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Naloxone Decreases Intake of 10% Sucrose in Preweanling Rats

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PHILOPENA, J., D. GREENBERG AND G. P. SMITH. *Naloxone decreases intake of 10% sucrose in preweanling rats.* PHARMACOL BIOCHEM BEHAV **54**(2) 333-337, 1996.—To investigate the role of opioids in the mediation of sucrose intake in the preweanling rat pup, we measured the effect of naloxone on intake of pups licking 10% sucrose from the floor of a beaker (independent ingestion test) and of pups ingesting 10% sucrose that was continuously infused through an anterior, sublingual oral catheter (oral catheter test). Pups were tested only once to eliminate the possible effect of test experience. Pups were tested in the second postnatal week (PN7, 9, 10, 11, and 14 days) with naloxone (1 mg/kg) or vehicle controls. Fourteen-day-old pups were also tested with 0.1 and 0.5 mg/kg. Naloxone began to be efficacious for inhibiting intake on PN10 in the oral catheter test and on PN11 in the independent ingestion test. On PN14, the inhibition of intake was dose related and naloxone was more potent for inhibiting intake in independent ingestion tests than in oral catheter tests. Naloxone not only decreased intake, it also decreased the incidence of licking, increased mouthing and resting, and had no significant effect on locomotion. The site of the inhibitory effect of naloxone on intake was in the central nervous system, presumably in the brain, because naloxonemethiodide, an analogue of naloxone that does not cross the blood-brain barrier, did not inhibit sucrose in either test. These results demonstrate that the intake of 10% sucrose depends on endogenous opioids as early as PN10 and that this opioid mechanism operates when pups have not had prior test experience and in a test (oral catheter test) where intake is not dependent on appetitive behaviors.

Naloxonemethiodide Ontogeny of the control of food intake Appetitive behavior Consummatory behavior
Opioid antagonists Orosensory control Eating

CENTRAL opioids are necessary for the integration of ingestive behaviors (11). A critical part of the evidence for this conclusion is that naloxone, an opioid antagonist that penetrates the blood-brain barrier, decreases food and water intake under a variety of experimental conditions while quartenary-substituted forms of opioid antagonists that do not pass the blood-brain barrier have no or little effect on ingestion (12).

In the case of eating, the effect of naloxone decreases the positive feedback effect of the orosensory stimuli of carbohydrates and other food stimuli that maintain eating (9,14). Because adult rats are adapted to experimental conditions, it is uncertain whether central opioids are necessary for the conditioned or unconditioned controls of eating. To avoid any effects of adaptation on ingestion, we measured the effect of naloxone on intake in the preweanling rat in an eating situation in which the rat pup has had no prior experience. Hall and his colleagues devised two tests for preweanling rats that obtain all of their nutrients by suckling their dam. Both of these tests require the isolated pup to ingest in the absence of the dam or siblings. Pups lick nutrients from the floor of the test chamber in one test (7), and they ingest nutrients infused through an oral catheter in the other test (5). The intake during the first experience in either of these tests is a measure of the unconditioned controls of intake that are functioning on that postnatal day. There is now considerable evidence that the controls of intake in these ingestion tests also operate in adult rats eating alone and that these controls appear sequentially during the preweanling period (6).

There are three relevant reports. Aroyewun and Barr (1,2) observed that naloxone decreased milk intake in both types of tests on postnatal day (PN) 14, but not at earlier ages. In

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contrast, Blass et al. (3) demonstrated that naltrexone, another opioid antagonist, decreased milk intake on PN10 in both types of tests; younger rats were not tested.

In this article, we report that naloxone decreased intake of 10% sucrose during the first experience in both of these tests in pups during the second postnatal week. This is evidence that central opioids are necessary for the unconditioned control of sucrose intake in rats. A preliminary report has appeared (13).

METHOD

Subjects were the offspring of male and female Sprague–Dawley rats (Taconic Farms, Germantown, NY) mated in our laboratory. Pregnant females were individually housed in Plexiglas cages on corncob bedding and were maintained on a 12 L : 12 D schedule with the light phase occurring between 0700 and 1900 h. Purina 5012 Formulab chow (Ralston, NC) and water were available ad lib, and ambient room temperature was kept at 21 ± 3°C. Litters were culled to a maximum of 10 pups (5 male and 5 female if possible) 24–48 h after birth. Pups were not handled until the day of testing except for weekly maintenance. Pups were tested only once; each pup received only one injection (drug or vehicle) prior to one of the two ingestion tests. No more than one male and one female pup from a litter received the same treatment.

Independent Ingestion Test

This procedure was initially described by Hall (7). Pups on PN7, 9, 10, 11, and 14, were removed from the dam 4 h prior to the test and placed in individual 1-liter Nalgene beakers and kept in an incubator maintained at 32 ± 1°C for 3.5 h. Thus, each pup was deprived of food, water, and contact with the dam and siblings. The pups were then brought into the testing area where they were voided of urine and feces by lightly stroking the anogenital region with a cotton Curity sponge (The Kendall Co., Boston, MA) and the urethral meatus was sealed with cyanoacrylate glue (Krazy Glue, Inc., Columbus, OH) to prevent further excretion. An intraperitoneal injection of drug or vehicle (0.15 M sodium chloride) was administered 15 min before the test. Pups were weighed in a 1-liter Nalgene beaker to the nearest 0.01 g (XT Top Loading Balance, Fisher Scientific Co., Fair Lawn, NJ) immediately after the injection. They were then placed onto dry Kimwipes (Kimberly Clark Corp., Roswell, GA) in clean 1-liter Nalgene beakers in a humid test chamber (a 15-gallon glass aquarium with Plexiglas top) at 38 ± 1°C. At the start of the 20-min intake test, 4 ml of the 10% sucrose solution (w/v reagent grade, Fisher Chemicals, Fairlawn, NJ) prewarmed in the test chamber was squirted onto the Kimwipe next to the pup. The pups mouthed and licked at the soaked Kimwipe to ingest the solution.

At the end of the ingestion test, each pup was removed from the chamber, dried with a Kimwipe, and weighed again to the nearest 0.01 g. Intake was measured by the change in body weight and expressed as the percent body weight gained (% BWG).

Oral Catheter Test

Pups were removed from the mother 4 h before the presentation of the 10% sucrose test diet. Each pup was anesthetized with ether and an anterior oral catheter was implanted sublingually according to the procedure of Hall (5). Animals were then individually placed into 1-liter Nalgene beakers and kept in an incubator at 32 ± 1°C for 3.5 h. The pups were then brought to the testing area. Pups were voided of urine and feces, and the anogenital region was occluded as previously described. An IP injection of drug or vehicle (0.15 M sodium chloride) was given 15 min before the intake test. The pups were promptly weighed and placed into clean 1-liter Nalgene beakers in the humid test chamber kept at 38 ± 1°C. The oral catheters were connected to 5-ml infusion syringes by a piece of Silastic Brand tubing (i.d. = 0.025 in., Dow Corning Corp., Midland, MI) or with PE-50 tubing (Clay Adams Polyethylene Tubing, Thomas Scientific, Swedesboro, NJ). The 10% sucrose, prewarmed in the test chamber, was infused (Harvard Infusion Pumps model 975, Harvard Apparatus, Natick, MA) for 20 min. The infusion rate for PN7–14 pups was 0.072 ml/min for a total of 1.44 ml. The second set of PN14 pups tested for a dose–response effect of naloxone received the sucrose at a rate of 0.140 ml/min for a total of 2.80 ml. The rates used were based on previous work by Tyrka and Smith (16). During the intraoral infusion, the pup controls intake by ingesting the sucrose or allowing it to flow out of its mouth. All pups began mouthing as soon as the solution reached their mouths. At the end of the infusion, the pups were dried with tissue paper and weighed again to the nearest 0.01 g. Change in body weight was the measure of intake and was expressed as % BWG.

Behavioral Scoring

On PN14 a fourth group of pups were behaviorally scored in the Independent Ingestion test. Latencies to begin licking at the soaked tissue with 10% sucrose were recorded. Individual behavior was recorded for 5 s each minute for the duration of the 20-min test. The pups were scored as exhibiting one of the following eight behaviors: licking (the pup's tongue was visibly in contact with the soaked tissue), mouthing (the pup's head was raised above the beaker floor and the pup exhibited jaw movements), locomotion (the pup was moving on all four paws about the beaker), stretching (the pup raised itself up on its hind quarters against the beaker wall), immobile (the pup was motionless with its eyes open), head grooming (forepaws groomed at the head), body grooming (pup groomed any part of its body except its head), and resting (the pup was motionless and its eyes were closed). Although these behaviors were usually mutually exclusive, sometimes mouthing and another kind of movement occurred together. When this happened, both behaviors were scored.

Drug Administration

Drugs were administered IP 15 min prior to the presentation of the 10% sucrose test solution. The volumes injected through a 30 gauge, 1/2 inch needle were 0.10 ml per rat on PN7, 9, 10, and 11, and 0.12 ml per rat on PN14. In the first group in both ingestion tests, all pups were injected with naloxone (1 mg/kg) or with its vehicle (0.15 M sodium chloride). On PN14, the second group of pups in both ingestion tests was injected with naloxone (0.1, 0.5, or 1.0 mg/kg). The third group of pups on PN14, tested in both ingestion tests, was injected with naloxonemethiodide (1.0 mg/kg), or with vehicle (0.15 M sodium chloride). Prior to behavioral scoring, the fourth group of PN14 rats was injected with 1.0 mg/kg naloxone or with vehicle. Naloxone was purchased from the Sigma Chemical Co. (St. Louis, MO), and naloxonemethiodide from Research Biochemicals Inc. (RBI, Natick, MA).

Statistical Analyses

In each age group the data from male and female rats were pooled because a two-way ANOVA (sex × drug) failed to

find a significant difference in intake between the sexes or a significant interaction between sex and drug. The ingestion data on different PN days were analyzed by comparing the mean intakes after vehicle and naloxone or naloxonemethiodide treatment for each PN day by a one-way ANOVA followed by Tukey's honestly significant difference (HSD) test. The three doses of naloxone administered on PN14 were analyzed by a one-way ANOVA followed by Tukey's honestly significant difference (HSD) tests for differences in mean intakes after vehicle treatment or between drug doses.

The incidence of locomotion, resting, mouthing, and licking in the four 5-min intervals of the test was analyzed by a three-way, repeated measures ANOVA with treatment, sex, and time as the main factors and with time as the repeated factor. When a significant overall interaction between treatment and time was found, follow-up analysis of treatment effects in each of the four intervals was performed by *t*-test. Because pups rarely stretched, groomed, or were immobile, the incidence of these behaviors was not analyzed.

All analyses were performed with the Statistical Analysis System [(SAS), Cary, NC].

RESULTS

Naloxone (1 mg/kg) reduced sucrose intake significantly beginning on PN10 in the oral catheter test (Fig. 1) and on PN11 in the independent ingestion test (Fig. 2). Naloxone significantly decreased the intake of sucrose on PN14 in a dose-related manner in both types of ingestion tests (Figs. 3 and 4). The threshold dose on PN14 was 0.1 mg/kg in the independent ingestion tests and was 0.5 mg/kg in the oral catheter tests. In addition to this difference in threshold dose, when the potency of naloxone was expressed as percent inhibition, naloxone was significantly more potent in independent ingestion tests than in oral catheter tests, $F(1, 2) = 6.1$, $p = 0.02$. Naloxonemethiodide (1 mg/kg), a peripheral opioid receptor antagonist that does not cross the blood-brain barrier, did not have any effect on intake in 14-day-old pups in either test (Table 1).

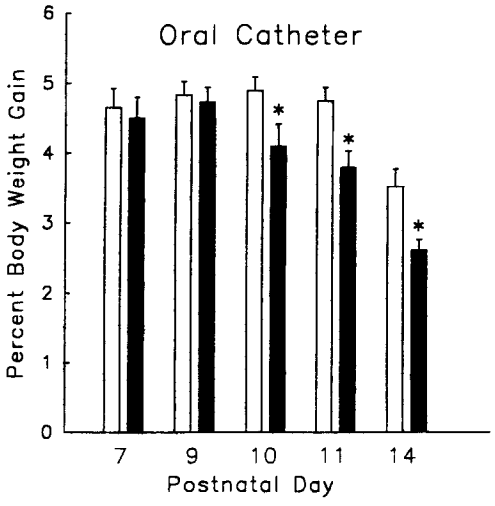

FIG. 1. Mean ± SE percent body weight gain after vehicle (open bars) or naloxone (1 mg/kg, filled bars) treatment in the oral catheter test. The number of pups tested with each treatment on each PN day was 21–24. Percent body weight gain was significantly less than vehicle on PN10, $F(1, 45) = 4.76$, $p = 0.05$, PN11, $F(1, 44) = 9.76$, $p = 0.003$, and PN14, $F(1, 46) = 8.79$, $p = 0.005$. Note that naloxone decreased intake in the oral catheter test on PN10 but did not decrease intake in the independent ingestion test until PN11 (see Fig. 2).

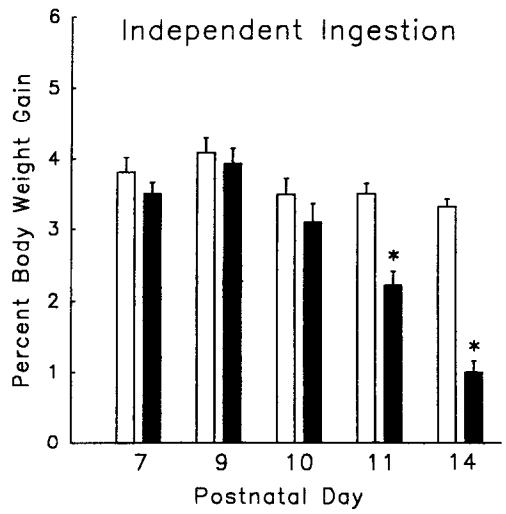

FIG. 2. Mean ± SE percent body weight gain after vehicle (open bars) or naloxone (1 mg/kg, filled bars) treatment in the independent ingestion test. The number of pups tested on each PN day with each treatment was 20–26. Percent body weight gain was significantly less than vehicle on PN11, $F(1, 49) = 28.32$, $p = 0.0001$, and PN14, $F(1, 44) = 145.53$, $p = 0.0001$.

Naloxone had three significant behavioral effects in 14-day-old pups: naloxone decreased licking, $F(1, 42) = 22.3$, $p = 0.0001$, increased mouthing, $F(1, 42) = 33.7$, $p = 0.0001$, and increased resting, $F(1, 42) = 5.5$, $p = 0.02$. Naloxone had no significant effect on locomotion (Figs. 5 and 6).

There was a significant interaction between the effects of naloxone and time on licking, $F(3, 126) = 3.3$, $p = 0.02$, and mouthing, $F(3, 126) = 3.9$, $p = 0.01$. Naloxone decreased licking in the first 10 min of the test ($p \leq 0.002$), but not in the second 10 min ($p > 0.1$). In contrast, naloxone increased mouthing in the first 15 min of the test ($p \leq 0.02$), but not in the last 5 min ($p = 0.055$).

In addition to these effects of naloxone, the incidence of all of the behaviors changed over time during the tests. There was significantly more licking during the 6–10-min interval than during 16–20-min interval ($p < 0.05$), more locomotion during the 16–20-min than the 6–10-min interval ($p < 0.05$), and more resting during the last 5 min than in any of the

TABLE 1

EFFECT OF NALOXONEMETHIODIDE ON INTAKE IN 14-DAY-OLD PUPS

Test	Percent Body Weight Gain	
	Saline	Naloxonemethiodide
Independent Ingestion	3.2 ± 0.19	3.1 ± 0.15
Oral Catheter	5.6 ± 0.18	5.6 ± 0.25

Data are mean ± SE. N = 23–24 pups in each condition for each test. The dose of naloxonemethiodide was 1 mg/kg.

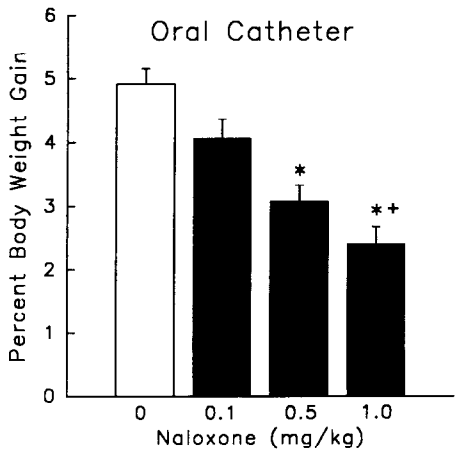

FIG. 3. Mean ± SE percent body weight gain in the oral catheter test as a function of the dose of naloxone for pups on PN14. n = 20–40 pups per dose. Naloxone decreased percent body weight gain significantly in a dose-related manner, $F(3, 101) = 18.05$, $p = 0.0001$.* Significantly different from vehicle treatment, $p = 0.05$. +Significantly different from 0.1 mg/kg. Note that 0.1 mg/kg naloxone did not decrease intake significantly in the oral catheter test, but did in the independent ingestion test (compare with Fig. 4).

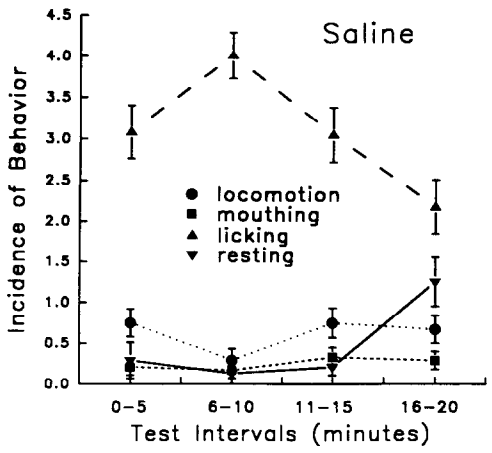

FIG. 5. Data are mean (±SE) incidence of four behaviors during independent ingestion tests on PN14 after administration of saline (IP). n = 24 pups.

preceding 5-min intervals ($p < 0.05$). Although there was an overall effect of time on the incidence of mouthing, no comparison of the intervals was significant when analyzed by Tukey's HSD test. Thus, at the end of the tests, pups licked less and locomoted and rested more. This pattern could represent decreased intake and increasing satiation at the end of the tests, but because intake was not measured at 5-min intervals, the interpretation of the temporal changes of behavior is uncertain.

DISCUSSION

The major result of these experiments is that naloxone decreased intake of 10% sucrose in pups toward the end of the second postnatal week during the first experience with eating in independent ingestion or oral infusion tests (Figs. 1 and 2). The inhibitory effect of naloxone is in the brain because a peripherally acting antagonist did not change intake (Table 1). These results extend previous reports that naloxone inhibited intake of milk in the same two types of tests in pups on PN10 (3) or PN14 (1,2). Taken together, these data provide compelling evidence that central opioids are necessary for the unconditioned control of eating in preweanling pups as early as PN10. We found no significant effect of naloxone on intake

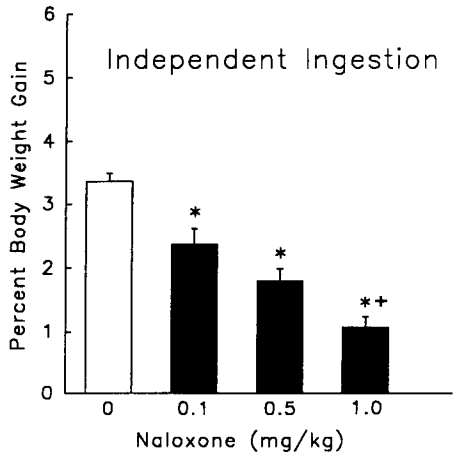

FIG. 4. Mean ± SE percent body weight gain in the independent ingestion test as a function of the dose of naloxone for pups on PN14. n = 21–36 pups per dose. Naloxone significantly decreased percent body weight gain in a dose-related manner, $F(3, 100) = 34.82$, $p = 0.0001$.* Significantly different from vehicle treatment, $p = 0.05$. +Significantly different from 0.1 and 0.5 mg/kg, $p = 0.05$.

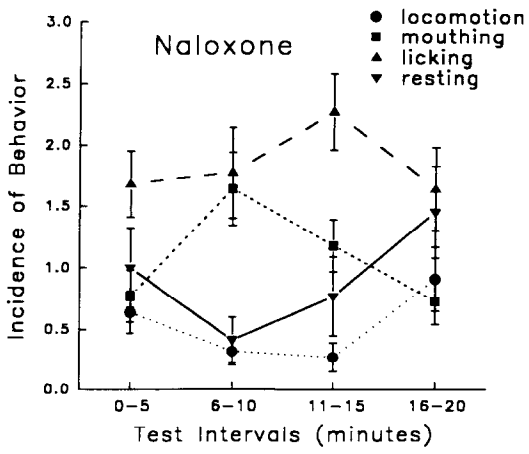

FIG. 6. Data are mean (+SE) incidence of four behaviors during independent ingestion tests on PN14 after administration of naloxone (1 mg/kg, IP). n = 22 pups. Compare with Fig. 5 and note that the ordinate scale is expanded in Fig. 6 compared to Fig. 5. Naloxone decreased licking, increased mouthing and resting, and did not change locomotion (see text).

in either test on PN7 and 9, and Capuano et al. (4) observed no effect of naltrexone on milk intake in the independent ingestion test on PN5.

Because the controls of eating in these tests at this age appear to be involved in the control of eating in the adult rat (6), it is possible that the inhibitory effect of naloxone on intake in adult rats in a variety of conditions is the result of naloxone's effect on this unconditioned control. But there is no decisive evidence for this because experiments in adult rats have not been designed to distinguish between naloxone's effect on conditioned and unconditioned controls.

Note that opioid antagonists other than naloxone have been reported to decrease intake during suckling as early as the first postnatal day (8,10), but naloxone had no effect on intake during suckling in pups from PN9 to PN18 (3). Although the reason for these different results is not clear, the results are further evidence that the controls of suckling in pups during the first 2 postnatal weeks are different from the controls of ingestion in pups ingesting away from the dam (6).

Two aspects of our results on PN14 deserve comment. The first is that naloxone was significantly more potent for decreasing intake in the independent ingestion test than in the oral catheter test (compare Figs. 3 and 4). Our result differs from reports of equal potency of naloxone for decreasing milk intake in both tests on PN14 (1) and equal potency of naltrexone on milk intake in both tests on PN10 (3).

The second aspect of the experiments is the behavioral results. We observed that naloxone decreased licking, increased mouthing and resting, but had no significant effect on locomotion (Figs. 5 and 6). Aroyewun and Barr (1) reported that naloxone (5 mg/kg, IP) decreased licking (they referred to this behavior as mouthing), but increased a composite score for nonlicking activity. In contrast, Blass et al. (3) observed no behavioral effect of naltrexone in doses (0.25, 0.5, or 1.0 mg/kg, IP) that decreased intake 20–30% in either test. Despite the different results, all of the data are evidence that the inhibition of intake was not due to a general inhibition of activity.

The results of these experiments are also interesting to compare with the results of experiments of identical design in which dopamine antagonists, raclopride and SCH 23390, were administered (15,16). There were two major differences from the results with naloxone: first, the dopamine antagonists decreased intake of 10% sucrose in the independent ingestion test on PN7, the earliest date tested. Second, the dopamine antagonists had little or no effect on intake in the oral catheter tests on PN7 or PN14. Because oral infusions eliminate appetitive movements prior to the initial contact with 10% sucrose as well as the appetitive movements required to reinitiate contact with 10% sucrose during the meal, this differential efficacy of naloxone and the dopaminergic antagonists in the oral catheter tests is evidence that central opioid mechanisms are necessary for the consummatory phase of ingestion, but dopaminergic mechanisms are not. In contrast, both mechanisms are necessary for the appetitive movements required for ingestion of 10% sucrose in the independent ingestion tests.

Furthermore, both of the differences between naloxone and the dopamine antagonists are evidence that the central dopaminergic and opioid mechanisms exert independent control of eating in the first two postnatal weeks. This may also be true of adult rats, but that is not clear.

In summary, these results are evidence that in the latter half of the second postnatal week, central opioid mechanisms are necessary for the normal, unconditioned control of the appetitive and consummatory phases of eating 10% sucrose. The central sites of this action and the type(s) of opioid receptors involved remain to be determined.

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