Physiology & Behavior 101 (2010) 40–44

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Morphine treatment during pregnancy modulates behavioral selection in lactating rats

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ARTICLE INFO

Article history:
Received 18 January 2010
Received in revised form 5 April 2010
Accepted 14 April 2010

Keywords:
Opioid
Morphine
Drug abuse
Periaqueductal gray
Maternal behavior
Sensitization
Foraging

ABSTRACT

Previous studies have demonstrated that treatment of postpartum female rats with morphine inhibits maternal behavior and stimulates foraging. Exposure to drugs of abuse may result in a progressive enhancement of their reinforcing effects. Puerperal treatment with morphine leads to reverse tolerance to this drug. The present study investigated whether repeated morphine treatment during late pregnancy may influence the effects of different morphine dosages on behavioral selection in lactating rats. Females were simultaneously exposed to pups and insects, and the choice between taking care of the pups and hunting insects was observed. Female Wistar rats were treated with morphine (3.5 mg/kg/day, subcutaneous [s.c.]) or saline for 5 days beginning on pregnancy day 17. On day 5 of lactation, animals were acutely challenged with morphine (0.5, 1.0, or 1.5 mg/kg, s.c.; MM0.5, MM1.0, and MM1.5 groups, respectively) or saline (MS group) and tested for predatory hunting and maternal behavior. Control groups were pretreated with saline and challenged with morphine (SM0.5, SM1.0, and SM1.5 groups) or saline (SS group). Animals treated with morphine during late pregnancy and acutely challenged with 1.0 mg/kg morphine (MM1.0 group) exhibited significantly decreased maternal behavior and enhanced hunting. This effect was not evident with the 0.5 mg/kg dose. The 1.5 mg/kg morphine dose decreased maternal behavior and increased hunting in both the MM1.5 group and in animals challenged with morphine after previous saline treatment (SM1.5 group). These results provide evidence of plasticity of the opioidergic role in behavioral selection during lactation.

1. Introduction

Maternal behavior in female rats is under the inhibitory influence of opiates [1,14]. β-endorphin, an endogenous opioid, infused into the ventricular system of lactating rats dose-dependently blocked normal maternal behavior, suggesting that the changes in endogenous opiate levels alter maternal responsiveness in rats [2,13]. Nonsedative doses of morphine disrupt and naloxone restores maternal responsiveness in female rats [1,4,15,25].

While some studies reported that morphine prevented the onset of maternal behavior in female rats, other reports showed that this drug facilitates maternal behavior when injected into the ventral tegmental area [21] and that naloxone fails to facilitate maternal care when injected in male and female juvenile rats [27].

Exposure to some drugs of abuse, particularly morphine, may result in a progressive and enduring enhancement of their reinforcing behavioral effects [3]. This progressive increase in responsiveness to a drug is referred to as reverse tolerance. In animals subjected to morphine-induced reverse tolerance, we and others have demonstrated that maternal behavior may be inhibited by low doses of morphine that are otherwise ineffective at inducing such inhibition in morphine-naive lactating rats [15,23,26]. In dams tested exclusively with pups, with no other simultaneous challenge, acute injection of 5.0 mg/kg morphine inhibited maternal behavior. A dose of 3.0 mg/kg morphine did not inhibit maternal behavior unless the dam was subjected to opioidergic stimulation during late pregnancy [1,15,17,18,26].

In a previous study examining the neural basis of inhibition of maternal behavior in response to low morphine doses in morphine-experienced dams, we found that morphine treatment induced a behavioral switch from maternal to predatory behavior. Morphine-challenged dams that were tested in an environment containing both pups and roaches (which served as prey) clearly preferred hunting rather than nursing [24,25].

Our current working hypothesis was that repeated administration of morphine modifies the subsequent inhibitory effects of morphine on hunting *vs.* maternal behavior even at doses lower than those used previously. To test this hypothesis, we examined repeated morphine treatment during late pregnancy influences the effects of morphine on

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doi:10.1016/j.physbeh.2010.04.013

behavioral selection in rats. These findings support an important role for opiates in the context of modulating the dam's motivational drive to forage and hunt and suggest that the brain structures involved in behavioral selection are plastic and adaptive.

2. Materials and Methods

2.1. Animals and housing

Subjects were adult Wistar nulliparous female rats, weighing 190–220 g and approximately 90 days of age at the beginning of the study. In all experiments, females were mated by placing them with sexually experienced males. The day when sperm was observed in the vaginal cytology was designated pregnancy day 1. Pregnant females were individually housed in opaque polypropylene cages (41×34×16 cm) containing approximately 1.0 L of medium-grade pine flakes. Food and water were available *ad libitum* in light- (0600–1800 h) and temperature- (23–25 °C) controlled testing rooms. On lactation day 2, females were left with their litters (culled to eight pups on day 2 of lactation; four males and four females) until testing on lactation day 5.

2.2. Pretreatment

Rats were injected subcutaneously with saline or 3.5 mg/kg morphine (Cristália Laboratory™, São Paulo, Brazil) daily for 5 days beginning on pregnancy day 17. The total volume injected into each animal was 1 ml/kg/day.

Parturition was considered day 0 of lactation. On postpartum days 3 and 4, dams were placed into the experimental cage for 30 min/day for adaptation without their pups. The experimental cage was identical to the home cage, with the exception that the experimental cage had no wood flakes and was made of Plexiglas to permit videotaping animal behavior. Animals were maintained in accordance with the guidelines of the Committee on Animals of the Colégio Brasileiro de Experimentação Animal and the Committee on the Care and Use of Laboratory Animal Resources, National Research Council, USA. In all experiments, animals were tested only once.

2.3. Behavioral observations

On the morning of postpartum day 5 or 6, dams were tested simultaneously for maternal care toward the pups and predatory hunting behavior. Dams were placed into the experimental cage. Sixty minutes later, they received an acute injection of saline or morphine (0.5, 1.0, or 1.5 mg/kg). Thirty minutes later, eight of each dam's own pups and five live mature cockroaches (*Periplaneta americana*) were introduced into the cage for behavioral testing. During the 30 min trial, dams were observed for maternal and insect-hunting behaviors.

The time spent ambulating or rearing was considered to reflect exploratory behavior. The number of times the dam contacted the pups was also quantified. The following parameters were recorded for maternal behavior: (*i*) retrieving pups (a pup may be retrieved more than once to be carried to another part of the cage), (*ii*) grouping and licking pups, and (*iii*) after retrieving and grouping at least five pups, splaying her legs and arching over the pups, which then attached to her nipples.

Predatory hunting behavior was evaluated by the percentage of rats that captured the five insects. Videotaped behavior was analyzed with Etholog 2.2 [20]. Each animal was tested only once.

2.4. Effects of morphine sulfate on choice in lactating rats: care for pups vs. predatory insect-hunting

Thirty minutes before behavioral testing, lactating rats received an acute subcutaneous injection of morphine sulfate (0.5 mg/kg, $n=10$; 1.0 mg/kg, $n=10$; 1.5 mg/kg, $n=10$) or saline ($n=10$). The following groups were used (Table 1): (*i*) animals treated for 5 days with morphine sulfate (3.5 mg/kg/day beginning on pregnancy day 17) and acutely challenged with morphine on lactation day 5 (MM0.5 group, 0.5 mg/kg morphine, $n=10$; MM1.0 group, 1.0 mg/kg morphine, $n=10$; MM1.5 group, 1.5 mg/kg morphine, $n=10$), (*ii*) animals treated with morphine sulfate and acutely challenged with saline (MS group, $n=10$), (*iii*) animals treated for 5 days with saline and acutely challenged with morphine sulfate (SM0.5 group, 0.5 mg/kg morphine, $n=9$; SM1.0 group, 1.0 mg/kg morphine, $n=10$; SM1.5 group, 1.5 mg/kg morphine, $n=10$), and (*iv*) animals treated with saline for 5 days and acutely challenged with saline (SS group, $n=10$).

Table 1
Specification of groups according to the pretreatment and challenge.

Groups	Pretreatment (pregnancy day 17 until 21)	Challenge (day 5 of lactation)
SS	Saline 0.9%	Saline 0.9%
MS	Morphine 3.5 mg/kg	Saline 0.9%
SM0.5	Saline 0.9%	Morphine 0.5 mg/kg
MM0.5	Morphine 3.5 mg/kg	Morphine 0.5 mg/kg
SM1.0	Saline 0.9%	Morphine 1.0 mg/kg
MM1.0	Morphine 3.5 mg/kg	Morphine 1.0 mg/kg
SM1.5	Saline 0.9%	Morphine 1.5 mg/kg
MM1.5	Morphine 3.5 mg/kg	Morphine 1.5 mg/kg

The behavioral test was performed as described above. In all experiments, the test cage was washed with a water–alcohol (5%) solution before behavioral testing to eliminate possible bias due to odors left by previous subjects. To minimize possible circadian influences on rat behavior, experimental and control observations were inter-mixed. Each animal was tested only once.

2.5. Statistical analysis

Comparisons among groups were analyzed by two-way parametric analysis of variance (ANOVA) using pregnancy morphine (yes or no) as one factor and dose of morphine during test (0.0, 0.5, 1.0, and 1.5 mg/kg) as the other factor, followed by Tukey's *post hoc* test for number of contacts with pups, number of retrievals, and time spent engaging in exploratory behavior. Fisher's Exact Probability Test was used to compare the number of animals displaying grouping and licking behavior, arched-back nursing, and the percentage of animals that caught all five insects during the test. A probability of $p<0.05$ was considered significant for all comparisons.

3. Results

In all cases each MM group was compared to the respective SM group and to SS and MS groups. The percentage of rats from the MM0.5 group that displayed grouping and licking behavior during the test was lower than the SS and SM0.5 groups (Fisher's Exact Probability Test, $p=0.020$, Fig. 1A). The percentage of rats from the MM1.0 group that displayed grouping and licking behavior during the test was lower than the SS and SM1.0 groups (Fisher's Exact Probability Test, $p=0.003$, Fig. 1A). The percentage of rats from the MM1.5 and SM1.5 groups that displayed grouping and licking behavior during the test was lower than the SS group (Fisher's Exact Probability Test, MM1.5×SS, $p=0.003$; SM1.5×SS, $p=0.020$; Fig. 1A). No other significant differences were found in displaying of grouping and licking behavior among groups.

The percentage of animals displaying arched-back nursing was reduced in the MM0.5 group compared with the SM0.5 group (Fisher's Exact Probability Test, $p=0.006$, Fig. 1B). The percentage of animals displaying arched-back nursing was reduced in the MM1.0