, and 800 nmol/kg doses of raclopride according to the regimen described previously.

### *Results*

*CS+ and CS− intake during training.* Significant differences in sham intakes were observed between the CS+ and CS− conditions, $F(1, 8) = 21.08$, $p < 0.002$, but were not observed between training days, $F(1, 8) = 0.16$, NS or for the interaction between days and conditions, $F(1, 8) = 0.46$, NS. Overall, the rats drank nearly three times more of the CS+ solution (26.8 ml) than the CS− solution (8.3 ml) during retraining; both intakes remained stable over the two retraining days (Fig. 2A).

*Raclopride and conditioned flavor preferences.* All doses of raclopride significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 32) = 14.59$, $p < 0.0001$. Statistically similar reductions in total intake were noted following all doses of raclopride (Fig. 2B). Overall, the rats consumed more of the CS+ (10.5 ml) than of the CS− (6.5 ml) solutions during these tests, $F(1, 8) = 9.50$, $p < 0.015$. However, there was a significant interaction between raclopride doses and CS solutions, $F(4, 32) = 15.94$, $p < 0.0001$. Raclopride failed to alter intake of the CS− solution, but significantly reduced intake of the CS+ solutions following all doses relative to vehicle treatment (Fig. 2C). Whereas CS+ intake was significantly higher than CS− intake following vehicle, CS+ and CS− intake failed to differ from each other following all doses of raclopride. Raclopride also significantly reduced the percent CS+ intake, $F(4, 32) = 10.21$, $p < 0.0001$. The percent CS+ intake following vehicle (75.8%) was significantly reduced by the 50 (55.1%), 200 (53.4%), 400 (57.1%), and 800 (48.6%) nmol/kg doses of raclopride (Fig. 2D).

### *Discussion*

As in Experiment 1A, raclopride reduced total intakes of the sucrose + saccharin solutions and the preference for the CS+ flavor in the two-bottle, sham-feeding tests. The drug effect appeared more potent under the ad lib feeding condition of this experiment than the food-restricted condition of the initial experiment. Thus, the two lowest doses completely blocked expression of the CS+ preference in the nondeprived rats, but only attenuated the preferences in the food-restricted rats. However, the order of testing was not counterbalanced, so that the influence of deprivation state on preference reduction by raclopride requires further study.

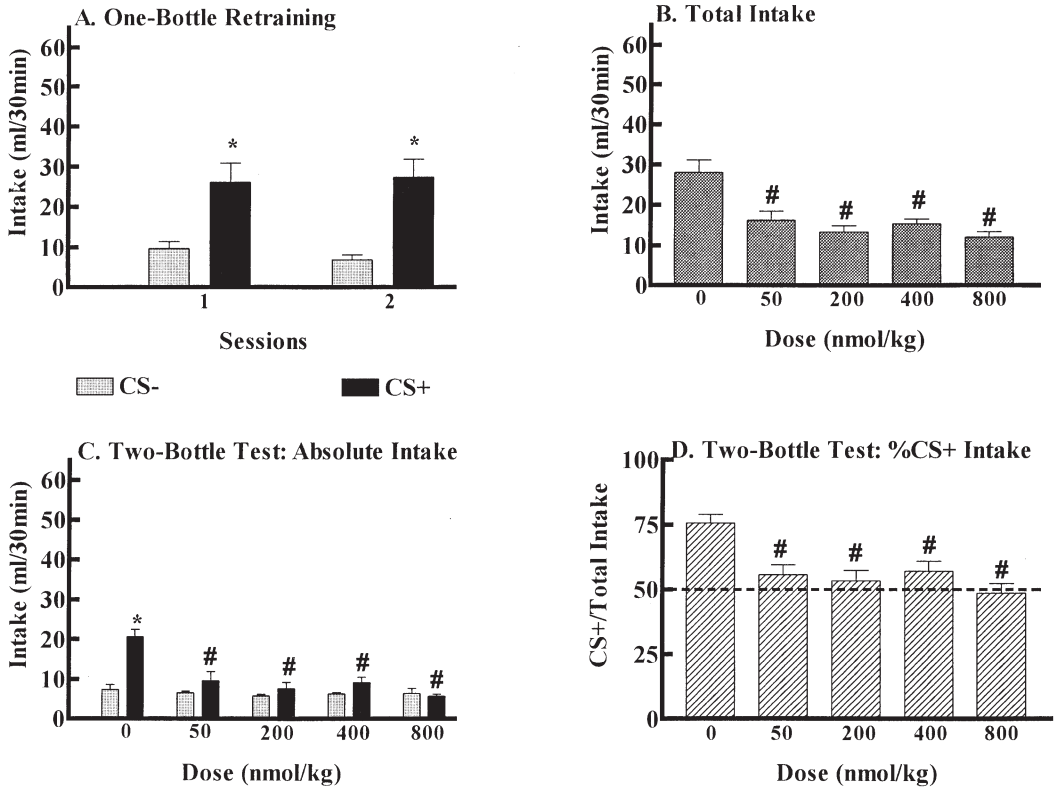


FIG. 2. Experiment 1B, Raclopride: Conditioned flavor preferences in ad lib-fed, sham-feeding rats. (A) Sham-feeding intakes (mean ± SEM) in one-bottle retraining tests (30 min) with the CS−/saccharin and CS+/sucrose solutions in ad lib-feeding rats. (B) Sham-feeding total intakes (mean ± SEM, 30 min) of a saccharin + sucrose solution in two-bottle tests with the CS+ and CS− flavors, respectively, following raclopride. (C) Sham-feeding intakes of the CS+ flavored and CS− flavored solutions following raclopride relative to vehicle treatment. (D) Percentage CS+ intake following raclopride relative to vehicle treatment.

## EXPERIMENT 2A

Both $D_2$ and $D_1$ receptors have been implicated in mediating the effects of reward upon behavior with antagonists directed at either receptor subtype decreasing the ability of rewarding stimuli to control responding [see reviews: (4,6–8,34,60)]. Both $D_1$ (SCH23390) and $D_2$ (raclopride) antagonists decreased sham feeding of sucrose solutions by affecting maintenance of intake rather than latency to initiate sham feeding (43–47). Microstructural analysis of these antagonist effects revealed that both dopaminergic antagonists reduced the rate of licking in sham-feeding animals in a pattern similar to reducing the concentration of the sucrose (43,55). To assess whether dopaminergic effects upon the expression of conditioned flavor preferences also involve $D_1$ receptor mediation, the second experiment evaluated whether equimolar doses of the selective $D_1$ receptor antagonist, SCH23390 (13,27,54), would alter the expression of a flavor preference conditioned by the taste of sucrose in sham-feeding rats under food-restricted (Experiment 2A) and ad lib feeding (Experiment 2B) conditions.

### *Method*

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

*CS+/CS− training procedure.* The rats were given 10 one-bottle, sham-feeding training sessions (30 min/day) with the CS+/sucrose and CS− saccharin solutions as in Experiment 1.

Following training, the rats were given two-bottle preference tests with the CS+ and CS− presented in a sucrose + saccharin mixture as in Experiment 1. There were eight two-bottle sham-feeding test sessions (30 min/day) with unlimited access to the solutions. Rats were exposed to four vehicle tests (1 ml/kg, SC) and one test each following SCH23390 (Research Biochemicals Intl.) doses of 50, 200, 400, and 800 nmol/kg 30 min prior to the test sessions according to the regimen described in Experiment 1A.

### *Results*

*CS+ and CS− intake during training.* Significant differences in sham intakes were observed across training days, $F(4, 36) = 15.90$, $p < 0.0001$, between the CS+ and CS− conditions, $F(1, 9) = 39.65$, $p < 0.0001$, and for the interaction between days and conditions, $F(4, 36) = 10.06$, $p < 0.0001$. Overall, the rats drank nearly four and one-half times more of the CS+ solution (31.9 ml) than the CS− solution (6.9 ml) during training (Fig. 3A). Whereas intake of the CS− solution remained stable over the 5 days of training, intake of the CS+ solution significantly increased over the last 4 days of training (Fig. 3A).

*SCH23390 and conditioned flavor preferences.* All doses of SCH23390 significantly reduced total intakes during the two-

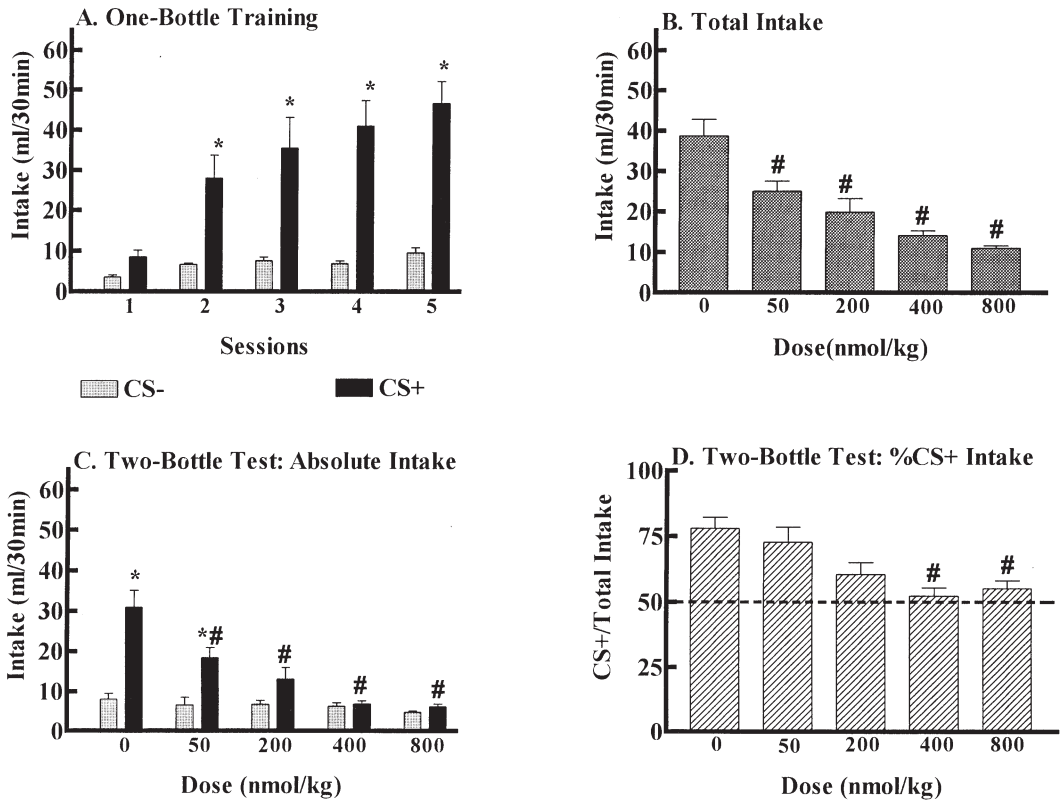


FIG. 3. Experiment 2A, SCH23390: Conditioned flavor preferences in food-restricted, sham-feeding rats. (A) Sham-feeding intakes (mean ± SEM) in one-bottle training tests (30 min) with the CS−/saccharin and CS+/sucrose solutions in food-restricted rats. (B) Sham-feeding total intakes (mean ± SEM, 30 min) of a saccharin + sucrose solution in two-bottle tests with the CS+ and CS− flavors, respectively, following SCH23390 at doses of 50, 200, 400, or 800 nmol/kg. (C) Sham-feeding intakes of the CS+ flavored and CS− flavored solutions following SCH23390 relative to vehicle treatment. (D) Percentage CS+ intake following SCH23390 relative to vehicle treatment.

bottle sham-feeding tests relative to the vehicle treatment, $F(4, 36) = 20.38$, $p < 0.0001$; the two higher (400 and 800 nmol/kg) SCH23390 doses produced significantly greater reductions than the two lower (50 and 200 nmol/kg) doses (Fig. 3B). Reductions in total intake were noted following all doses of SCH23390. Overall, the rats consumed more of the CS+ (15.1 ml) than of the CS− (6.5 ml) solutions during these tests, $F(1, 9) = 29.74$, $p < 0.0004$. However, there was a significant interaction between SCH23390 doses and CS solutions, $F(4, 36) = 10.06$, $p < 0.0001$. SCH23390 did not alter CS− intakes, but significantly reduced CS intakes following all doses relative to vehicle treatment (Fig. 3C). Whereas CS+ intakes were significantly higher than CS− intakes following the vehicle and 50 nmol/kg dose of SCH23390, CS+ and CS− intake did not differ from each other following the three higher doses of SCH23390. SCH23390 also significantly reduced the percent CS+ intake, $F(4, 36) = 7.88$, $p < 0.0001$. The percent CS+ intake following vehicle (78.0%) was significantly reduced by the two higher 400 (52.4%) and 800 (55.3%) nmol/kg doses of SCH23390 (Fig. 3D).

*Discussion*

The $D_1$ antagonist, SCH23390, significantly and dose dependently reduced intake of a sucrose + saccharin solutions in two-bottle sham-feeding tests to the same degree and with similar potency as the $D_2$ antagonist, raclopride. These data confirm previous studies demonstrating that both $D_1$ and $D_2$ antagonists reduce sucrose sham feeding (43–47,55). The novel finding is that SCH23390 also blocked the expression of a flavor preference conditioned by sucrose with a magnitude and potency that were similar to that observed following raclopride. Thus, both $D_1$ and $D_2$ receptors appear to be involved in the expression of flavor–flavor conditioned preferences. The second phase of this experiment examined the ability of SCH23390 to inhibit sucrose + saccharin intake and CS+ preference in rats given ad lib access to food and water.

EXPERIMENT 2B

*Method*

At the end of Experiment 2A, 9 of the 10 rats were given ad lib access to food and water for 2 weeks. They were then given four retraining sessions with the CS+/sucrose and the CS−/saccharin solutions; water bottles were also available during these sessions. Following retraining, the rats were given two-bottle preference tests with the CS+ and CS− flavors presented in the sucrose + saccharin mixture. They received four vehicle injections, and one injection each of the 50, 200, 400, and 800 nmol/kg doses of SCH23390 according to the regimen described previously.

*Results*

*CS+ and CS− intake during training.* Significant differences in sham intakes were observed between the CS+ and CS−

conditions, $F(1, 8) = 26.18$, $p < 0.0009$, but were not observed between training days, $F(1, 8) = 4.19$, NS or for the interaction between days and conditions, $F(1, 8) = 0.21$, NS. Overall, the rats drank nearly three and one-half times more of the CS+ solution (32.7 ml) than the CS− solution (9.4 ml) during training; both intakes remained stable over the two retraining days (Fig. 4A).

*SCH23390 and conditioned flavor preferences.* SCH23390 significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 32) = 13.67$, $p < 0.0001$. Statistically similar reductions in total intake were noted following the 200, 400, and 800 nmol/kg doses of SCH23390 (Fig. 4B). Overall, the rats consumed more of the CS+ (9.6 ml) than of the CS− (6.2 ml) solutions during these tests, $F(1, 8) = 12.36$, $p < 0.008$. However, there was a significant interaction between SCH23390 doses and CS solutions, $F(4, 32) = 3.90$, $p < 0.011$. SCH23390 did not alter CS− intakes, but dose dependently and significantly reduced CS+ intakes following the 200, 400, and 800 nmol/kg doses relative to vehicle treatment (Fig. 4C). Whereas CS+ intakes were significantly higher than CS− intakes following the vehicle and 50 nmol/kg dose of SCH23390, CS+ and CS− intakes did not differ from each other following the three higher doses of SCH23390. SCH23390 also significantly reduced the percent CS+ intake, $F(4, 32) = 4.63$, $p < 0.005$. The percent CS+ intake following vehicle (70.3%) was significantly reduced by the 200 (52.6%), 400 (48.5%), and 800 (49.2%) nmol/kg doses of SCH23390 (Fig. 4D).

*Discussion*

The experiment demonstrated that ad lib-fed rats, like food-restricted rats, displayed reductions in total sucrose + saccharin intake and CS+ preference following administration of the $D_1$ antagonist SCH23390. The results are very similar to those obtained with raclopride in Experiment 1B except that SCH23390 was somewhat less effective than raclopride in reducing CS+ preference at the 50 nmol/kg dose. Otherwise, the data from Experiments 1 and 2 indicate that both the $D_1$ antagonist, SCH23390, and the $D_2$ antagonist, raclopride, are potent in eliminating the expression of sucrose-conditioned flavor preferences in sham-feeding animals.

## EXPERIMENT 3

In the first two experiments, the rats were initially trained and tested to sham feed the flavored sucrose and saccharin solutions while food restricted. The third experiment determined if water-restricted rats would acquire a preference for a flavor paired with sucrose during sham-drinking training sessions, and if this CS+ preference would also be attenuated by raclopride and SCH23390. This would establish the generality of the flavor–flavor preference conditioning paradigm as well as the drug effects reported previously.

A second purpose for this experiment was to reduce the difference in the intakes of the CS+ and CS−-solutions during training. The food-restricted rats of Experiments 1 and

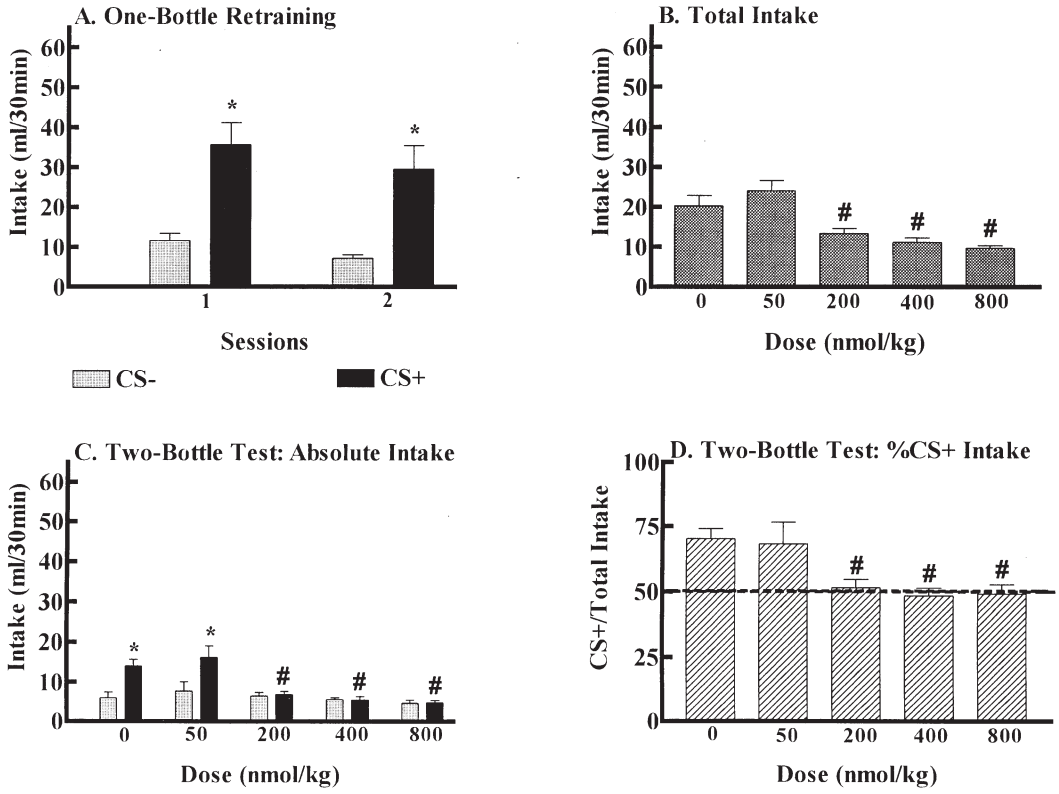


FIG. 4. Experiment 2B, SCH23390: Conditioned flavor preferences in ad lib-fed, sham-feeding rats. (A) Sham-feeding intakes (mean ± SEM) in one-bottle retraining tests (30 min) with the CS−/saccharin and CS+/sucrose solutions in ad lib-feeding rats. (B) Sham-feeding total intakes (mean ± SEM, 30 min) of a saccharin + sucrose solution in two-bottle tests with the CS+ and CS− flavors, respectively, following SCH23390. (C) Sham-feeding intakes of the CS+ flavored and CS− flavored solutions following SCH23390 relative to vehicle treatment. (D) Percentage CS+ intake following SCH23390 relative to vehicle treatment.

2 consumed over four times more CS+/sucrose than CS−/saccharin in the one-bottle training sessions. This is consistent with prior data showing that sugar solutions stimulate much more sham-feeding than do saccharin solutions in food-restricted rats (51). In contrast, water-restricted rats were observed to sham drink (now referred to as sham drinking rather than sham feeding) substantial amounts of a saccharin solution (51). Based on these findings, we expected that with water-restricted rats, the intakes of the CS+/sucrose and CS−/saccharin solutions would be similar during one-bottle training. However, this latter expected result proved not to be the case.

*Method*

*Subjects and initial training.* Twenty naive male rats were fitted with gastric cannulae as in Experiment 1. They were placed on a water-restriction schedule in which water was unavailable from 1600 h of one day to 1200 h of the next day. As in the prior experiments, these water-restricted rats were initially trained to drink a 8% maltodextrin solution with their gastric fistula open. Sham-drinking training continued until all rats approached the sipper tubes with short (<1 min) latency, typically within 5 days.

*One bottle training.* The rats were given 10 one-bottle, sham-drinking training sessions (30 min/day) with unlimited access to the CS training solutions at the end of the 20-h daily water restriction. As described previously, the CS− was presented an odd-numbered days, and the CS+ was presented on even-numbered days. Food was unavailable during all test times. On days 7–10, the rats received vehicle treatment (1 ml normal saline/kg body weight, SC) 30 min prior to the training session, during which they had access to two sipper tubes, one containing the CS− and CS+ solution, and the other containing water.

*Two bottle tests.* Following training, eight two-bottle preference tests were conducted with the flavored sucrose + saccharin solutions as in Experiment 1. Ten rats were exposed to four vehicle tests (1 ml/kg, SC) and one test each following SCH23390 doses of either 50, 200, 400, or 800 nmol/kg 30 min prior to the test sessions. The remaining 10 rats were similarly treated except that they were given equimolar doses of raclopride.

*Retraining and testing.* After 1 week, all rats were given four retraining sessions with the CS+/sucrose and the CS−/saccharin solutions. Water bottles were also available during these sessions. Following retraining, the rats were given two-bottle preference tests with the CS+ and CS− flavors presented in the sucrose + saccharin solutions. Rats initially treated with SCH23390 received four vehicle injections, and one injection each of the 50, 200, 400, and 800 nmol/kg doses of raclopride. Rats initially treated with raclopride received four vehicle injections, and one injection each of the 50, 200, 400, and 800 nmol/kg doses of SCH23390 according to the regimen described previously. Because there was no effect of order on drug testing, the data from these two tests were combined.

*Results*

*CS+ and CS− intake during training.* Because water intakes in the two-bottle training sessions (days 7–10) were substantial in this experiment, intake data from the one-bottle (days 1–6) and two-bottle (days 7–10) sessions were evaluated separately. Significant differences in sham intakes were observed between the CS+ and CS− conditions, $F(1, 18) = 78.70$, $p < 0.0001$, across the first three pairs of training days, $F(2, 36) = 23.52$, $p < 0.0001$, and for the interaction between days and conditions, $F(2, 36) = 17.95$, $p < 0.0001$. Overall, the rats drank nearly four times more of the CS+/sucrose solution (30.3 ml) than the CS−/saccharin solution (7.5 ml) during training. Intake of the CS+, but not the CS− solution significantly increased on the second and third training days (Fig. 5A). On days 7 and 9, the rats had access to the CS− solution and water, and on days 8 and 10 to the CS+ solution and water. Evaluation of these training data revealed significant differences in sham intakes between CS− and CS+ days, $F(1, 18) = 30.50$, $p < 0.0001$, between CS solution and water intakes, $F(1, 18) = 35.48$, $p < 0.0001$, and for the interaction between days and solutions, $F(1, 18) = 19.21$, $p < 0.0004$. Total intakes (CS and water) were significantly higher ($p < 0.001$) on the CS+ days (65.6 ml) than CS− days (49.0 ml) days. Furthermore, on CS+ training days, intakes of the CS+/sucrose solution and water were comparable (32.7 vs. 33.0 ml), whereas on CS− training days, the rats consumed substantially more water than the CS−/saccharin solution (42.3 vs. 6.8 ml) (Fig. 5B).

A similar intake pattern was observed during the two retraining days with significant differences in sham intakes between CS− and CS+ days, $F(1, 18) = 5.08$, $p < 0.039$, between CS solution and water intakes, $F(1, 18) = 24.55$, $p < 0.0001$, and for the interaction between days and solutions, $F(1, 18) = 19.63$, $p < 0.0004$. There was a small, but significant ($p < 0.036$) difference in total intakes on CS− (70.6 ml) and CS+ (67.1 ml) training days. However, on CS+ training days, the rats consumed comparable amounts of CS+/sucrose and water (34.1 vs. 33.0 ml), whereas on CS− training days, they consumed significantly more water than the CS−/saccharin solution (57.3 vs. 13.3 ml) (Fig. 5C).

*Raclopride and conditioned flavor preferences.* Raclopride significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 64) = 71.11$, $p < 0.0001$. Dose-dependent reductions in total intake were noted following the 200, 400, and 800 nmol/kg doses (Fig. 6A). Overall, the rats consumed more of the CS+ (24.9 ml) than of the CS− (12.5 ml) solutions during these tests, $F(1, 16) = 54.94$, $p < 0.0001$. There was a significant interaction between raclopride doses and CS solutions, $F(4, 64) = 5.59$, $p < 0.0006$. Whereas raclopride failed to alter intake of the CS− solutions relative to vehicle treatment, raclopride dose dependently and significantly reduced intake of the CS+ solutions following the 200, 400, and 800 nmol/kg doses relative to vehicle treatment (Fig. 6B). CS+ intakes were significantly higher than corresponding CS− intakes following the vehicle and 50 nmol/kg dose of raclopride, but CS+ and CS− intake did not differ from each other following the three higher doses of raclopride. Raclopride also significantly reduced the percent CS+ intake, $F(4, 64) = 2.83$, $p < 0.032$. The percent CS+ intake following vehicle (73.1%) as significantly reduced by the 800 (49.4%) nmol/kg dose of raclopride (Fig. 6C).

*SCH23390 and conditioned flavor preferences.* SCH23390 significantly reduced total intakes during the two-bottle sham-feeding tests relative to the vehicle treatment, $F(4, 72) = 55.37$, $p < 0.0001$. Reductions in total intake were noted following all doses of SCH23390, with the two higher doses significantly more effective than the two lower doses (Fig. 7A). Overall, the rats consumed more of the CS+ (20.3 ml) than of the CS− (11.9 ml) solutions during these tests, $F(1, 18) = 13.46$, $p < 0.002$. There was a significant interaction between SCH23390 doses and CS solutions, $F(4, 72) = 7.83$, $p < 0.0001$. SCH23390 significantly reduced intake of the CS− solutions following the 400 and 800 nmol/kg doses relative to ve-

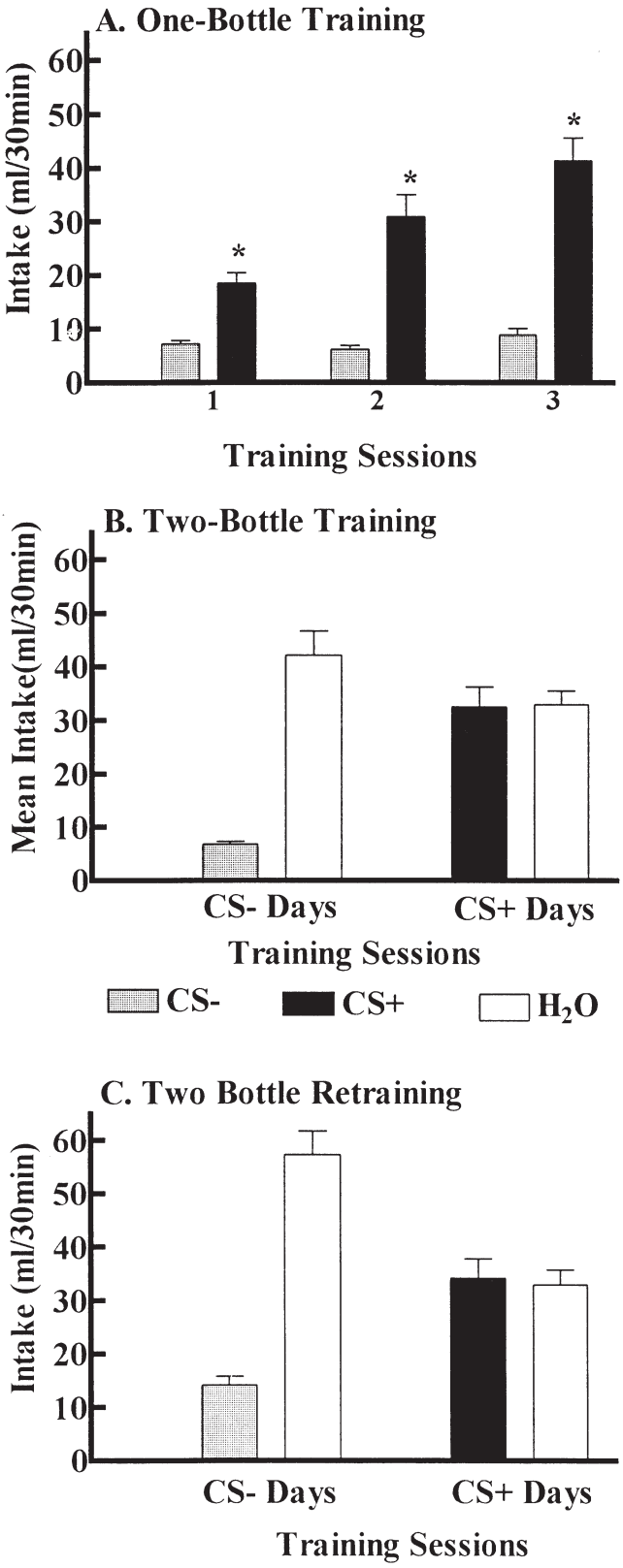


FIG. 5. Experiment 3: Training and retraining for conditioned flavor preferences in water-restricted, sham-drinking rats. (A) Sham-feeding intakes (mean ± SEM) in one-bottle training tests (30 min) with the CS−/saccharin and CS+/sucrose solutions in rats water-restricted for 20 h prior to testing, and allowed real-drinking access to water for 4 h after testing. (B) Sham-feeding intakes (mean ± SEM) in two-bottle-training tests (30 min) with either the CS−/saccharin and water as choices on the days 7 and 9 training sessions, or the CS+/sucrose and water as choices on the days 8 and 10 training sessions in water-restricted rats. (C) Sham-feeding intakes (mean ± SEM) in two-bottle-retraining tests (30 min) with either the CS−/saccharin and water as choices on the days 1 and 3 retraining sessions, or the CS+/sucrose and water as choices on the days 2 and 4 retraining sessions in water-restricted rats. Note that increased sham-drinking of water occurred when it was paired with the CS−/saccharin solution, and that equal amounts of sham-drinking intake occurred for the water and CS+/sucrose solutions.

hicle treatment (Fig. 7B). However, SCH23390 significantly reduced intake of the CS+ solutions following all doses relative to vehicle treatment (Fig. 7B). Whereas CS+ intakes was significantly higher than corresponding CS− intakes following the vehicle and 50 nmol/kg dose of SCH23390, CS+ and CS− intake did not differ from each other following the three higher doses of SCH23390. SCH23390 also significantly reduced the percent CS+ intake, $F(4, 72) = 5.20, p < 0.001$. The percent CS+ intake following vehicle (70.8%) was significantly reduced by the 200 (54.7%), 400 (56.9%), and 800 (47.6%) nmol/kg doses of SCH23390 (Fig. 7C).

*Discussion*

The $D_1$ antagonist, SCH23390, and the $D_2$ antagonist, raclopride, each significantly and dose dependently reduced total sucrose + saccharin intake and blocked the expression of a conditioned flavor preference in sham-drinking rats that were water restricted for 20 h prior to the test. The effects of the two antagonists were similar both in terms of potency and magnitude of effect. The respective abilities of SCH23390 and raclopride to block the expression of a flavor preference conditioned by sucrose in sham feeding (Experiments 1 and 2) and sham drinking (present experiment) argue convincingly that $D_1$ and $D_2$ receptors are involved in the mediation of the expression of the orosensory component of conditioned flavor preferences.

An unexpected finding was that the water-restricted rats drank relatively little CS−/saccharin solution in the one-bottle training sessions. In fact, their CS−/saccharin intake was not much higher than that of the food-restricted rats in Experiments 1 and 2. This conflicts with a prior report of substantial saccharin sham drinking in water-restricted rats (51). Furthermore, when water was also available during the training sessions, the water-restricted rats sham drank considerably more water than CS−/saccharin. Yet, prior work indicates that water-restricted rats prefer 0.1% saccharin to water during two-bottle, sham-drinking tests (16). The reduced sham-intake preference that the present rats showed for the CS−/saccharin solution, relative to water, may be related to the inclusion of the Kool-Aid flavors, and/or their exposure to the CS+/sucrose solution during training. That is, the availability of the more-preferred sucrose solution on alternate training days may have reduced the relative attractiveness of the CS−/saccharin solution (20). Further research is needed to resolve this issue.

Regardless of the reason why CS−/saccharin intake was low in the present study, one of the aims of the experiment was to reduce the differences in intake between CS+ and CS− solutions during training. Because this was not achieved,

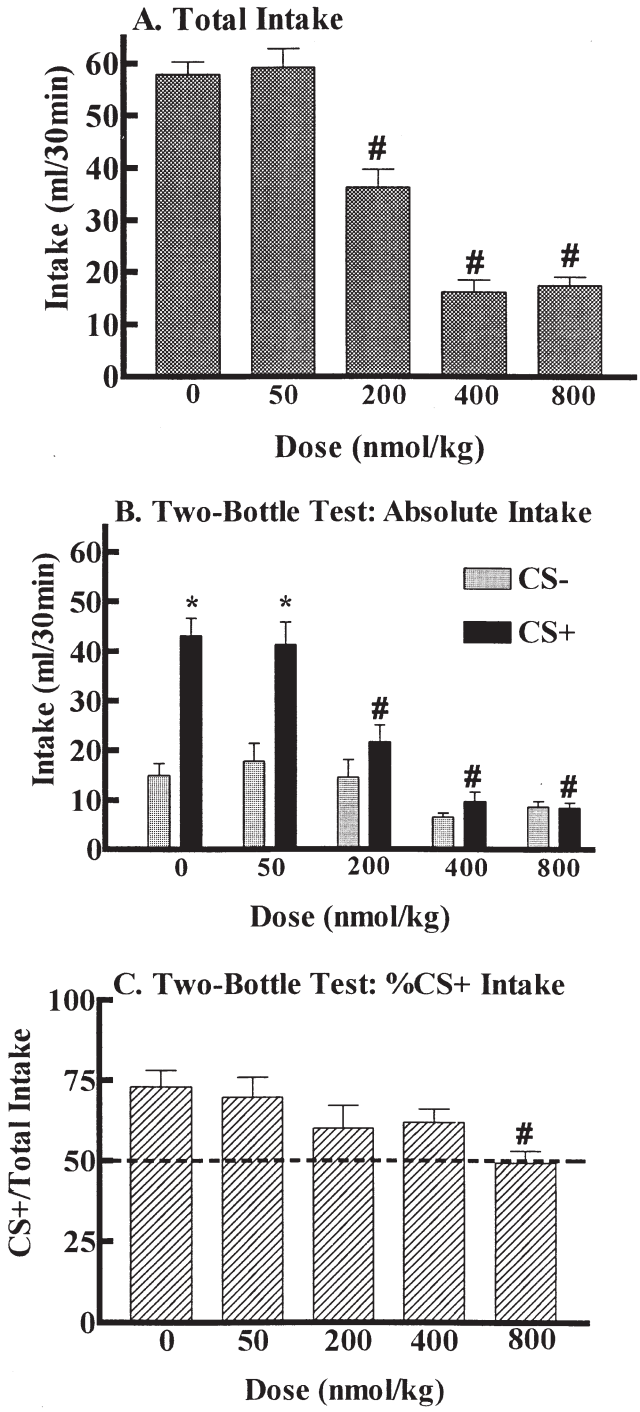


FIG. 6. Experiment 3, Raclopride: Conditioned flavor preferences in water-restricted, sham-drinking rats. (A) Sham-drinking total intakes (mean ± SEM, 30 min) of a saccharin + sucrose solution in two-bottle tests with the CS+ and CS− flavors, respectively, following raclopride at doses of 50, 200, 400, or 800 nmol/kg. (B) Sham-drinking intakes of the CS+ flavored and CS− flavored solutions following raclopride relative to vehicle treatment. (C) Percentage CS+ intake following raclopride relative to vehicle treatment.

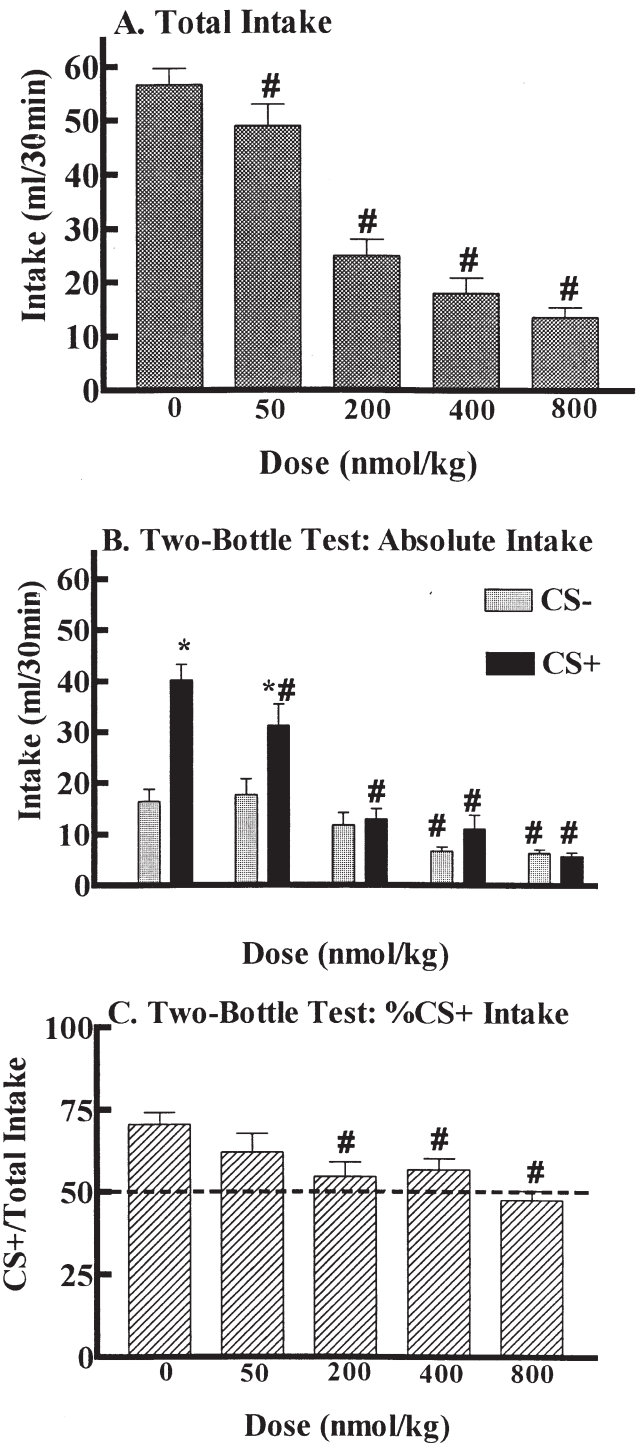


FIG. 7. Experiment 3, SCH23390: Conditioned flavor preferences in water-restricted, sham-drinking rats. (A) Sham-drinking total intakes (mean ± SEM, 30 min) of a saccharin + sucrose solution in two-bottle tests with the CS+ and CS− flavors, respectively, following SCH23390 at doses of 50, 200, 400, or 800 nmol/kg. (B) Sham-drinking intakes of the CS+ flavored and CS− flavored solutions following SCH23390 relative to vehicle treatment. (C) Percentage CS+ intake following SCH23390 relative to vehicle treatment.

it is not known what contribution, if any, differential intakes of the CS+ and CS− solutions during training had to the present sets of results.

## GENERAL DISCUSSION

The present study found that both $D_1$ (SCH23390) and $D_2$ (raclopride) receptor antagonists produced potent and dose-dependent reductions in sucrose–saccharin intake, and blocked the expression of a conditioned flavor preference under sham-feeding conditions in either food-restricted and ad lib-fed rats, and sham drinking in water-restricted rats. These data imply that these dopaminergic effects are acting through orosensory mechanisms because the sham-feeding procedure minimizes the postingestive consequences of sucrose consumption. These data agree with previous observations that $D_1$ and $D_2$ antagonists reduce sucrose intake in sham-fed animals (21,43–47,55), and that a $D_2$ antagonist reduces a sucrose-conditioned flavor presence in real-fed animals (26).

There appeared to be a great deal of similarity in terms of both magnitude and potency between the two dopamine receptor antagonists in reducing sucrose-conditioned flavor preferences. Both $D_1$ and $D_2$ receptor antagonists also reduce instrumental responding for food reward in different paradigms (5,17,41,42,59). The ability of the $D_1$ antagonist, SCH23390 to reduce sucrose-conditioned flavor preferences between doses of 200 and 800 nmol/kg (65–260 μg/kg) is quite comparable to the reductions by SCH23390 in operant food responding observed by the laboratories of Sanger [30–100 μ/kg: (42)], Beninger [50–100 μg/kg; (5)] and Salamone [50–150 μg/kg: (17)]. Although different $D_2$ antagonists were employed in our (raclopride) and other [metoclopramide: (5,42); sulpiride: (17)] studies, the dose ranges and patterns of effects were quite similar. The similarities in the potencies and magnitude of the raclopride and SCH23390 effects obtained in our study suggests that $D_1$ and $D_2$ receptors are functionally equivalent with regards to sucrose-conditioned flavor preferences. There are multiple forms of both types of receptors (22), and there is considerable overlap in the localization of both receptor subtypes in the brain as revealed by autoradiographic techniques [e.g., (11)]. Although one could potentially explain both effects by $D_1$–$D_2$ receptor interactions [e.g., (23)], this can only be determined using selective antagonists in discrete brain areas, and confirming these behavioral effects with biochemical measures.

In contrast, a previous study in our laboratory (65) found that although the general opiate antagonist, naltrexone significantly reduced the intake of sweet solutions, it had little or no effect on the acquisition or expression of flavor preferences conditioned by sucrose in sham-feeding rats. Naltrexone blocks μ and δ, and to a lesser degree, κ opioid receptor [see reviews: (36, 66)]. It is important to confirm naltrexone's failure to affect sucrose-conditioned flavor preferences using selective antagonists for each receptor subtype. This proviso notwithstanding, it appears that flavor–flavor conditioned preferences are mediated by dopamine, but not general opioid, receptor antagonists. This pattern differs from the observations that both neurotransmitters may be involved in modulating sucrose intake as well as place preferences conditioned by sucrose. For example, we (Delamater, Sclafani, and Bodnar, submitted) have observed that in food-restricted rats, naltrexone is effective in reducing the expression, but not the learning, of conditioned place preferences reinforced by sucrose. Moreover, other investigators (3) have reported that dopamine antagonists can interfere with the learning of sucrose-reinforced place preferences. Still other research has suggested that the dopamine and opioid systems may interact in place preference conditioning. The conditioned place preference induced by opioid agonists into the ventral tegmental area could be blocked by either dopamine receptor antagonism or dopamine depletion (38), and morphine-induced place preferences could be blocked by pretreatment with $D_1$, but not $D_2$ antagonists (1,29,52,53). Such effects have been interpreted in terms of interactions between endogenous opioids and dopamine in mediating reward processes [see review: (15)]. However, the absence of opioid effects (65) in the face of potent $D_1$-mediated and $D_2$-mediated effects upon the expression of conditioned flavor preferences suggest that sucrose-conditioned flavor and place preferences may have different underlying substrates.

Berridge (10) has proposed that the response to food (and other) rewards involve two functional components: "liking," defined by the hedonic or palatable characteristics of food, and "wanting," defined by the appetitive or incentive motivation to gain food. Berridge (10) proposed that the mediation of food "liking" involves opioid and GABA/benzodiazepine systems, while the mediation of food "wanting" involves mesotelencephalic dopamine systems. Dopamine has been intimately implicated in reward processes (2–4,6–9,15,19,30,34, 40,55,60,61). Antagonists directed at either dopamine receptor subtype decrease the ability of rewarding stimuli to control responding [see reviews: (4,6–8,34,61)]. However, whereas dopamine receptor antagonists decrease the incentive or reward value of food [e.g., (55,61–63)], neither dopamine receptor antagonists nor dopamine depletion shifts hedonic tastes towards aversion in the taste reactivity test (8,37,57). According to Berridge's model (10), the findings that dopaminergic, but not opioid antagonists, block the expression of flavor–flavor conditioned preferences would indicate that this type of conditioning involves changes in incentive salience ("wanting") of the cue flavor, but not in the hedonic ("liking") response to the flavor. Yet, other data suggest that at least some forms of flavor–flavor conditioning involve hedonic shifts (12). Clearly, further work is needed to explain the pharmacological and psychological basis of flavor conditioning.

One final point concerns the possibility in the present studies that dopamine antagonist effects upon expression of a flavor preference might be explained by noting the difference between the conditions of training and testing. If flavor preference learning in the sham-feeding paradigm is state dependent [e.g., (35)], for example, then dose-dependent reductions in flavor preferences might be expected during the test. Although the present studies cannot rule out this possibility, it is noteworthy that in our prior research (65) with the opioid antagonist, naltrexone, no reductions in flavor preferences were observed. This dissociation between the effects of naltrexone and dopamine receptor subtype antagonists on flavor preferences is not obviously accounted for by an appeal to state-dependent learning.

In conclusion, the present series of experiments clearly demonstrate that $D_1$ and $D_2$ receptors are involved in the mediation of the expression of the orosensory component of flavor–flavor conditioned preferences. The generalizability of these effects were clearly demonstrated in food-restricted and ad lib-fed sham-feeding animals, and in water-restricted sham-drinking animals.

## ACKNOWLEDGEMENTS

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