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Opiate Involvement in Postpartum Aggression in Rats

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KINSLEY, C. H. AND R. S. BRIDGES. *Opiate involvement in postpartum aggression in rats.* PHARMACOL BIOCHEM BEHAV **25**(5) 1007–1011.—Opiates and the endogenous opioids mediate maternal behavior and various forms of aggression. The present study sought to investigate the role of opiates in postpartum aggression (PPA), an intense form of agonistic behavior displayed by lactating females. Primiparous rats were screened for their PPA against adult males on day seven postpartum. They were then randomly assigned to one of four treatment groups [morphine, 5.0 mg/kg; naloxone alone, 0.5 mg/kg; morphine (5.0 mg/kg) plus naloxone (0.5 mg/kg); and saline] and tested for PPA on postpartum days eight and nine following the respective treatments. Morphine significantly lowered PPA, and naloxone antagonized the effect. Whereas the morphine plus naloxone, naloxone alone, and saline groups exhibited higher levels of PPA than that shown by the morphine group, there were no differences in PPA found among the morphine plus naloxone, naloxone alone, or saline groups. These results, in conjunction with evidence describing the state of the endogenous opioid system in the postpartum rat, suggest that some aspect of the endogenous opioid system may be involved in another form of maternal behavior, postpartum aggression.

Aggression Maternal behavior Opiates Opioids

MATERNAL behavior in the rodent consists of two phases, the onset and the maintenance phases. The former is mediated primarily by the dramatic endocrine changes which accompany pregnancy in the rodent; the latter, by stimuli provided by the offspring [24]. Of the many behaviors which comprise both the onset and maintenance phases of maternal behavior, some of the least understood are the agonistic behaviors displayed by the female. During the postpartum period, when confronted by an adult, intruder male, the female will engage in explosive bouts of lunging and attacking with the purpose of driving him from the vicinity of the nest. This aggression, referred to as postpartum aggression (see [28, 29, 33] for reviews), is characterized by a rapid onset of a series of biting attacks directed at the back and flanks of the male. It functions, presumably, as both a population regulatory mechanism [14] and as a nest defense strategy ([29]; cf., however, [6]). The behavior, heavily tied to suckling stimulation [32], is most intense during early to middle lactation in both the mouse [28,31] and rat [5,6].

Recently, attention has been focussed on the possible role of the endogenous opioid peptides (EOP) and opiates in the expression of maternal behavior. For example, it was shown that morphine disrupted maternal behavior and that naloxone reversed those effects [1,11]. In a postpartum lactating female the presence of the EOP's—as measured by both hypothalamic and pituitary content of beta-endorphin and Met-enkephalin [10, 20, 23], and a behavioral assay such as pain threshold [9]—appears low. This reduced neural content may be of importance for the exhibition of maternal behavior and postpartum aggression. Opiates generally suppress aggression [16], whereas opiate antagonists facilitate or enhance such behaviors [7,8]. Inasmuch as opiates, and possibly the endogenous opioids, mediate certain aspects of maternal behavior, of which, thus far, pup retrieval and other pup-directed behaviors are the only example, and opiates suppress aggression, it was the aim of the present paper to investigate whether or not and to what degree opiates are involved in the exhibition of another important aspect of maternal behavior, postpartum aggression. It is hypothesized that opiates are inhibitory to the expression of postpartum aggression, as they have been found to suppress other forms of aggression [16] as well as maternal behavior [1]. Therefore, by treating postpartum females with morphine, at a time when their levels of aggression are highest [28] and brain endorphins and enkephalins are low ([20]; Hammer and Bridges, submitted), it should be possible to cause a disruption in the maintenance of that aggressive behavior similar to those reported for pup-directed behaviors [1,11].

METHOD

Subjects

Adult virgin female Sprague-Dawley rats (225–250 grams;

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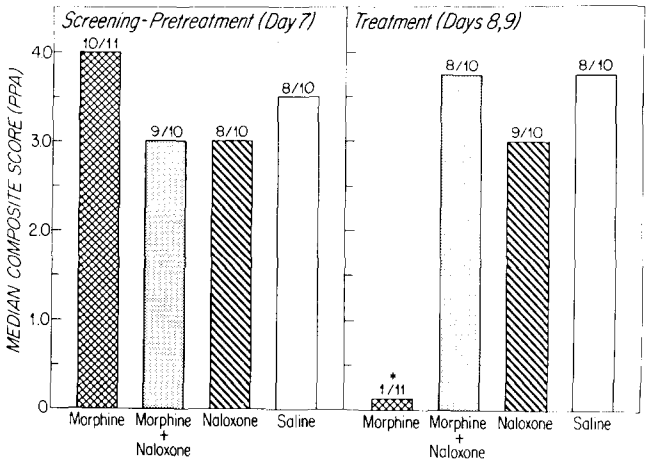

FIG. 1. Median composite aggression score (No. lunges + No. attacks) for postpartum females treated with morphine, morphine plus naloxone, naloxone alone, or saline. The numbers within each histogram represent the proportion of animals exhibiting aggression out of the total number of animals tested. On day seven postpartum each female was screened for her aggressive behavior in the absence of any treatment, then randomly assigned to one of the four treatment groups. For two consecutive days each female was tested for her aggression against an unfamiliar, adult male rat. The values for Treatment were summed over the two treatment days and divided by two, the number of test days, to arrive at each animal's composite score. *Both the proportion and intensity of postpartum aggression were significantly reduced in morphine-treated females relative to the remaining three treatment groups.

TABLE 1

PROPORTION (%) EXHIBITING EITHER LUNGES AND ATTACKS, AND MEDIAN NUMBER OF LUNGES AND ATTACKS AT SCREENING AND FOLLOWING TREATMENT FOR GROUPS MORPHINE, MORPHINE PLUS NALOXONE, NALOXONE ALONE, AND SALINE

	Screening (Day 7 Postpartum)		Treatment (Days 8 and 9 Postpartum)	
Group	Lunges	Attacks	Lunges	Attacks
Morphine	10/11	7/11	1/11	0/11
	(91%)	(63%)	(9%)	(0%)
	2.0	2.0	*	†
Morphine plus Naloxone	9/10	6/10	7/10	4/10
	(90%)	(60%)	(70%)	(40%)
	2.0	1.5	4.5	2.75
Naloxone alone	8/10	3/10	9/10	4/10
	(80%)	(30%)	(90%)	(40%)
	2.0	2.0	2.5	3.25
Saline	7/10	5/10	8/10	6/10
	(70%)	(50%)	(80%)	(60%)
	3.0	2.0	2.5	2.0

All females were screened for their aggression against an adult male intruder on Day 7 postpartum, randomly assigned to one of the four treatment groups, and tested for postpartum aggression on two consecutive days (Days 8 and 9). The values for Treatments were obtained averaging each animal's responses over the two test days (for only those animals that fought. *†Too few animals fought to calculate a median. See text for significance levels).

Charles River Laboratories, Inc., Wilmington, MA) were used as experimental animals, and adult males of the same strain (225–250 grams) for studs and opponents for testing (see below). The animals were housed in polypropylene cages (25 by 45 by 20 cm) and provided with food (Purina Rat Chow) and water, ad lib. All animals were kept in temperature (21–24°C) and light (on, 0700–1900 hr) controlled rooms throughout the study. Animal facilities are AAALAC approved and conform to the guidelines set forth by the NIH for the care and treatment of laboratory animals.

Procedure for Generating Experimental Animals

A single stud male was introduced into the homecage of the virgin female and the two allowed to mate. Following determination of mating (viz., presence of sperm in the vaginal lavage), the male was removed and the female provided with nesting material. Aside from routine maintenance, the females were left undisturbed until the completion of delivery (approximately 22 days) at which time each female was moved into a new cage, her litter was removed, and she was given six mixed-sex foster pups. Tests for postpartum aggression were conducted on days seven to nine, a time when the levels of aggression have been reported to be maximal [5,6]. All animals were screened for postpartum aggression at 0800–1000 hr on postpartum day seven, then randomly assigned to one of four treatment groups.

Postpartum Aggression Behavioral Testing Procedures

The tests for postpartum aggression (PPA), an adaptation of Svare [28,29] and Erskine *et al.* [5,6], consists of removing the young from the nest at the time the females are injected, thereby ensuring that the pups will not be harmed or cause any interference. (It has been shown that removal of the young for short periods of time, three hours or less, has no effect upon levels of aggression [30].) An adult male is then placed into the female's cage at a point furthest from her. All opponents were adult virgin, untreated males of the same strain (approximately 60–80 days of age), having had no prior contact with the test females. The tests were for fifteen minutes, and the following behaviors were recorded: Lunges—these are defined as a rapid thrust toward the opponent, failing contact. This behavior is usually accompanied by threatening squeals by the female and tail rattling; Attack—defined in the present examination as biting and grappling with the opponent, consisting usually of repeated attempts at latching onto and holding him by his rump; throwing her hips in a sideways attitude, accompanied occasionally by kicking; and engaging in an upright "boxing posture," flailing away at the opponent with her forepaws. The female and the opponent will often wrestle around the cage as the female continues to attack him. Despite repeated submissive postures by the male—frequently within the first few seconds of the encounter—the female continues to attack. For purposes of comparison, an overall aggression score, the composite aggression score, was calculated for each animal. It is simply the total number of lunges plus the total number of attacks for a particular animal. Any unusual behavior on the part of either the female or the male, e.g., mounting attempts by the female, the opponent fighting

back, etc., were all monitored and recorded. All treatment groups were coded; therefore, the experimenter was blind to the particular treatment to which an animal was assigned.

Design and Method

Following the screening test on postpartum day seven females were randomly divided into four groups (N=10–11/group). On the morning of postpartum days eight and nine, beginning at 0800 hr, each group received subcutaneous (SC) injections of one of the following four treatments: morphine sulphate (5.0 mg/kg), naloxone hydrochloride (0.5 mg/kg), naloxone hydrochloride (0.5 mg/kg) plus morphine sulphate (5.0 mg/kg), or physiological saline (0.5 ml). At the time of the injection the pups were removed from the homecage and the mothers placed back into their nests. The test for postpartum aggression was conducted 60 minutes later. Immediately following the completion of testing on days 7–9, the rat young were returned to each test female and informal observations of the mothers' maternal behavior were made. Since tests for PPA following treatment were conducted on two consecutive days (postpartum days 8 and 9) the data were averaged across the two days by taking the total and dividing by two, the number of test days. This allows for a more reliable index of an animal's behavior as opposed to a single test on a single day.

RESULTS

Figure 1 depicts the composite postpartum aggression score of lactating rats administered morphine, morphine plus naloxone, naloxone alone, or saline prior to and following treatment (left-hand portion of Fig. 1 and right-hand portion of Fig. 1, respectively). Morphine administration suppressed postpartum aggression, whereas naloxone antagonized this effect in the morphine plus naloxone group. The values within each histogram represent the proportion of animals engaging in aggression. Chi square analyses (with Yate's correction) on these proportions showed that significantly fewer of the morphine-treated females exhibited any type of aggression when compared to the other three treatment groups, [vs. morphine plus naloxone, $\chi^2(1)=10.93$, $p<0.001$; vs. naloxone, $\chi^2(1)=13.93$, $p<0.0002$; vs. saline, $\chi^2(1)=10.93$, $p<0.001$]. The latter three treatment groups did not differ among themselves. There was no difference in any of the groups between test days eight and nine and we therefore collapsed the data for each group across these two days.

The treatment also affected the intensity of the aggression. A Kruskal-Wallis nonparametric analysis of variance (ANOVA) on the pre-treatment screening composite scores showed that, as expected, there was no difference among the four groups in the level of aggression exhibited prior to administration of the drugs. Following administration, however, the Kruskal-Wallis test revealed a significant effect of treatment, $H=155.05$, $p<0.001$. Follow-up comparisons using the Mann-Whitney U showed that the morphine group exhibited significantly less aggression than all three groups, (vs. morphine plus naloxone, $U=14.5$, $p<0.02$; vs. naloxone, $U=8.0$, $p<0.002$; vs. saline, $U=13$, $p<0.02$). There was no difference among morphine plus naloxone, naloxone alone, or saline in their composite aggression scores.

Table 1 depicts a breakdown of the composite aggression score into lunges and attacks. Chi square analysis of the proportion of females exhibiting lunging revealed that significantly fewer morphine-treated females exhibited lunges as compared to the other three groups, [vs. morphine plus naloxone, $\chi^2(1)=8.41$, $p<0.004$; vs. naloxone alone, $\chi^2(1)=13.93$, $p<0.0002$; vs. saline, $\chi^2(1)=10.93$, $p<0.001$]. Analysis of the proportion of animals exhibiting attacks (with the Fisher's Exact Probability test) revealed a similar effect as significantly fewer morphine-treated females attacked the opponent as compared to the remaining three groups, (vs. morphine plus naloxone, $p<0.035$; vs. naloxone alone, $p<0.035$; vs. saline, $p<0.004$).

Analysis of the intensity measures with the Kruskal-Wallis ANOVA showed, for lunges, a significant effect of treatment, $H=148.51$, $p<0.001$. A follow-up with the Mann-Whitney U showed that the morphine group exhibited significantly fewer lunges than the other three groups, which did not differ among themselves, (vs. morphine plus naloxone, $U=20.0$, $p<0.02$; vs. naloxone, $U=10$, $p<0.002$; vs. saline, $U=14$, $p<0.02$). For analysis of the attacks, the Kruskal-Wallis showed that there was a significant effect of treatment, $H=138.59$, $p<0.001$. (The morphine group did not exhibit any attacks; their inclusion in this analysis was for the sake of completeness. For the remaining three groups, therefore, the Kruskal-Wallis was performed and revealed no difference, though naloxone alone animals had a slightly higher median number of attacks.)

Finally, our informal observations of the females' maternal behavior indicated that within several hours of the injection and behavioral test, all females (regardless of treatment group) eventually retrieved their young and acted maternally toward their litters.

DISCUSSION

Normally, a postpartum female exhibits high levels of aggression toward an intruder male. Morphine administration suppresses this level of aggression, and naloxone, a potent narcotic antagonist, can reverse the effect. Furthermore, the action of morphine on the postpartum female does not appear to affect general maternal behavior as all females eventually retrieve and crouch over their litters. The present results suggest, therefore, that endogenous opioids may mediate postpartum aggression.

Gintzler [9] reported that postpartum maternal females exhibit a transient hyperalgesia 7–20 days postpartum. It is possible that this hyperalgesia may be of importance for the elicitation of PPA. Indeed, if one was to plot the curve for the onset and intensity of PPA previously reported by others [5, 6, 33], and that observed in the present study, the curve would be nearly the direct inverse of that shown for the postpartum pain thresholds in Gintzler's paper ([9], p. 194), suggesting decreased opiate activity. Wardlaw and Frantz [34] measured hypothalamic beta-endorphin in seven day postpartum females and found significant reductions in opioid content, as did Panerai *et al.* [20] who reported significant reductions in beta-endorphin in the hypothalamus and midbrain of day eight postpartum females. Hammer, Ronsheim and Bridges [12] have recently reported decreased opiate receptors in the postpartum, lactating rat. Together, these data indicate the presence of fewer receptors or less ligand availability (or both) in the postpartum female, a condition which may have behavioral relevance in terms of both pup-directed [1,11] and maternal aggressive behaviors. How this may translate into effects on aggressive behavior is suggested by the findings of Bridges and Ronsheim [2] and Mayer and Rosenblatt [15]. The former showed that hypo-

thalamic levels of beta-endorphin decreased as the pregnant animal approached term, and Mayer and Rosenblatt [15] reported that female rats in the late prepartum period (3.5 hours prior to delivery) were more likely to attack intruder males than at any other point earlier in pregnancy. These data, then, support the notion that reductions in endogenous opioid activity may facilitate both maternal behavior and maternal aggression.

The present findings are the first to implicate a role for opiates in the aggressive behavior displayed by the postpartum female. Evidence for other neurochemical mediation of PPA was provided by Ieni and Thurmond [13] who showed that antagonism of serotonin (5-HT) through depletion of 5-HT stores (with PCPA) or 5-HT receptor antagonists reduced the aggressive behavior of postpartum female mice. To what extent morphine may have had indirect effects on this particular neurotransmitter system is unclear. To date, manipulations of morphine-5-HT interactions have met with inconclusive and often opposite results as acute administration of morphine has been shown to both increase and decrease 5-HT function [26].

It could be argued that one reason morphine-treated postpartum females fail to show aggression may be the drug's effects on the females' general activity. Based upon findings from previous reports from our laboratory, however, this interpretation is unlikely. Bridges and Grimm [1], who found that morphine disrupted pup-oriented maternal responsiveness, evaluated possible morphine effects on activity. They reported that morphine-treated females displayed levels of activity in an open field comparable to those shown by saline-treated control females. Their activity data indicate that morphine, at least in the dose employed in this study, (which was the same as that employed in the latter report [1]), does not reduce postpartum aggression simply by depressing the females' general activity. It should be kept in mind, however, that an animal's activity in an open field may not reflect that displayed in its homecage.

As to the central effects of morphine on postpartum aggression, it has been shown that morphine placement directly into the MPOA disrupts maternal behavior [25]. Because the MPOA is involved in maternal behaviors [17–19], and the POA in general has been implicated in aggressive behaviors [3,22], it is interesting to speculate that the POA may also be a site involved in postpartum aggression. Another feasible central site of morphine's action on postpartum aggression is the periaqueductal grey. This region receives fibers from the POA [4], is a site of high gonadal steroid [27] and opiate receptor content [21], and is involved in other forms of aggressive behavior [3]. Experiments to determine the central locale of opioid mediation of postpartum aggression are topics of ongoing studies in our laboratory.

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