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Potency of SCH 23390 for Decreasing Sucrose Intake in Rat Pups Depends on Mode of Ingestion

A. TYRKA AND G. P. SMITH[1]

Department of Psychiatry, Cornell University Medical College
and Edward W. Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center
21 Bloomingdale Road, White Plains, NY 10605

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TYRKA, A. AND G. P. SMITH. *Potency of SCH 23390 for decreasing sucrose intake in rat pups depends on mode of ingestion.* PHARMACOL BIOCHEM BEHAV **39**(4) 955–961, 1991.—To investigate the role of dopaminergic mediation of the positive reinforcing effect of sucrose during development, we tested the effects of the D-1 antagonist, SCH 23390, on the intake of 10% sucrose in 7-, 14-, and 21-day-old rats during independent ingestion (pups lick sucrose from the floor of a beaker) or during continuous intraoral infusion of sucrose. SCH 23390 inhibited intake more in independent ingestion tests than in intraoral catheter tests. At 7 and 14 days this difference was qualitative—SCH 23390 was efficacious in independent ingestion tests, but not in intraoral catheter tests. At 21 days the difference was quantitative—SCH 23390 decreased intake in both tests, but was more potent in independent ingestion tests. SCH 23390 decreased intake during independent ingestion tests without changing latency to eat or time-sampled activity scores at 7 and 14 days, but not at 21 days; thus the inhibition of intake is not accounted for by a generalized motor deficit at 7 and 14 days. Possible explanations for the differential potency include density of reinforcement, pattern of central dopamine metabolism, and interference with the appetitive movements required to maintain contact with the sucrose in the independent ingestion test.

Dopamine Ontogeny of ingestion Positive reinforcement D-1 receptor Sweet taste Food reward
Ontogeny of dopamine function Incentive motivation Dopamine antagonist Independent ingestion
Dopamine and motor function Intraoral catheter

AN extensive body of pharmacological research demonstrates that ascending brain dopamine (DA) systems play a critical role in the positive reinforcing effect of a variety of stimuli. For example, the dopaminergic antagonist, pimozide, attenuates lever pressing for the positive-reinforcing stimuli of intracranial electrical self-stimulation (ICSS) and of self-administration of psychotropic drugs by adult rats (19,20). Pimozide also decreases the intake of food and sweet solutions in doses that do not produce obvious motor disabilities (6, 22, 23). Wise has suggested that pimozide decreases intake by attenuating the positive reinforcing effect of food stimuli that maintains eating (19, 21, 22).

Our laboratory has been interested in the possibility that central dopaminergic activity mediates the positive reinforcing effect of the gustatory stimulation produced by sucrose that maintains sham feeding (17). Schneider and her colleagues have demonstrated that SCH 23390, a D-1 receptor antagonist, and raclopride, a D-2 receptor antagonist, decrease sucrose intake during sham feeding by adult rats in a dose-related manner (15). This decrease of intake is not due to effects of the antagonists on licking movements, but is due to an inhibitory effect on the central processing of the hedonic or sensory effect of oral sucrose (13,14).

To determine if dopaminergic mediation of the hedonic or sensory effect of oral sucrose stimulation in adult rats is innate or acquired, we investigated this problem in preweanling rats. Preweanling rats ingest more sucrose than water within the first postnatal week, and by the end of the second postnatal week, intake is a direct function of sucrose concentration (1, 8, 9, 18). We have reported preliminary results that are consistent with the possibility that the dopaminergic mediation of the positive reinforcing effect of sucrose is innate. First, ingestion of 10% sucrose in rats as young as 14 days increased dopaminergic metabolism significantly in the hypothalamus, olfactory tubercle, amygdala-piriform cortex and the caudate (3). Second, the D-2 antagonist, raclopride, decreased intake of 10% sucrose in rats as early as postnatal day 7 during independent ingestion tests in which pups lick sucrose from the floor of a beaker, but not during intraoral infusion of sucrose (16).

In the present study, we provide further evidence for innate dopaminergic mediation of the central hedonic or sensory effect of gustatory stimulation by sucrose, by demonstrating the inhibitory effect of a D-1 antagonist, SCH 23390, on the ingestion of 10% sucrose by 7-, 14-, and 21-day-old rats in independent ingestion and intraoral catheter tests.

METHOD

Subjects were the offspring of timed-pregnancy, Sprague-Dawley rats obtained from Taconic Farms (Germantown, NY). Pregnant females were housed individually in Plexiglas cages on

[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.

corn cob 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 hours. Litters were culled 24–48 hours after birth to a maximum of 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 included more than one male and one female from a single litter.

Independent Ingestion Test

This ingestion test was first described by Hall and Bryan (7). Pups were removed from their home cages in the morning, four hours before the intake test, and placed individually in 1-liter (7-day and 14-day pups) or 2-liter (21-day pups) Nalgene beakers into a 32°C incubator (Precision Scientific Group, Model 818, Chicago, IL). After approximately 3.5 hours, 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.). An IP injection of SCH 23390 or its vehicle (0.15 M sodium chloride) was administered 15 minutes before the start of the intake test. At the end of the 4-hour deprivation, pups were weighed to 0.01g (XT Top Loading Balance, Fisher Scientific Co.) and placed into individual beakers in a humid, 38°C test chamber (a 15-gallon glass aquarium with a Plexiglas top) for the 20-minute intake test. Tissue paper (Kimwipes, Kimberly-Clark Corp.; Roswell, GA) that had been soaked with 4 ml of 10% (w/v) sucrose solution covered the floor of each beaker. Note that the sucrose solution was warmed to 38°C in the test chamber prior to the test.

Latency (seconds) for each pup to initiate mouthing of the tissue paper was recorded. If this did not occur within 300 seconds, the latency was considered to be 300 seconds for the purpose of data analysis. In addition, the behavior and activity of each animal were observed for 5 seconds every 4 minutes. Degree of activity was scored according to the method of Robinson et al. (12). Mouthing and grooming behavior were also noted; the activity associated with these behaviors was included in the activity score.

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).

The latency, activity, and behavior measures were included, to enable examination of the specificity of a drug effect on ingestion. Pups were further tested for evidence of drug-induced motor impairment after the intake test. The right hindlimb was placed on a horizontal bar that was elevated above the test surface by 3 mm for 7-day pups, 5 mm for 14-day pups, and 8 mm for the 21-day pups, and the seconds that elapsed before the rat removed the limb were recorded. The left hindlimb was tested in the same manner, and then both hindlimbs were tested again in the same sequence. If a hindlimb was not removed from the horizontal bar within 120 seconds, the latency score was considered to be 120 seconds. The mean latency of the four tests was used for analysis of the data.

Intraoral Catheter Test

Four hours before the start of this 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 (8). Following catheter implantation, animals were placed individually in 1-liter (7-day and 14-day pups) or 2-liter (21-day pups) Nalgene beakers into a 32°C incubator.

After approximately 3.5 hours, the animals were moved to room temperature, voided of urine and feces, and the anogenital region was sealed as described above. An IP injection of SCH 23390 or its vehicle (0.15 M sodium chloride) was administerred fifteen minutes before the start of the intake test. At the end of the 4-hour deprivation period, the 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 minutes via a Harvard Infusion Pump (Model 975). When solution is infused in this manner, a pup either swallows the liquid or allows it to flow out of its mouth. Rates of infusion were chosen on the basis of previous work to avoid ceiling effects, and were 0.072 ml/min for the 7-day-old rats, 0.14 ml/min for the 14-day-old rats, and 0.20 ml/min for the 21-day-old rats.

Activity measures and behavioral observations were made for 5 seconds at 4-minute intervals as described above. Latency was not measured in this paradigm, because all pups began mouthing as soon as the sucrose infusion began. Following the ingestion test, pups were dried and weighed, and tested for evidence of a drug effect on hindlimb withdrawal as described above.

Injection volumes in both ingestion tests were 0.10 ml for 7-day-old pups, 0.12 ml for 14-day-old pups, and 0.16 ml for the 21-day-old pups. All injections were administered IP with a 30 gauge, ½ inch needle. Doses of SCH 23390 were: 30, 60, 120, and 240 μg/kg for the 7-day-old animals, 14.3, 28.6, 57.1, 114.3, and 228.6 μg/kg for 14-day-old pups, and 16.7, 33.3, 66.7, 133.3, and 266.7 μg/kg for the 21-day-old pups.

Body weights of the pups were 16.90 ± 0.13 g at 7 days, 35.70 ± 0.27 g at 14 days, and 56.71 ± 0.43 g at 21 days. The number of animals used in the independent ingestion test was 152 males and 138 females; 107 males and 104 females were tested in the intraoral catheter test. Note that not all behavioral measures were made on all rats (see tables for individual n's).

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 21-day animals pretreated with 133.3 μg/kg in the independent ingestion test (males ingested significantly less than females, $p<0.05$). The ingestion data, latency for mouthing and for hindlimb withdrawal measures, and total activity scores were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range and Tukey's HSD tests. Since Tukey's HSD test gives a more conservative estimate of significant differences in post hoc tests than Duncan's Multiple Range test, some significant differences were obtained with Duncan's test, but not Tukey's. When this occurred, we specified that difference with D. The 4-minute activity measures were analyzed by two-way analysis of variance (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

SCH 23390 decreased intake of sucrose significantly in independent ingestion tests at the three ages tested (Fig. 1). The minimum dose required for a significant inhibition of intake was 30 μg/kg at 7 days, 14 μg/kg at 14 days, and 67 μg/kg at 21 days. Inhibition of intake became a more linear function of dose with age. Although SCH 23390 also inhibited intake of sucrose

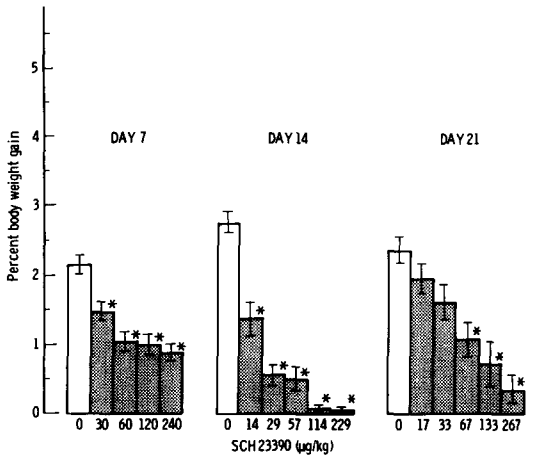

FIG. 1. Data are %BWG (mean ± SE) for 12–31 pups per dose in the independent ingestion test. ANOVA was significant for 7-day-old, $F(4,109) = 18.99$, $p = 0.0001$, 14-day-old, $F(5,96) = 36.75$, $p = 0.0001$, and 21-day-old, $F(5,68) = 9.00$, $p = 0.0001$, pups. Doses significantly different from vehicle, $^*p < 0.05$, Tukey's HSD test. SCH 23390 decreased intake at every age in a dose-related manner. Compare with results in the intraoral catheter test (Fig. 2).

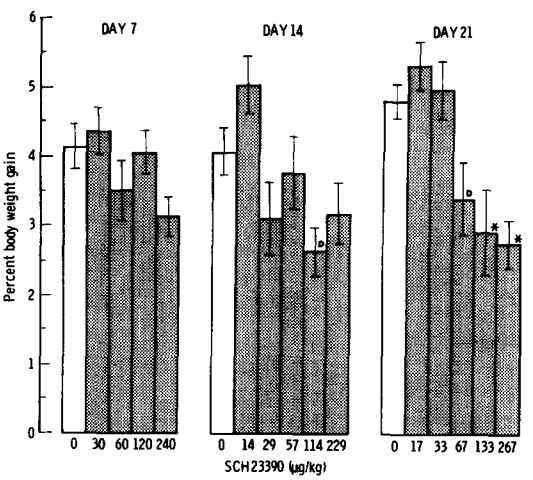

FIG. 2. Data are %BWG (mean ± SE) for 12–15 pups per dose in the intraoral catheter test. ANOVA was significant for 14 day-old, $F(5,67) = 3.90$, $p < 0.01$, and 21 day-old, $F(5,69) = 7.40$, $p = 0.0001$, pups. Doses significantly different from vehicle, $D = p < 0.05$, Duncan's Multiple Range Test; $^*p < 0.05$, Tukey's HSD test. Note that in 14-day-old pups, SCH 23390 significantly decreased intake with only one dose, 114 μg/kg; a larger dose had no significant effect. In 21-day-old pups, intake was decreased with doses ≥67 μg/kg.

significantly in intraoral catheter tests, it was much less potent (Fig. 2). Note that no dose of SCH 23390 was efficacious at 7 days; only 114 μg/kg produced a statistically significant difference at 14 days, and the biological significance of this effect is undermined by the fact that intake was not inhibited when the dose was doubled. At 21 days, however, the threshold dose for inhibition of intake in intraoral catheter tests was equivalent to that observed in independent ingestion tests (compare Figs. 1 and 2). But at this age, SCH 23390 was still more potent in independent ingestion tests than in intraoral catheter tests.

The effect of SCH 23390 on the latency to initiate mouthing in the independent ingestion test varied with age (Table 1). For 7-day pups, only 240 μg/kg of SCH 23390, the largest dose tested, resulted in a significantly longer latency than that of the saline-treated animals ($p < 0.05$). None of the doses tested increased latency significantly at 14 days. We have no explanation for the longer latency after vehicle injection at this age than at 7 or 21 days. In contrast to the results at earlier ages, doses ≥33 μg/kg produced significantly longer latencies in 21-day-old pups ($p < 0.0001$).

The latency for hindlimb withdrawal at each age in both ingestion tests is shown in Table 2. For the 7-day animals, latencies following treatment with SCH 23390 in the catheter test were not significantly different from those following saline except at the highest dose tested, 240 μg/kg ($p < 0.05$). No dose of SCH 23390 increased the latency significantly in the independent ingestion test. Similarly, in the 14-day-old pups, only the largest dose of SCH 23390 increased latency for hindlimb withdrawal in the intraoral catheter and independent ingestion tests ($p < 0.05$). In the 21-day-old pups, only the largest dose of SCH 23390 produced a significantly longer latency in the independent ingestion test ($p < 0.05$); no dose changed latency after intraoral catheter tests.

The total activity scores of 7-day animals were not affected by any dose of SCH 23390 in either intake test (Table 3). With 14-day-old pups, however, all doses of SCH 23390 decreased total activity in the intraoral catheter test ($p < 0.05$) and all doses >14 μg/kg decreased total activity in the independent ingestion test ($p < 0.05$). In 21-day-old pups, SCH 23390 also decreased total activity significantly with doses >33 μg/kg in the intraoral catheter test ($p < 0.05$) and >17 μg/kg in the independent ingestion test ($p < 0.05$).

TABLE 1

EFFECT OF SCH 23390 ON LATENCY TO INITIATE MOUTHING IN INDEPENDENT INGESTION TEST

Dose (μg/kg)	7-Day Pups
0	88 ± 15 (8)
30	91 ± 10 (8)
60	99 ± 17 (8)
120	136 ± 33 (7)
240	194 ± 30 (8)*
Dose (μg/kg)	**14-Day Pups**
0	163 ± 46 (5)
14	168 ± 29 (11)
29	
57	190 ± 50 (2)
114	259 ± 27 (8)
229	300 ± 0 (3)
Dose (μg/kg)	**21-Day Pups**
0	53 ± 9 (9)
17	64 ± 15 (4)
33	151 ± 31 (12)D
67	174 ± 28 (14)*
133	234 ± 28 (12)*
267	270 ± 24 (12)*

Data are seconds (mean ± SE) to initiate mouthing in the independent ingestion test; numbers of rats are in parentheses. ANOVA was significant at 7 days, $F(4,34) = 4.09$, $p < 0.01$, and at 21 days, $F(5,57) = 8.55$, $p = 0.0001$. Doses significantly different from value after vehicle, $D = p < 0.05$, Duncan's Multiple Range test; $^*p < 0.05$, Tukey's HSD test.

TABLE 2

EFFECT OF SCH 23390 ON LATENCY FOR HINDLIMB WITHDRAWAL

Dose (μg/kg)	Catheter	Independent Ingestion
7-Day Pups		
0	8.0 ± 4.1 (15)	9.8 ± 4.6 (8)
30	3.7 ± 1.2 (12)	17.1 ± 14.6 (6)
60	5.2 ± 1.6 (12)	20.8 ± 16.8 (2)
120	10.8 ± 3.4 (12)	16.8 ± 7.9 (7)
240	28.1 ± 8.3 (12)*	17.0 ± 4.7 (8)
14-Day Pups		
0	2.6 ± 0.7 (12)	1.6 ± 0.6 (5)
14	3.1 ± 1.0 (12)	2.8 ± 0.7 (11)
29	6.3 ± 3.0 (12)	
57	4.3 ± 1.2 (12)	2.1 ± 1.3 (2)
114	3.4 ± 0.8 (13)	3.9 ± 1.2 (7)
229	15.7 ± 5.4 (11)*	25.5 ± 10.0 (3)*
21-Day Pups		
0	0.6 ± 0.1 (15)	0.7 ± 0.1 (7)
17	0.8 ± 0.2 (12)	
33	0.5 ± 0.1 (12)	0.5 ± 0.1 (12)
67	0.8 ± 0.3 (11)	0.9 ± 0.2 (14)
133	0.6 ± 0.1 (12)	1.0 ± 0.2 (12)
267	3.7 ± 2.5 (12)	7.8 ± 3.7 (12)D

Data are seconds (mean ± SE) from 4 tests on each rat, numbers of rats are in parentheses. ANOVA was significant for 7-day-old rats in the intraoral catheter test, $F(4,58)=4.67$, $p<0.01$, for 14-day-old rats in the intraoral catheter, $F(5,66)=3.82$, $p<0.01$, and the independent ingestion tests, $F(4,23)=11.19$, $p=0.0001$, and for 21-day-old rats in the independent ingestion test, $F(4,52)=3.34$, $p<0.05$. Doses significantly different from value after vehicle, D = $p<0.05$, Duncan's Multiple Range test; *$p<0.05$, Tukey's HSD test.

TABLE 3

EFFECT OF SCH 23390 ON TOTAL ACTIVITY

Dose (μg/kg)	Catheter	Independent Ingestion
7-Day Pups		
0	3.7 ± 0.3 (14)	3.3 ± 0.8 (11)
30	3.4 ± 0.3 (12)	
60	3.9 ± 0.2 (12)	1.3 ± 0.4 (10)
120	4.2 ± 0.4 (10)	2.9 ± 0.3 (4)
240	3.5 ± 0.4 (12)	1.5 ± 0.7 (6)
14-Day Pups		
0	6.2 ± 1.2 (11)	6.8 ± 0.5 (12)
14	4.0 ± 0.5 (12)D	4.5 ± 1.2 (12)
29	2.4 ± 0.6 (12)*	2.1 ± 1.2 (6)*
57	2.9 ± 0.4 (12)*	1.8 ± 0.7 (8)*
114	2.8 ± 0.6 (13)*	0.8 ± 0.5 (8)*
229	3.3 ± 0.9 (12)D	0.2 ± 0.2 (3)*
21-Day Pups		
0	9.8 ± 1.0 (15)	7.4 ± 0.8 (12)
17	7.8 ± 1.0 (12)	5.3 ± 0.9 (12)
33	7.6 ± 1.0 (12)	4.0 ± 0.6 (12)*
67	4.9 ± 0.9 (12)*	4.1 ± 0.8 (14)*
133	2.8 ± 0.5 (12)*	2.6 ± 0.9 (12)*
267	3.5 ± 0.5 (12)*	1.8 ± 0.7 (12)*

Data are total activity scores (mean ± SE), numbers of rats are in parentheses. ANOVA was significant for independent ingestion data at 14 days, $F(5,43)=7.16$, $p=0.0001$, and 21 days $F(5,68)=6.36$, $p=0.0001$. For the intraoral catheter data, ANOVA was significant at 14 days, $F(5,66)=3.51$, $p<0.01$, and 21 days, $F(5,69)=10.12$, $p=0.0001$. Doses significantly different from vehicle, D = $p<0.05$, Duncan's Multiple Range test; *$p<0.05$, Tukey's HSD test.

SCH 23390 in doses approximately equal to ID_{50} (60 μg/kg at 7 days, 14 μg/kg at 14 days, and 67 μg/kg at 21 days) also significantly reduced the percent of animals that were observed to be mouthing at each observation interval in the independent ingestion tests. There was a main effect of drug at 7 days, $F(1,19)=5.79$, $p<0.05$, 14 days, $F(1,21)=6.28$, $p<0.05$, and 21 days, $F(1,24)=20.02$, $p<0.001$. There was also a main effect of time in the 7-day-old animals, $F(4,76)=5.64$, $p<0.001$, significantly fewer animals were observed to be mouthing at the end of the test than at the beginning of the intake test. There was no drug × time interaction.

In the intraoral catheter test, the same doses of SCH 23390 decreased mouthing at each observation interval at 21 days, $F(1,25)=4.44$, $p<0.05$, but not at 7 or 14 days.

Thus the effect of SCH 23390 on mouthing was correlated with the inhibition of intake in both tests.

Note that in contrast to the independent ingestion tests, there was also a main effect of time on percent mouthing in intraoral catheter tests at 7 days, $F(4,100)=5.41$, $p<0.001$, 14 days, $F(4,88)=7.13$, $p=0.001$, and 21 days, $F(4,100)=3.63$, $p<0.01$. There were, however, no drug × time interactions.

Since the total activity scores included the activity associated with mouthing and SCH 23390 decreasd the incidence of mouthing, we analyzed the activity scores of animals that were mouthing during the 5-second observation periods separately from the activity scores of those that were not mouthing during the 5-second period. Note that a single rat pup could be observed to be mouthing during one 5-second observation period and not observed to be mouthing during a subsequent 5-second observation period.

The activity scores of pups that were treated with the approximate ID_{50} doses of SCH 23390 and that were mouthing during observation periods in the independent ingestion tests were not different from the saline-treated pups that were mouthing at 7 or 14 days (Fig. 3). There was also no effect of time during the test. It was not possible to make these comparisons in the 21-day-old pups, because the number of pups mouthing in the antagonist-treated group at each interval was very small.

In nonmouthing pups, however, SCH 23390 decreased activity significantly at 7 and 21 days ($p<0.05$, Fig. 4). At 14 days, this effect was borderline ($p=0.055$).

There was also a main effect of time in the nonmouthing pups at 14 days ($p<0.05$); activity scores were smaller at 16 and 20 minutes than at 12 minutes ($p<0.05$, Duncan's Multiple Range test). At 21 days, there was also a main effect of time on activity scores ($p<0.01$). Activity scores were significantly smaller at 16 and 20 minutes than at 4, 8 or 12 minutes ($p<0.05$, Tukey's HSD test). Note that SCH 23390 did not change this pattern of activity in the nonmouthing pups—there was no drug × time interaction. Thus, when pups were observed to be mouthing in independent ingestion tests, SCH 23390 had no effect on activity scores. When pups were not observed to be mouthing, however, SCH 23390 decreased activity scores.

We also analyzed the activity scores of pups that were mouthing and not mouthing during the intraoral catheter test after the same three doses of SCH 23390. The activity scores of

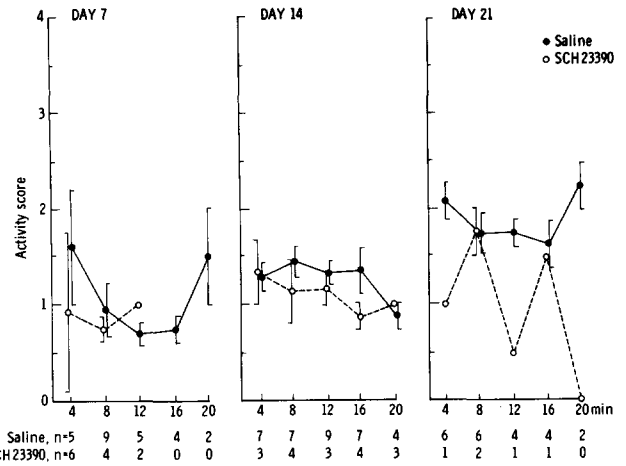

FIG. 3. Data are mean ± SE activity scores for pups that were observed to be mouthing during a 5-second period at 4-minute intervals throughout the independent ingestion test. Closed circles represent pups given an IP injection of vehicle; open circles represent pups pretreated with SCH 23390 (dose was 60 μg/kg at 7 days, 14 μg/kg at 14 days, and 67 μg/kg at 21 days; these doses were approximately ID_{50}'s). Numbers of pups that were mouthing are shown below each time period. Pretreatment with the antagonist did not affect activity scores of 7- or 14-day-old pups that were mouthing during the observation periods. It was not possible to make this comparison with the 21-day-old data, because the number of pups that were mouthing was too small.

the antagonist-treated pups that were mouthing during observation periods were not significantly different from the activity scores of the saline-treated pups at 7 or 14 days, but SCH 23390

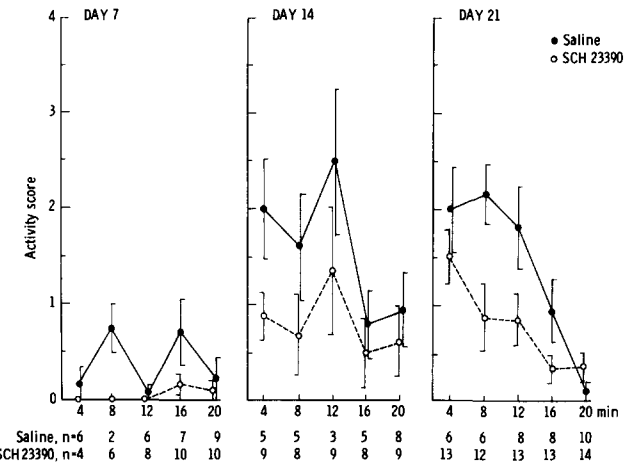

FIG. 4. Data are mean ± SE activity scores for pups not observed to be mouthing during a 5-second period at 4-minute intervals throughout the independent ingestion test. The doses of SCH 23390 used and the format of the figure are identical to those of Fig. 3. SCH 23390 significantly decreased activity when pups were not observed to be mouthing at 7 days, $F(1,19)=5.65$, $p<0.05$, and at 21 days, $F(1,24)=4.99$, $p<0.05$. At 14 days, this difference was not statistically significant, $F(1,21)=4.12$, $p=0.055$. There was a main effect of time at 14 days, $F(4,33)=2.90$, $p<0.05$, and at 21 days, $F(4,69)=10.78$, $p=0.0001$, but there was not a drug × time interaction.

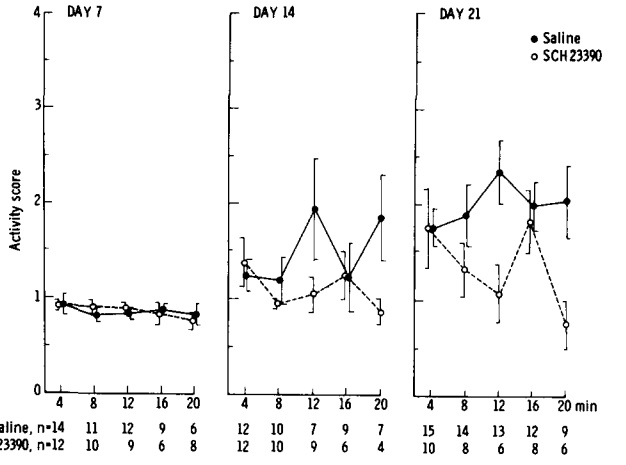

FIG. 5. Data are mean ± SE activity scores for pups that were observed to be mouthing during a 5-second period at 4-minute intervals throughout the intraoral catheter test. The doses of SCH 23390 used and the format of the figure are identical to that of Fig. 3. SCH 23390 did not affect activity of pups that were mouthing at 7 or 14 days. At 21 days, activity scores of pups pretreated with SCH 23390 were significantly lower than those of the vehicle-treated pups, $F(1,25)=4.36$, $p<0.05$.

significantly decreased the activity score of 21-day-old pups ($p<0.05$, Fig. 5).

When pups were not mouthing, however, SCH 23390 decreased activity scores significantly at 14 days ($p<0.05$) and at 21 days ($p<0.01$), but not at 7 days (Fig. 6). At 21 days, there was also a main effect of time ($p<0.01$) and a drug × time interaction ($p=0.0001$).

DISCUSSION

The major result of these experiments was that SCH 23390 was more potent for decreasing intake of 10% sucrose in inde-

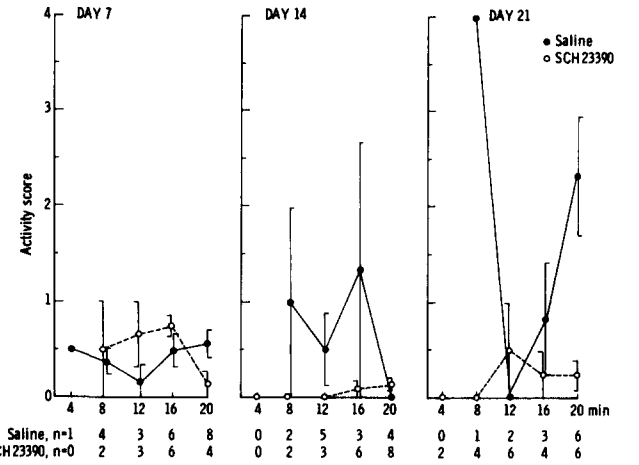

FIG. 6. Data are mean ± SE activity scores for pups not observed to be mouthing during a 5-second period at 4-minute intervals throughout the intraoral catheter test. The doses of SCH 23390 used and the format of the figure are identical to that of Fig. 3. SCH 23390 significantly decreased activity when pups were not observed to be mouthing at 14 days, $F(1,16)=4.78$, $p<0.05$, and at 21 days, $F(1,16)=13.57$, $p<0.01$, but not at 7 days. At 21 days, there was also a main effect of time, $F(4,9)=10.75$, $p<0.01$, and a drug × time interaction, $F(3,9)=25.55$, $p=0.0001$.

pendent ingestion tests than in intraoral catheter tests. The differential potency depended on age. At 7 and 14 days, the difference was qualitative: SCH 23390 decreased intake of 10% sucrose in a dose-dependent manner in independent ingestion tests, but had little or no effect in intraoral catheter tests (Figs. 1 and 2). At 21 days the difference was quantitative: SCH 23390 decreased intake in intraoral catheter tests, but the relationship of inhibition to dose was less orderly, and the magnitude of inhibition was smaller than in the independent ingestion tests (Figs. 1 and 2).

What accounts for this marked difference in the potency of SCH 23390? Since the route of administration and doses of SCH 23390, the gustatory stimulant (10% sucrose), the age and sex of the pups, and general test conditions were not different, the difference in potency seems to depend on the difference in the intraoral delivery of 10% sucrose in the two tests. In the intraoral catheter test, 10% sucrose was infused into the mouth continuously throughout the test. Thus, the contact of 10% sucrose with the sweet receptors in the mouth, the critical event that drives intake under these conditions, is not contingent on any nonoral movements of the pup. This is in marked contrast to the independent ingestion test, where contact of 10% sucrose with the sweet receptors in the mouth depends on movements of the pup, such as probing and licking, that are required to get sucrose from the tissues on the floor of the test beaker into the mouth. This ingestion test produces significantly less intake than the intraoral catheter test (Figs. 1 and 2).

Given that 10% sucrose is the reinforcing stimulus for ingestion in both tests, it is clear that reinforcement is more dense in the intraoral catheter tests than in the independent ingestion tests. Thus, in these experiments, the inhibitory potency of SCH 23390 on intake was inversely related to density of reinforcement. Nakajima (11) observed this same inverse relationship in adult rats bar pressing for food pellets—SCH 23390 was more potent on VI-30 s and VI-90 s schedules than on a CRF schedule. The potency difference Nakajima observed was quantitative, like the data in the 21-day-old rats (Figs. 1 and 2). Within the framework of the DA hypothesis of positive reinforcement of sucrose (13,17), such a quantitative difference could be the result of more dopamine being released as a consequence of more reinforcing stimulation and, because SCH 23390 is a competitive antagonist, there would be more competition by DA with SCH 23390 at D-1 sites. But this hypothetical explanation seems inadequate to account for the lack of efficacy of very large doses of SCH 23390 in 7- and 14-day-old pups in intraoral catheter tests (Fig. 2).

This lack of efficacy also cannot be explained by a lack of functional D-1 receptors at these ages (10,24), or by a failure of SCH 23390 to contact the central D-1 receptors, because SCH 23390 gained access to central D-1 receptors in sufficient amounts to produce dose-related inhibitions of intake in the independent ingestion tests in pups of the same age (Fig. 1).

The possibility that intraoral infusion of 10% sucrose does not stimulate the central dopamine system in 14-day-old pups can also be rejected, because we observed increased dopamine metabolism in the hypothalamus, olfactory tubercle, amygdala-piriform cortex, and caudate as a result of intraoral infusions of 10% sucrose for 20 minutes in 14-day-old pups (3). There are, however, regional differences in the metabolite (DOPAC or HVA) that is increased by intraoral infusion and independent ingestion.

Finally, the possibility that the brief etherization and insertion of the oral catheter 4 hours prior to the test, and the presence of the oral catheter during the test, were responsible for the different responses to SCH 23390, was tested by a follow-up experiment. SCH 23390 (60 μg/kg, IP) or saline was administered to 7-day-old pups that had an oral catheter implanted under ether anesthesia 4 hours before an II test. SCH 23390 was as efficacious in these pups [% BWG after saline = 2.01 ± 0.29 (n = 10), after SCH 23390 = 1.06 ± 0.27 (n = 9), $t = 2.41$, $p < 0.05$] as it had been in pups without oral catheters (Fig. 1).

Thus, a difference in density of reinforcement, or a difference in the pattern of regional DA metabolism, remain potential explanations for the lack of inhibitory potency of SCH 23390 in 7- and 14-day-old pups. Both possibilities require further investigation.

Another possible explanation is that the inhibition of intake in independent ingestion tests is due to impairment of the licking and probing movements necessary to get 10% sucrose into the mouth. The results with doses of SCH 23390 that inhibit intake provide four kinds of evidence against this explanation: first, SCH 23390 did not increase latency to initiate mouthing of 10% sucrose except at the largest dose tested at 7 days (Table 1). Second, SCH 23390 did not prolong the latency to withdraw the hindlimbs from an elevated bar except at the largest dose tested at 14 days. Third, SCH 23390 had no effect on the time-sampled measure of total activity in 7-day-old pups. At 14 days, the dose of SCH 23390 that decreased intake by approximately 50% had no statistically significant effect on total activity, but larger doses did decrease total activity significantly (Table 3). [But note that decreased activity can be dissociated from decreased intake. For example, at 21 days, SCH 23390 decreased total activity at a dose (33 μg/kg) that did not decrease intake significantly (Table 3 and Fig. 1).] Fourth, when pups were mouthing during independent ingestion tests, there was no difference in activity scores between pups treated with SCH 23390 and pups treated with saline (Fig. 3).

Thus the results of these four measures provide converging evidence against the possibility that SCH 23390 decreased intake by producing a generalized inhibition of motor activity. Furthermore, that latency to initiate mouthing was normal after doses of SCH 23390 that decreased intake by approximately 50% at 7 and 14 days (Fig. 1), is important evidence that the inhibitory effect of SCH 23390 on intake begins *after* pups make initial contact with 10% sucrose in the independent ingestion tests.

Such a defect in the maintenance of ingesting 10% sucrose is consistent with the classical, operational description of neuroleptic action on behavior—neuroleptics interfere with the sensory control of behavior (4). Dopaminergic antagonists have also been described as interfering with the motivational impact of reinforcing stimuli (21), and as altering the incentive value of primary or secondary reinforcing stimuli (2). Although originally used to describe the action of mixed D-1 and D-2 antagonists, the effect of SCH 23390 in these experiments certainly fits these descriptions.

Since SCH 23390 did not inhibit the oral, consumatory movements required for the much larger intakes of 10% sucrose in the intraoral catheter tests, we assume that SCH 23390 did not change the ability to execute these consumatory movements in the independent ingestion tests. This suggests that SCH 23390 decreased the appetitive movements, such as licking and probing, required *after* the initial mouthing of 10% sucrose to obtain 10% sucrose for ingestion. One of our results is consistent with this suggestion: when 7- and 14-day-old pups were not observed to be mouthing, those treated with SCH 23390 were significantly less active than saline-treated pups (Fig. 4). Such decreased activity could indicate a decrease in sensory or hedonic (arousal) effects of sucrose, and this could correlate with decreased appetitive movements to obtain more 10% sucrose. We have, however, no direct evidence for this.

Thus, as a working hypothesis, we suggest that the potency

and efficacy of SCH 23390 in independent ingestion tests in 7- and 14-day-old pups is due to a decrease in the control by 10% sucrose of the appetitive movements required for the maintenance of ingestion. Further experiments will be required to test this hypothesis, and these will require a more detailed analysis of motor capacities, abilities, and sequences after the initial ingestion of 10% sucrose has occurred than we obtained in these experiments.

Although we focussed this discussion on the results from the ingestion tests in 7- and 14-day-old pups because the results from the two ingestion tests were qualitatively different, we wish to emphasize two changes in the actions of SCH 23390 that occur by 21 days: first, SCH 23390 is efficacious, although less potent, in the intraoral catheter tests. Second, no dose of SCH 23390 decreased intake without decreasing total activity, and prolonging the latency to initiate mouthing. The reason for the increased potency of SCH 23390 for these motor effects is not known, but it may be relevant that the cholinergic control of movement matures during the third postnatal week, and this is known to contribute to the motor effects of dopaminergic antagonists (5).

In summary, we measured the effects of SCH 23390 on ingestion of 10% sucrose and some aspects of movement in two types of ingestion tests in 7-, 14-, and 21-day-old pups. The major result was that SCH 23390 inhibited intake in 7- and 14-day-old pups in a dose-related manner in independent ingestion tests, but not in intraoral catheter tests. Although the results do not permit a clear conclusion and further experiments are necessary to evaluate the relative roles of reinforcement density, pattern of regional dopamine metabolism, and subtle motor effects, we suggest, as a working hypothesis, that the differential efficacy is due to SCH 23390 decreasing the sensory control by 10% sucrose of the appetitive movements required to *maintain* ingestion of sucrose during independent ingestion tests. This hypothesis explains the lack of efficacy of SCH 23390 in intraoral catheter tests at these ages by the fact that such appetitive movements are not required to maintain intake in those tests. Some additional explanation is required for the changes in the results observed at 21 days, an age at which SCH 23390 was efficacious, though less potent, in the intraoral catheter tests, and inhibition of intake was always correlated with inhibition of activity and an abnormally long latency to initiate mouthing.

In conclusion, we wish to note that ingestion of sucrose solutions by preweanling pups appears to provide a reliable and sensitive test system for the developmental analysis of the neural mechanisms of the innate, positive reinforcing effect of stimulation of sweet receptors in the mouth on ingestive behavior.

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