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Raclopride Decreases Sucrose Intake of Rat Pups in Independent Ingestion Tests

A. TYRKA, C. GAYLE AND G. P. SMITH[1]

Department of Psychiatry, Cornell University Medical College, New York, NY 10021
Edward W. Bourne Behavioral Research Laboratory,
The New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605

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TYRKA, A., C. GAYLE AND G. P. SMITH. *Raclopride decreases sucrose intake of rat pups in independent ingestion tests.* PHARMACOL BIOCHEM BEHAV **43**(3) 863-869, 1992.—To investigate the role of dopaminergic activity at D_2 receptors in the mediation of the positive reinforcing effect of sucrose on ingestion in preweanling rats, we tested the effects of the D_2 antagonist, raclopride, on the intake of 10% sucrose of rats on postnatal days (PN) 7, 14, and 21. Intake was measured during independent ingestion tests in which pups licked sucrose from the floor of a beaker and during oral catheter tests in which sucrose was continuously infused through an anterior, sublingual, oral catheter. Rats were tested once to eliminate the possibility that repeated test experience would affect the response to raclopride. Pretreatment with raclopride resulted in decreased intake in independent ingestion (II) tests, but not in oral catheter (OC) tests on PN 7, 14, and 21. The inhibition of intake was not due to a generalized motor deficit because raclopride did not affect latency to eat, time-sampled activity scores, or latency to withdraw the hindlimb from a raised position. These results demonstrate that dopaminergic activity at D_2 receptors is necessary for the positive reinforcing effect of sucrose that maintains ingestion in the II test but not in the OC test.

Ontogeny of ingestion Sweet taste Ontogeny of dopamine function Dopamine antagonist
Positive reinforcement Food reward Dopamine antagonist Independent ingestion
D_2 receptor Intraoral catheter Incentive motivation

WE recently reported (7) that SCH 23390, a D_1 antagonist, was more potent for inhibiting intake in preweanling rats when pups were licking 10% sucrose from a tissue on the bottom of a beaker [independent ingestion test (II)] than when 10% sucrose was continuously infused into the mouth through an anterior, sublingual catheter [oral catheter test (OC)]. On postnatal days (PN) 7 and 14, SCH 23390 decreased intake in the II test but not in the OC test. On PN 21, SCH 23390 decreased intake in both tests but was more potent in the II test. The inhibitory effects of SCH 23390 on intake in the II test on PN 7 and 14 did not appear to be due to motor effects of SCH 23390 because doses of the drug that inhibited intake did not change the latency to initiate ingestion or the latency to hindlimb withdrawal. On PN 21, however, motor effects may have contributed to the inhibition of intake because all doses that inhibited intake also prolonged the latency to initiate eating and decreased total activity.

The present report extends the investigation of the role of central dopaminergic activity in the positive reinforcing effect of sucrose stimulation during development to an examination of the effects of D_2 receptor blockade in preweanling rats. We tested the effects of the selective D_2 antagonist, raclopride, on ingestion of 10% sucrose on PN 7, 14, and 21 in intraoral catheter and independent ingestion tests. A preliminary report has appeared (6).

METHOD

Subjects

Subjects were the offspring of timed-pregnancy, Sprague-Dawley rats that had been obtained from Taconic Farms. Pregnant females were housed individually in Plexiglas cages on corncob bedding with water and Purina 5012 Formulab chow available ad lib. Ambient temperature was maintained at 23 ± 2°C; the light phase occurred between 0600 and 1800 h. Litters were culled at 24-48 h after birth to a maximum of

[1] Requests for reprints should be addressed to G. P. Smith, M.D., Bourne Laboratory, New York Hospital-Cornell Medical Center, 21 Bloomingdale Road, White Plains, NY 10605.

10 pups. Animals were not handled again until the time of testing except during weekly maintenance. Animals were tested only once and no treatment condition used more than one male and one female from a single litter.

Independent Ingestion Test

This ingestion test was first described by Hall and Bryan (3). Pups were removed from their home cages in the morning, 4 h before the intake test, and placed individually in 1-l (PN 7 and 14) or 2-l (PN 21) Nalgene beakers into a 32°C incubator (Precision Scientific Group, Model 818, Chicago, IL). After approximately 3.5 h, pups were moved to the laboratory room; urination and defecation were induced by manual stroking of the anogenital region with a cotton swab. The urethral meatus was then occluded with cyanoacrylate glue (Krazy Glue, Inc., Columbus, OH). An intraperitoneal injection of raclopride or its vehicle (0.15 M sodium chloride) was administered 15 min before the start of the intake test. At the end of the 4-h deprivation, pups were weighed to 0.01 g (XT Top Loading Balance, Fisher Scientific Co., Fair Lawn, NJ) and placed into individual beakers in a humid, 38°C test chamber (a 15-gal glass aquarium with a Plexiglas top) for the 20-min intake test. Tissue paper (Kimwipes, Kimberly-Clark Corp., Roswell, GA) that had been soaked with 4 ml 10% (w/v) sucrose solution covered the floor of each beaker. The sucrose solution had been warmed to 38°C in the test chamber prior to the test.

Following the ingestion test, pups were dried and weighed again to 0.01 g. The difference in body weight from the beginning to the end of the test was the measure of intake and was expressed as percent body weight gain (%BWG).

Intraoral Catheter Test

Four hours before the start of the intake test, pups were lightly anesthetized with ether and anterior oral catheters (heat-flared PE-10) were implanted sublingually according to the procedure of Hall (2). Following catheter implantation, animals were placed individually in 1-l (PN 7 and 14) or 2-l (PN 21) Nalgene beakers into the 32°C incubator.

After approximately 3.5 h, animals were moved to room temperature, voided of urine and feces, and the anogenital region was sealed as described above. At the end of the 4-h deprivation period, pups were weighed and placed into individual beakers in the test incubator at 32°C. The catheters were connected to 5-ml infusion syringes and the 10% sucrose solution was infused continuously for 20 min via a Harvard Infusion Pump (Harvard Apparatus, Natick, MA, Model 975). When solution is infused in this manner, pups either swallow the liquid or allow it to flow out of the mouth. Rates of infusion were chosen on the basis of previous work to avoid ceiling effects and were 0.072 ml/min for PN 7 rats, 0.14 ml/min for PN 14 rats, and 0.20 ml/min for PN 21 rats. Following the ingestion test, pups were dried and weighed.

Drug Administration

An IP injection of raclopride or its vehicle (0.15 M sodium chloride) was administered 15 min before the start of an intake test. Injection volumes in both ingestion tests were: 0.10 ml for PN 7, 0.12 ml for PN 14, and 0.16 for PN 21 rats. These were administered with a 30-ga, $\frac{1}{2}$-in. needle. Doses of raclopride tested in II tests were: 194, 394, 781, 1,563, and 3,125 μg/kg on PN 7; 53, 106, 208, 417, 1,667, and 3,333 μg/kg on PN 14; and 45, 89, 179, 357, 1,429, and 2,857 μg/kg on PN 21. Doses tested in OC tests were 63, 313, and 3,125 μg/kg on PN 7; 106, 208, 417, 833, 1,667, and 3,333 μg/kg on PN 14; and 1,429 and 2,857 μg/kg on PN 21. There were 135 females and 138 males used in II tests, and 102 males and 100 females were used in OC tests. The body weight (means g ± SEM) was 16.5 ± 0.13 on PN 7, 35.4 ± 0.30 on PN 14, and 55.6 ± 0.56 on PN 21.

Measurement of Motor Effects

To enable examination of the specificity of an inhibitory effect of the drug on ingestion in II tests, separate groups of eight female and eight male pups on PN 14 and eight male and seven female pups on PN 21 were treated with the approximate D_{50} of raclopride for inhibiting intake in II tests on PN 14 and 21 or its vehicle, and several measures of movement were made during the II tests. The test procedures for the intake tests were exactly the same as described above and three measurements of movement were made.

First, the latency (seconds) for each pup to initiate mouthing of the tissue paper was recorded. If this did not occur within 300 s, the latency was considered 300 s for the purpose of data analysis.

Second, the behavior and activity of each animal were observed for a period of 5 s every 4 min. Degree of activity was scored according to the method of Robinson et al. (4). Total activity was the sum of the five 4-min activity scores during the test. Mouthing and grooming behavior were also noted; the activity associated with these behaviors was included in the activity score.

Third, pups were tested for evidence of motor impairment after the intake test: The right hindlimb was placed on a horizontal bar that was elevated above the test surface by 5 mm for PN 14 and 8 mm for PN 21 pups, and the number of seconds that elapsed before the rat removed the limb was recorded. The left hindlimb was then tested. This procedure was repeated with both hindlimbs. If a hindlimb was not removed from the horizontal bar within 120 s, the latency score was considered 120 s. The mean latency of the four tests was used for data analysis.

Statistical Analyses

Data from male and female rats were pooled because *t*-tests performed on the intake (%BWG) data revealed a significant sex effect only in the PN 14 pups pretreated with saline in II tests [$t(44) = 2.06$, $p < 0.05$, females ingested more than males]; there was not a sex difference in any other condition at PN 7, 14, or 21. The ingestion data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range and Tukey's honestly significant difference (HSD) tests. Cases where Duncan's multiple-range test yielded a significant difference, but the more conservative Tukey's HSD test did not, are indicated in the tables and figures by a "D." The latency, hindlimb withdrawal, total activity, and intake data for the second group of rats were analyzed by *t*-tests. The 4-min activity measures were analyzed by two-way ANOVA (drug × time, with time as a repeated factor) followed by Duncan's multiple-range and Tukey's HSD tests. All analyses were performed with the Statistical Analysis System [(SAS), Cary, NC].

RESULTS

Raclopride decreased intake of sucrose significantly in II tests at each of the three ages tested (Fig. 1). Inhibition of

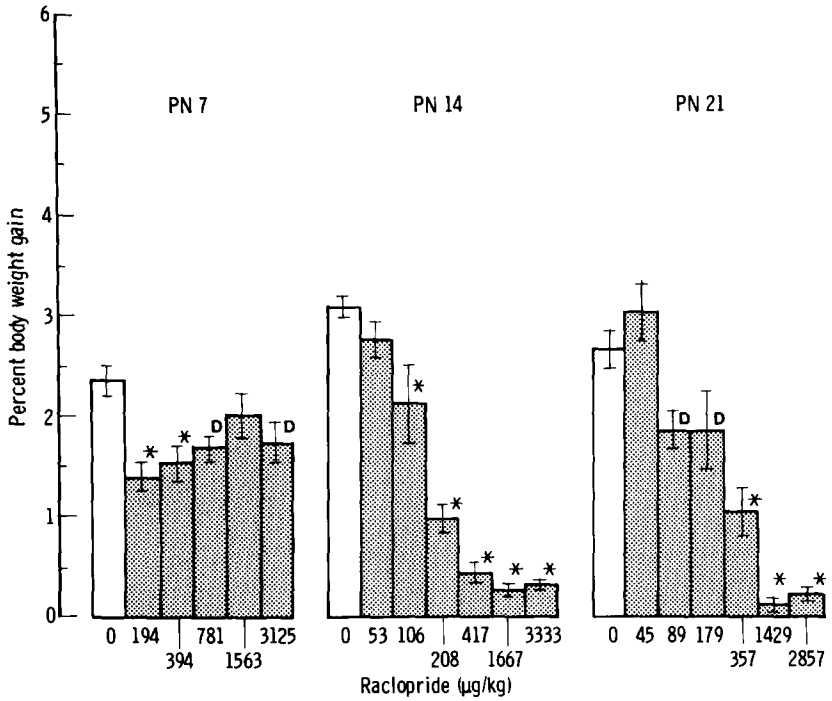

FIG. 1. Data are percent body weight gain (mean ± SE) in independent ingestion tests from 10–30 pups on postnatal day (PN) 7, 10–46 pups on PN 14, and 10–25 pups on PN 21. Raclopride decreased intake significantly at every age: Analysis of variance was significant on PN 7, $F(5, 74) = 4.56$, $p < 0.01$, PN 14, $F(7, 100) = 50.31$, $p = 0.0001$, and PN 21, $F(6, 78) = 22.91$, $p = 0.0001$. Doses of raclopride were significantly different from vehicle: $^{D}p < 0.05$, Duncan's multiple-range test; $^{*}p < 0.05$, Tukey's honestly significant difference (HSD) test. Compare with results in the intraoral catheter test (Fig. 2).

intake was a monotonic function of raclopride dose on PN 14 and 21; the minimum doses that decreased intake were 106 and 89 μg/kg, respectively. On PN 7, however, inhibition of intake was a flat function of raclopride dose (Fig. 1). In contrast to the results in II tests, no dose of raclopride decreased intake significantly in OC tests on PN 7 or 21, and only the largest dose (3,333 μg/kg) decreased intake significantly on PN 14 (Fig. 2).

Doses of raclopride that inhibited intake >50% had no significant effect on latency to initiate mouthing, total activity, or latency to hindlimb withdrawal in II tests on PN 14 or 21 (Table 1).

Consistent with the inhibition of intake, pretreatment with raclopride significantly reduced the percent of animals observed to be mouthing on PN 14, $F(1, 14) = 5.04$, $p < 0.05$, and PN 21, $F(1, 13) = 8.09$, $p < 0.05$. There was also a drug × time interaction on PN 14, $F(4, 56) = 2.56$, $p < 0.05$; while the percent of animals observed to be mouthing was an inverted-U function in both the antagonist and saline conditions, the peak of this curve was at 12 min for saline-treated rats and at 16 min for raclopride-treated rats. On PN 21, the percent of pups observed to be mouthing decreased with time, but this main effect of time was not statistically significant, $F(4, 52) = 2.35$, $p = 0.07$.

Because the activity score included the level of activity associated with mouthing, and because the percent of animals observed to be mouthing was reduced for antagonist-treated pups, we analyzed the activity scores of animals that were mouthing during the 5-s observation periods separately from the activity scores of those that were not mouthing during the 5-s period. Note that a single rat pup could be observed to be mouthing during one 5-s observation period and observed to not be mouthing during a subsequent 5-s observation period. PN 14 and 21 raclopride-treated pups were compared to saline-treated pups within each of these categories (mouthing and not mouthing).

There were no significant main effects of drug on activity on PN 14 or 21 when pups were either mouthing (Fig. 3) or not mouthing (Fig. 4). While raclopride-treated PN 14 pups that were not observed to be mouthing appeared to exhibit lower activity levels than their saline-treated counterparts (Fig. 4), this difference was not significant, $F(1, 10) = 4.20$, $p = 0.07$. Activity of PN 14 pups that were mouthing decreased during the test, but this main effect of time was of borderline significance, $F(4, 25) = 2.75$, $p = 0.0505$.

On PN 21, pups were significantly less active at 16 and 20 min when they were not observed to be mouthing, $F(4, 30) = 5.48$, $p < 0.01$ (Fig. 4). There was not a main effect of drug nor a drug × time interaction for this measure on PN 21.

DISCUSSION

Raclopride decreased intake of sucrose solution in II tests on PN 7, 14, and 21 but had no effect on intake in OC tests, except in pups pretreated with a huge dose of the antagonist on PN 14 (Figs. 1 and 2). The inhibition of intake in II tests was a monotonic function of raclopride dose on PN 14 and 21 but not on PN 7.

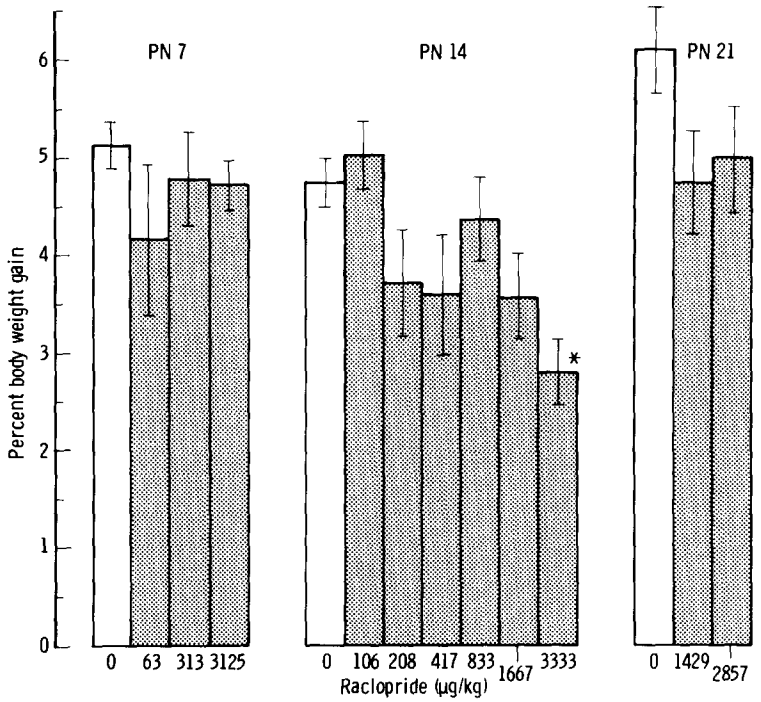

FIG. 2. Data are percent body weight gain (mean ± SE) in intraoral catheter tests from 6-28 pups on postnatal day (PN) 7, 4-43 pups on PN 14, and 10-12 pups on PN 21. Note that raclopride significantly decreased intake only at 14 days, $F(6, 90) = 3.73, p < 0.01$, and only a huge dose, 3,333 µg/kg, was effective: *$p < 0.05$, Tukey's honestly significant difference (HSD) test.

Because the procedures employed in the two tests were identical for such variables as distribution of subjects, deprivation procedures, drug dose and route of administration, gustatory stimulant (10% sucrose), and length of the test, these factors are not responsible for the differential effect. Ambient temperature of the test chamber was higher during II tests (38°C) than during OC tests (32°C), but this difference was not responsible for the different results because raclopride was also not efficacious in OC tests in which the ambient temperature was 38°C (unpublished observations).

Although pups had an oral catheter inserted under light ether anesthesia 4 h before the OC test but not before the II test, a follow-up experiment demonstrated that this difference was not responsible for the differential effect of raclopride in

TABLE 1

LACK OF AN EFFECT OF RACLOPRIDE ON MEASURES OF MOTOR PERFORMANCE IN INDEPENDENT INGESTION TESTS

	PN 14		PN 21	
Measure	Saline	Raclopride	Saline	Raclopride
Percent body weight gained	4.33 ± 0.36 (8)	1.43 ± 0.21* (8)	3.03 ± 0.45 (7)	1.42 ± 0.43* (8)
Latency to initiate ingestion (seconds)	124 ± 15 (8)	111 ± 18 (8)	119 ± 23 (7)	166 ± 32 (8)
Total activity score	8.1 ± 1.0 (8)	5.9 ± 1.4 (8)	7.1 ± 1.7 (7)	4.9 ± 0.5 (8)
Latency to hindlimb withdrawal (seconds)	1.0 ± 0.4 (8)	3.3 ± 1.2 (8)	0.4 ± 0.3 (7)	1.6 ± 1.1 (8)

Data are mean ± SE. The numbers in parentheses are the number of rats from which data were obtained. The activity score was obtained using the method of Robinson et al. (4).

*Raclopride decreased intake significantly on PN 14 (208 µg/kg, $t = 6.88, p = 0.0001$) and on PN 21 (357 µg/kg, $t = 2.58, p < 0.05$), but did not have a significant effect on these measures of motor performance at either age.

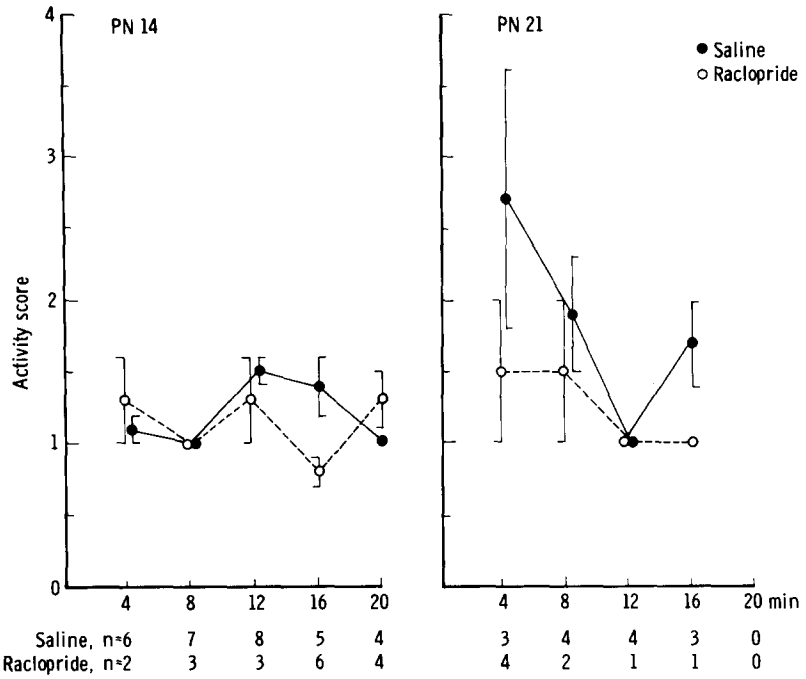

FIG. 3. Data are mean ± SE activity scores for postnatal day (PN) 14 and 21 pups that were observed to be mouthing during a 5-sec period at 4-min intervals throughout the independent ingestion test. (●), pups given an IP injection of vehicle; (○), pups pretreated with raclopride (208 μg/kg at 14 days, and 357 μg/kg at 21 days; these doses were approximate ID_{50}s). Numbers of pups that were mouthing are shown below each time period. Pretreatment with the antagonist did not affect activity scores on PN 14 or 21 when pups were mouthing during the observation periods. Activity decreased during the test on PN 14, but this main effect of time was of borderline significance, $F(4, 25) = 2.75$, $p = 0.0505$, and there was no drug × time interaction. On PN 21, there was not a main effect of time nor a drug × time interaction.

the two tests. The approximate D_{50} for decreasing intake in II tests (208 μg/kg) or saline was administered to PN 14 pups that had an oral catheter implanted under ether anesthesia 4 h prior to an II test. During the test, pups licked the sucrose from the tissue despite the presence of the oral catheter; raclopride significantly decreased intake under these conditions [%BWG after saline = 1.93 ± 0.32 (n = 6), after raclopride = 0.29 ± 0.08 (n = 7), $t = 5.01$, $p < 0.01$].

Because pups ingested significantly more sucrose during OC tests than during II tests (Figs. 1 and 2), it is possible that the greater orosensory, positive reinforcing stimulation produced by the constant infusion of sucrose in the OC tests was responsible for the lack of efficacy of raclopride. We tested this idea in recent, preliminary experiments by decreasing the rate of oral infusion so that the volume of sucrose ingested in the OC tests was not larger than the volume ingested in II tests. Under these conditions, however, raclopride still failed to inhibit intake in the OC tests (8).

The mode of delivery of sucrose was the major difference between the two ingestion tests and is probably the critical variable in the differential effect of raclopride. In OC tests, the sucrose solution is continuously infused into the mouth; the pup need only make oral consumatory movements involved in ingestion, or, conversely, the oral and postural movements necessary to allow the solution to spill out of the mouth when the pup does not ingest it (2).

Ingestion in the II test, on the other hand, requires a pup to probe, mouth, and lick the sucrose from the tissue paper on the floor of the test beaker to bring sucrose into contact with the oral receptors. Thus, while ingestion in the II test depends upon oral consumatory movements and oral and nonoral appetitive behaviors required to bring sucrose into the mouth, ingestion in the OC test requires only consumatory movements. Because raclopride did not decrease the consumatory-dependent, large intakes in the OC test, we conclude that dopaminergic action at D_2 receptors is not necessary for the normal oral consumatory movements of ingestion. This suggests that the inhibition of intake by raclopride in the II test is due to some effect on the appetitive movements required in that test. Thus, dopaminergic activity at D_2 receptors must be necessary for the normal performance of these behaviors in response to 10% sucrose.

We have not identified the specific change in the frequency or intensity of these movements produced by raclopride, but we have evidence that the effect of raclopride was not due to a generalized decrease in motor performance. Doses of raclopride that decreased intake significantly on PN 14 and 21 did not change the latency to initiate mouthing, activity scores, or latency to hindlimb withdrawal (Table 1). It is important to note that initiation of independent ingestion by raclopride-treated pups occurred with normal latency. Thus, raclopride decreased intake by interfering with the appetitive behaviors

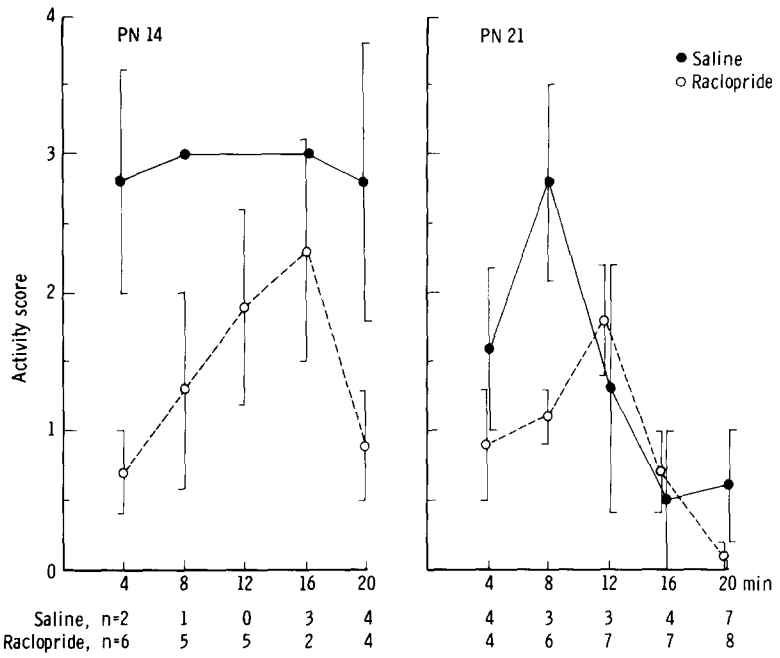

FIG. 4. Data are mean ± SE activity scores for pups not observed to be mouthing during a 5-sec period at 4-min intervals throughout the independent ingestion test. The doses of raclopride used and the format of the figure are identical to those of Fig. 3. On postnatal day (PN) 14, the decreased activity of antagonist-treated pups was not statistically significant, $F(1, 10) = 4.20, p = 0.07$. On PN 21, there was a main effect of time, $F(4, 30) = 5.48, p < 0.01$; activity was significantly decreased at 16 and 20 min. There was no drug × time interaction at either age.

necessary for the *maintenance* of ingestion but not with the appetitive behaviors necessary for the *initiation* of ingestion. This is evidence that raclopride-treated pups were capable of executing the movements necessary for independent ingestion at the beginning of the test; any generalized motor effects of raclopride should have been present by 15 min after drug administration.

The potency of raclopride for inhibiting intake of 10% sucrose in the II tests was equivalent to the potency of raclopride for inhibiting intake of 10% sucrose in sham-feeding tests in adult rats [D_{50} is approximately 200 μg/kg (5)]. The differential potency of raclopride in the two ingestion tests is similar to the differential potency of SCH 23390, a D_1 antagonist, in the same two tests (7). In contrast to the effects of raclopride, however, SCH 23390 decreased intake of rats on PN 21 in OC tests. That effect, however, may have been due to the motor effects of the antagonist because doses of SCH 23390 that inhibited intake also increased the latency to initiate ingestion and decreased activity. Until the contribution of these motor effects to the inhibition of intake by SCH 23390 can be clarified, the significance of the differential efficacy of SCH 23390 and of raclopride for inhibiting intake in OC tests on PN 21 will remain uncertain.

From the prior results with SCH 23390, we proposed the working hypothesis that the differential efficacy of SCH 23390 in the two ingestive tests was due to SCH 23390 decreasing the sensory control by 10% sucrose of the appetitive behaviors required to *maintain* ingestion of sucrose during II tests. The current results with raclopride are consistent with that hypothesis. The results with SCH 23390 and raclopride implicate dopaminergic D_1 and D_2 receptor mechanisms in the maintenance of the ingestion of sucrose in II tests as early as PN 7. Although D_1 and D_2 receptor mechanisms have been implicated in the maintenance of ingestion of sucrose (1,5) and other foods (9,10) in adult rats in previous studies, our results provide two new facts about the relationship between these receptor mechanisms and ingestion: First, the D_1 and D_2 mechanisms are necessary for the normal ingestive response as early as PN 7. Second, the D_1 and D_2 mechanisms are necessary for the normal ingestive response to the first exposure to sucrose. Taken together, these facts are evidence that dopaminergic action at D_1 and D_2 receptors (presumably in the brain) is necessary for the maintenance of the unconditioned ingestive response to sucrose in the rat. It remains to be determined whether this dopaminergic action facilitates the central processing of the sensory or positive reinforcing effects of sucrose. The facilitation of the postingestive, satiating effect of sucrose by SCH 23390 and raclopride is also possible, but we consider this less likely. Finally, the results suggest that the normal development of central dopaminergic mechanisms is necessary for the pup to engage in the independent ingestion that is an integral part of weaning.

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