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Raclopride Reduces Sucrose Preference in Rats

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HSIAO, S., AND G. P. SMITH. *Raclopride reduces sucrose preference in rats.* PHARMACOL BIOCHEM BEHAV **50**(1) 121–125, 1995.—Dopaminergic D_1 and D_2 antagonists decrease the intake of sweet solutions during sham feeding. Because the decreased intake of 10% sucrose produced by the D_1 and D_2 antagonists has been demonstrated to occur in the absence of significant deficits in the initiation of ingestion, or of its motor performance, we investigated the hypothesis that raclopride decreases intake by lowering the reinforcing potency of the orosensory stimulation provided by sucrose during sham feeding. Rats were adapted to ingest two differently flavored 10% sucrose solutions for 5 min in one-bottle tests. The flavored solution that rats preferred was paired with pretreatment with a dose of raclopride (400 μg/kg, IP, 15 min) that produced a mean decrease of intake of 55%. The other flavored 10% sucrose solution was paired with vehicle (0.15 M NaCl) injections. After three or six pairings with raclopride or vehicle injection, two two-bottle preference tests were given without raclopride pretreatment. Preference for the flavored 10% sucrose solution previously paired with raclopride decreased significantly in both tests. We interpret this decreased preference as evidence that raclopride decreased the reinforcing potency of flavored 10% sucrose during one-bottle tests. This is consistent with our hypothesis and with the more general hypothesis of Wise that central dopaminergic mechanisms mediate the reinforcing effect of food.

Flavor-association Lick pattern Hedonics Food reward Conditioned preference

DOPAMINERGIC (DA) antagonists decrease the intake of sucrose and other sweet solutions (7,14,17). This inhibitory effect is not dependent on postingestive satiating processes because it is still observed when postingestive effects are minimized or eliminated by sham feeding (3,10–12). This inhibitory effect can also be obtained without evidence of motor impairments in the initiation or rate of licking sucrose and with slight (10) or no change in the efficiency of licking (4,17). A response summation function analysis also found no deficit in motor capacity (1).

The failure of motor deficits or postingestive satiating effects to account for this inhibition, and the demonstration that the inhibition of intake produced by a fixed dose of a DA antagonist depended on the concentration of sucrose being sham fed, led us to suggest that the antagonist decreased intake by lowering the perceived sensory intensity or the reinforcing potency of a sucrose solution (3,9,13). That the effect of a dopaminergic antagonist on the intake of a sweet solution was functionally equivalent to reducing the concentration of the solution (3,17) did not resolve this issue because sensory intensity and reinforcing potency are tightly correlated across a broad range of sucrose concentrations.

Recently Willner et al. (16) reported that a dose of pimozide previously demonstrated to decrease intake of a sucrose solution did not change perceived intensity of very dilute sucrose solutions when they were used as discriminative stimuli in a T-maze task. This is strong evidence against the possibility that a DA antagonist decreases the intake of a sucrose solution by decreasing its perceived sensory intensity.

In contrast to this evidence against a decrease in perceived intensity as the explanation for the decreased intake produced by DA antagonists, three experiments support the hypothesis that DA antagonists decrease the reinforcing potency of sucrose solutions. Bailey et al. (1) used a reward summation function analysis to show that pimozide displaced the function to the right without changing the maximum response rate. In the other two experiments, Muscat and Willner (7) and Towell et al. (15) tested the effect of D_1 and D_2 antagonists on the

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TABLE 1
MICROSTRUCTURE OF LICKING BEHAVIOR: BURST-RELATED MEASURES

Sucrose Concentration	Treatment	Intake (ml)	Burst Size (No. licks)	Interburst Interval (s)	No. Bursts
10% (s)	None	9.9 (0.7)	49.4 (7.9)	1.4 (0.2)	38.5 (6.4)
5% (s)	None	8.1 (0.8)*	33.4 (5.5)*	4.0 (0.8)*	37.3 (3.7)
10% (s)	Saline	4.3 (0.9)	51.2 (19.5)	0.8 (0.1)	22.9 (6.9)
10% (s)	Raclopride	4.2 (0.9)	13.2 (2.1)*	3.8 (0.9)*	43.5 (8.3)*
10% (d)	Sal-paired	6.0 (0.7)	34.2 (4.3)	5.8 (1.0)	65.7 (10.3)
10% (d)	Rac-paired	3.9 (0.4)*	30.8 (5.3)	10.4 (2.3)	45.0 (6.5)

Data are means (±SE), (s) One-bottle test; (d) two-bottle test.
*$p < 0.05$ vs. 10% sucrose or 10% sucrose, saline-treated in one-bottle tests or 10% sucrose, saline-paired in two-bottle tests.

preference for five concentrations of sucrose relative to water in two-bottle tests. The major result was that the antagonists increased the preference for high concentrations of sucrose (34%), but decreased the preference for low concentrations (0.7%). The authors interpreted the results as consistent with a reduction in reinforcing potency of concentrated solutions. But because the preference tests lasted 1 h, the change in preference could have been influenced by the postingestive, negative feedback effects of the specific sucrose solution and of water that occurred during the test, as well as by the orosensory, positive reinforcing effects. Furthermore, in these experiments by Bailey et al., Muscat and Willner, and Towell et al., preference and reward summation function tests were done while the rats were under the influence of the DA antagonists.

We investigated this issue with a protocol that was designed to test the interpretation of Towell et al. (15), and to extend their observations. First, we measured preference in two-bottle tests that lasted five min; this minimized postingestive effects on preference. Second, we performed the preference test in the absence of a DA antagonist. Thus, any change in preference could not be accounted for by some effect of the DA antagonist on performance in the choice test. Third, we monitored the rate and pattern of licking with electronic lickometers to investigate whether the change in preference was correlated with a change in the microstructure of licking. We report that under these conditions, three or six pairings of raclopride, a D_2 antagonist, with a flavored 10% sucrose solution in one-bottle tests decreased the preference for that flavored 10% sucrose solution in a two-bottle test, relative to a differently flavored 10% sucrose solution not previously paired with raclopride.

METHOD

Subjects

Twelve Sprague-Dawley male albino rats (Taconic Farms, Germantown, NY), initially weighing 250-310 g, were used throughout this study. They were housed individually in standard wire mesh, hanging cages with water ad lib, except as noted. Purina rat chow (Ralston-Purina, St Louis, MO) was provided from 1700-1900 h. Food was left over a weekend when testing was not conducted. Animals were kept in a room with temperature at 21 ± 1°C and a 12 h : 12 h light-dark cycle (lights on at 0700 h).

Apparatus

Liquid intake was measured with tubes of 0.1 ml graduation, and licking responses were recorded with an eight-channel lickometer (Dilog Instruments, Tallahassee, FL) to study the microstructure of ingestive behavior. Testing was done in lickometer cages identical to the home cages except that in the front of a test cage a plastic block was attached with two openings separated by 4 cm for insertion of drinking spouts. The spout tip was slightly recessed such that the rat

TABLE 2
MICROSTRUCTURE OF LICKING BEHAVIOR: RATE-RELATED MEASURES

Sucrose Concentration	Treatment	Fast Lick Rate (Licks/s)		Fast Licks (% Total Licks)	No. Licks/ml
		Mean	SD		
10% (s)	None	6.58 (0.14)	0.55 (0.03)	96.5 (0.8)	155.2 (6.9)
5% (s)	None	6.65 (0.14)	0.57 (0.03)	94.5 (1.3)*	142.2 (7.9)
10% (s)	Saline	6.56 (0.04)	0.59 (0.04)	94.6 (1.5)	162.1 (8.7)
10% (s)	Raclopride	6.59 (0.09)	0.74 (0.05)*	89.4 (1.5)*	192.7 (16.3)*
10% (d)	Sal-paired	6.63 (0.12)	0.56 (0.03)	94.2 (1.0)	150.4 (6.8)
10% (d)	Rac-paired	6.64 (0.12)	0.56 (0.02)	93.5 (0.9)	159.4 (9.4)

Data are means (± SE). (s) One-bottle test; (d) two-bottle test. Fast lick rate is the number of licks/s within a burst, or with an interlick interval ≤ 0.25 s. The mean ± SE of SD is a measure of the variability of this rate of licking.
*$p < 0.05$ vs. 10% sucrose or 10% sucrose, saline-treated in one-bottle tests.

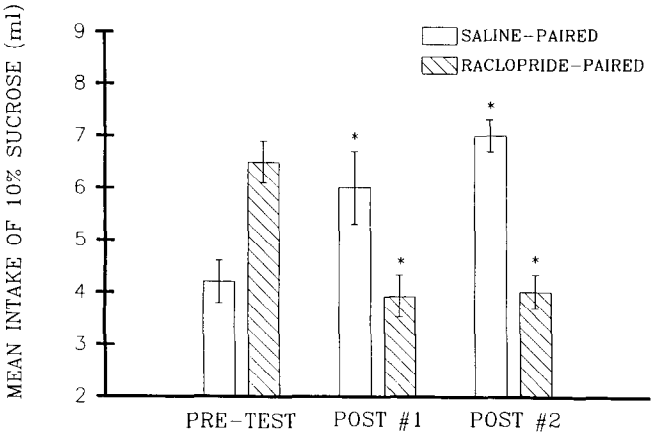

FIG. 1. Mean intakes (± SE) of the flavored 10% sucrose solutions before (PRE-TEST) or after (POST) having been paired with raclopride (400 μg/kg) or with saline injections in two-bottle tests. Post 1 is from two-bottle tests done on days 3 and 5 after two or three pairings with raclopride (Table 3). Post 2 is from two-bottle tests done on days 8 and 10 after two or three more pairings with raclopride (Table 3). *Significantly different from pretest intake, $p < 0.05$.

had to extend its tongue ~ 3 mm forward to reach the opening. This allowed distinctive licking responses to be measured. Guillotine doors blocked access of the spouts until the doors were lifted. A computer was programmed to record the timing between tongue contacts with the spout and analyze the lick patterns (see subsequent description).

Adaptation Procedure

The animals were water-deprived overnight and placed into the lickometer cages with two bottles of 10% sucrose solution for about 1 h in the morning (0900 h) and 1 h in the afternoon (1500 h). Time of testing did not affect intake in one- or two-bottle tests. When the animals drank at least 2 ml, water deprivation was discontinued. The rats were trained to lick the solution for 5 min. Training lasted until rats licked immediately from the spout placed at the right or left position and ingested at least 10 ml. This took about 10 days. To train the animals to lick from both spouts in two-bottle tests, whenever an animal licked from a spout for 20 s, the spout was withdrawn and then returned 10 s after the animal licked from the other spout for about 20 s. This was done during the first minute of training. After this training period, the rats were given one-bottle tests for 5 min with 5% and 10% sucrose. The measures of intake and licking were used in the microstructural analysis (Tables 1 and 2).

Differential Flavor Conditioning and Preference Testing

The rationale of our test protocol was to use two-bottle preference tests as a measure of the conditioned reinforcing potency of two 10%-sucrose solutions that had one of two flavors added. In a given rat, one flavor was paired with raclopride pretreatment and the other was paired with vehicle pretreatment. Because all preference tests were run without drug pretreatment, preference was a measure of the conditioned reinforcing potencies of the two flavors. Grape- and orange-flavored solutions were prepared from reagent grade sucrose, deionized water, and unsweetened Kool-Aid powder (General Foods, White Plains, NY) (0.05%). Two preference tests counterbalanced for positions were run before any drug treatment and were used as the baseline measure of preference between the flavored solutions.

Next, one flavored 10% sucrose (the flavor of at least equal or greater preference) (Figs. 1 and 2) was chosen to be paired with raclopride pretreatment (400 μg/kg, IP, at −15 min) in one-bottle intake tests. The other flavored 10% sucrose was paired with isovolumetric saline pretreatment in separate one-bottle tests on days 1–5 (Table 3). Because raclopride significantly decreased the intake of flavored 10% sucrose, we yoked the intake after saline pretreatment to the intake that occurred in the preceding test with raclopride to ensure that equivalent orosensory and postingestive effects of the flavored 10% sucrose solutions occurred after raclopride and saline pretreatment. To evaluate whether differential pairing of flavored 10% sucrose with raclopride or saline pretreatment produced a decrease in the reinforcing potency of the flavored 10% sucrose paired with raclopride, we performed two two-bottle preference tests in the absence of drug treatment on days 3 and 5 (Table 3). This was followed by further differential pairing of flavors with raclopride or saline in one-bottle tests on days 7 and 9, and another pair of two-bottle preference tests on days 8 and 10 (Table 3).

All tests lasted 5 min. Each pair of preference tests was counterbalanced for position of the spouts. If a rat did not lick from both spouts in the first 20 s of a preference test, it was forced to sample the second spout by the investigator who removed the first spout for 5–10 s. This was required in a minority of the tests. Two measures of preference were used: a) the intakes of the two solutions; and b) the preference ratio—that is, the intake of a solution as a percentage of the total fluid intake.

Microstructural Measures

A lick was a contact between the tongue and the spout, and the intervals between consecutive contacts were recorded. Rats show a pattern of lick intervals that is characterized by bursts of licks at a fast rate (5–8 licks/s) separated by pauses of varying duration. A burst is defined as a run of three or more

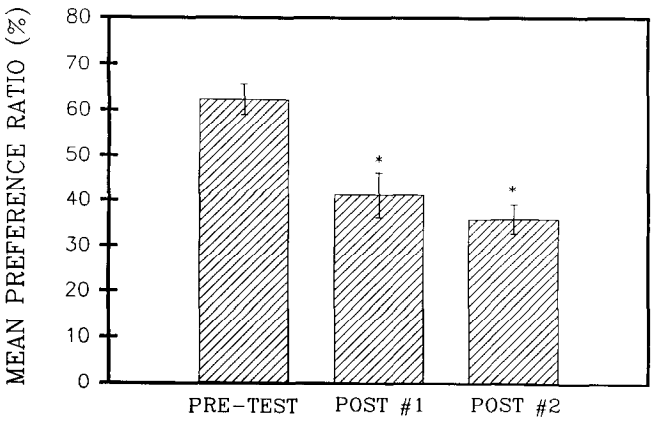

FIG. 2. Mean preference ratios (± SE) of the flavored 10% sucrose solutions paired with raclopride relative to differently flavored 10% sucrose solutions paired with saline injections measured in two-bottle tests done on days 3 and 5 (Post 1) and on days 8 and 10 (Post 2). Preference ratio is the intake of the solutions paired to raclopride treatment divided by the total intake expressed as a percentage. *Significantly different from pretest, $p < 0.05$.

TABLE 3
SEQUENCE OF TESTS

	0830 h		1500 h	
Day	Test Condition	Treatment	Test Condition	Treatment
1			one-bottle	Raclopride
2	one-bottle	Saline	one-bottle	Raclopride
3	one-bottle	Saline	two-bottle	None
4	one-bottle	Raclopride		
5	one-bottle	Saline	two-bottle	None
6			one-bottle	Raclopride
7	one-bottle	Saline	one-bottle	Raclopride
8			two-bottle	None
9	one-bottle	Saline	one-bottle	Raclopride
10	two-bottle	None		

The dose of raclopride was 400 μg/kg (IP).

consecutive interlick intervals ≤ 0.25 s (2); thus, burst size is the number of licks in a single burst. An interburst interval is the time between consecutive bursts. The mean burst size, mean interburst interval, and total number of bursts in a 5-min test were calculated for each rat in each test. The mean rate and standard deviation of the licks with interlick intervals of 0–0.25 s ("fast licks") were calculated for each rat per test, as well as the percentage of these licks of the total licks. The efficiency of licking was estimated by dividing the total number of licks by the milliliers ingested. The analysis of rate and pattern of licking was performed with the Quick-Lik Program of Davis and Smith (2).

Statistical Analysis

Wilcoxon's paired-comparison test was used to evaluate differences in measures of preference between preference tests done before and after differential conditioning.

To evaluate the effect of repeated raclopride pretreatment on one-bottle intakes, analysis of variance with repeated measures was used; α was set at $p = 0.05$.

RESULTS

Raclopride pretreatment that decreased the intake of a flavored 10% sucrose solution in one-bottle tests produced a significant decrease in the preference for that flavored 10% sucrose solution in two-bottle tests in the absence of drug treatment. The intake of the flavored 10% sucrose solution that had been paired with raclopride significantly decreased [$F(2, 22) = 7.97$; $p < 0.01$], but the intake of the flavored 10% sucrose solution that had been paired with saline significantly increased [$F(2, 22) = 5.70$; $p < 0.05$] (Fig. 1). Thus, the preference ratio of the flavored 10% sucrose paired with raclopride also significantly decreased [$F(2, 22) = 9.75$; $p < 0.01$] (Fig. 2). Note that the decreased preference was as large after two or three raclopride treatments (Post 1) as after five or six raclopride treatments (Post 2; Figs. 1 and 2).

In contrast to the development of the relative aversion for the flavored 10% sucrose paired with raclopride treatment in the preference tests, there was no evidence for the development of a conditioned taste aversion in the six one-bottle tests with raclopride pretreatment, because intake did not significantly change with repetitive testing (Fig. 3). Under our conditions, this dose of raclopride produced a mean decrease of intake of 55% ($p = 0.002$).

Although rats drank significantly less of the flavored 10% sucrose that had been paired with raclopride than of the flavored 10% sucrose that had been paired with saline (Fig. 1 and 2), microstructural analysis detected no significant differences in the rate or pattern of licking during the same preference tests (Tables 1 and 2). Because there is evidence that burst size is a function of sucrose concentration [10% vs. 5%; Table 1 and (2)], the lack of a difference in burst size during the two-bottle tests demonstrates that when rats licked the less preferred solution, the licking response was normal in the sense that they emitted a burst of licks appropriate for the concentration of sucrose.

The failure to find a significant difference in the pattern or rate of licking in the two-bottle tests when rats were not pretreated with drugs was not the result of insensitivity of the microstructural analysis because significant changes of pattern (Table 1), variance of rate of licking, percentage of fast licks, and efficiency of licking (Table 2) were detected in the one-bottle tests preceded by raclopride treatment.

DISCUSSION

In brief two-bottle preference tests, rats drank less flavored 10% sucrose solutions that had been paired with raclopride treatment in prior one-bottle tests than flavored 10% sucrose solutions that had been paired with saline treatment. This decrease in preference was not a direct effect of raclopride because raclopride was not administered on the day of the two-bottle tests.

This decreased preference was not accompanied by any significant change in the rate of licking (Table 2). Thus, the decreased preference was not due to a motor impairment of licking. When rats licked the flavored 10% sucrose that had been paired with raclopride in prior one-bottle tests, they had fewer licks in a burst, longer intervals between bursts, and a smaller number of bursts; but none of these changes was statistically significant (Table 2).

This decreased preference was not the result of a different amount of orosensory stimulation during one-bottle tests because we yoked the intake of flavored 10% sucrose preceded by a saline injection to the intake of the flavored 10% sucrose preceded by raclopride in the immediately previous test. How-

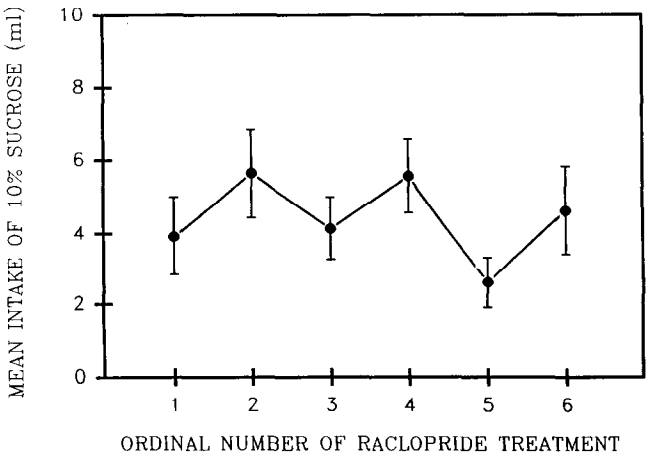

FIG. 3. Mean one-bottle intake (± SE) of flavored 10% sucrose solutions after an injection of raclopride (400 μg/kg, IP) 15 min before tests on days 1, 2, 4, 6, 7, and 9 (Table 1).

ever, given the significant differences in the microstructural analysis of the pattern of licking during one-bottle tests after raclopride or saline pretreatment (Table 1), it is possible that this differential pattern of orosensory stimulation produced differential reinforcement (see subsequent discussion).

The appearance of the decreased preference probably did not result from an aversive effect of raclopride in the one-bottle tests, because we observed no evidence of a conditioned taste aversion in the six one-bottle tests in which raclopride was administered (Fig. 3), and we did not observe a larger decrease in preference after five or six pairings of flavor and raclopride than after 2 or 3 pairings (Figs. 1 and 2). This is consistent with prior reports that haloperidol, another DA antagonist with potent binding to D_2 receptors, did not produce a conditioned taste aversion (8), that repeated administration of pimozide did not diminish licking responses (4) or operant responding (1) for sucrose solutions, and that pimozide did not produce aversive displays of mouth gaping, chin rubbing, or facial grooming in a taste reactivity test (6). That a raclopride-paired sucrose solution apparently did not become aversive can also be inferred from a recent result indicating that a rat's microstructure of licking would change if a sucrose solution were made aversive by adulterating it with quinine (5).

Having reviewed the reasons against other explanations, we suggest that the decreased preference for the flavored 10% sucrose solution occurred because raclopride decreased the positive reinforcing potency of the solution in the one-bottle tests. Thus, when tested in the two-bottle test in the absence of drug, the flavor previously paired with raclopride and now functioning as a CS for that drug-state was ingested less because a two-bottle preference test measures the relative reinforcing potency of the two solutions. This is the first demonstration of raclopride decreasing the reinforcing potency of a sweet solution in the absence of the drug and its potential effect on sensory processing or motor performance. This suggests that central D_2 mechanisms are necessary for the normal reward of ingesting 10% sucrose; but a contribution from raclopride's action at peripheral DA receptors cannot be excluded. Our results add to the mounting evidence (13) that is consistent with hypothesis of Wise and Rompre (17) that central dopaminergic mechanisms are involved in the mediation of food reward. Further work is required to identify the site(s) of D_2 receptors necessary for decreasing preference and to determine whether antagonists of other dopaminergic receptors also produce this effect.

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