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Opiate Antagonism Reduces Placentophagia and Pup Cleaning by Parturient Rats

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MAYER, A. D., P. L. FARIS, B. R. KOMISARUK AND J. S. ROSENBLATT. *Opiate antagonism reduces placentophagia and pup cleaning by parturient rats.* PHARMACOL BIOCHEM BEHAV 22(6) 1035–1044, 1985.—Since endogenous opiate mechanisms are activated during parturition, the present study examined in rats the effects of opiate antagonism on maternal care during and shortly after parturition. Endogenous opiate mechanisms were blocked in late pregnant rats by (1) naltrexone pellet implants (Experiment 1); (2) acute naloxone injections of 10 mg/kg (Experiment 2) or 0.1 mg/kg (Experirment 7); or (3) induction of opiate tolerance (Experiment 3). All methods resulted in a significant decrease in placentophagia and/or in cleaning pups of umbilical cords and birth fluids (Experiment 6). Other aspects of maternal care appeared relatively unaffected and 24 hr pup survival rates were lowered only by induction of morphine tolerance (probably via its effects on the young). In nonpregnant females, naloxone produced a small but significant decrease in placentophagia (Experiment 4) whereas morphine-tolerant nonpregnant females consumed placentas as readily as controls (Experiment 5). Thus the inhibition of placentophagia produced by opiate antagonism may be specific to conditions associated with parturition. These findings suggest that endogenous opiates support placenta eating and pup cleaning during and immediately after birth. Mediation may be via opiate effects on ingestive behavior, and/or via a reduction in the stress of parturition which otherwise can interfere with the female's ability to perform these tasks.

Parturition Placentophagia Tolerance Opiate antagonism

RECENT findings indicate that endogenous opiate systems are activated during late pregnancy and parturition: β-endorphin levels are elevated in human plasma during labor [7, 12, 13, 18, 20, 24, 27, 28, 34, 45], and are increased in rat brain (hypothalamus, midbrain and amygdala) throughout mid- to late-pregnancy, declining after parturition [55]. It has been suggested that endogenous opiates late in pregnancy facilitate the development of maternal responsiveness [33], and/or protect the female from the stress of parturition by altering pain perception [12, 13, 18, 20, 56]. In support of the latter hypothesis, rat flinch-jump thresholds to footshock have been reported to increase during pregnancy, first gradually and then steeply, shortly before parturition [25]. Nevertheless the role(s) of endogenous opiates in pregnancy and parturition remain more the subject of speculation than of research.

The present series of experiments was undertaken to determine whether blocking the actions of endogenous opiates (in pain and other systems) would alter the behavior of female rats during parturition, either as observed directly or as inferred from the conditions of their litters. We first treated females with naltrexone, an opiate antagonist, at a dose level reported to eliminate the prepartum rise in flinch-jump thresholds [25]. In exploratory work and the first experiment, naltrexone-treated females were observed continuously while giving birth, and their pups were inspected 30 min, 1 day and 1 week following delivery. Perhaps the most significant observation was negative in import: in most instances and in most respects the maternal behavior of the naltrexone-treated females could not be differentiated from that of sham-treated females and the survival of their litters was not compromised. There was, however, a striking difference between sham- and naltrexone-treated females in placentophagia during parturition; most sham-treated females ate each placenta shortly after it was delivered, whereas most naltrexone-treated females ate only a few or even none until several hours later. This difference seemed worthy of further investigation, and in subsequent experiments we determined that placenta-eating at parturition also was reduced by a second opiate receptor antagonist, and by induced tolerance to the analgesic effects of morphine. (Tolerance to morphine presumably renders the animal unresponsive to its endogenous opiates as well.) Finally we determined that the reduction in placentophagia at parturition by opiate blockade did not represent only an aversive response to placental tissue, but rather seemed to be the most readily observed and quantified manifestation of a more general reduction in licking and cleaning afterbirth products from the pups.

These studies to our knowledge constitute the first demonstration that opiate blockade influences certain aspects of maternal behavior at parturition, and thus the first evidence, albeit indirect, that endogenous opiates normally influence behavior at this time. We suggest that endogenous opiates support placentophagia and pup cleaning through involvement in ingestive behavior, and/or by reducing stress,

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thus permitting the female to more rapidly and effectively initiate maternal care.

EXPERIMENT 1. EFFECTS OF NALTREXONE ON PARTURITIONAL BEHAVIOR

Late pregnant females were housed in glass aquaria permitting close observation during parturition. Experimental females were implanted with naltrexone pellets which block prepartum increases in pain threshold [25]. Since pilot work had suggested that naltrexone decreased placentophagia, we particularly noted this behavior. However, we also looked for differences between naltrexone- and sham-treated females in a broad range of variables, including duration of parturition, condition of pups, and relative frequencies of 19 items of behavior observed during parturition.

METHOD

Animals

This and subsequent experiments used animals purchased as young adults from Charles River Laboratories, Wilmington, MA (CD strain, originally Sprague Dawley) or their offspring. Females were between 2.5 and 5.5 months of age at the time of testing. Prior to and during mating animals were housed on a reversed 12:12 light-dark cycle, lights on at 7 p.m. (EST); 6–8 days after mating females were moved to a 12:12 cycle, lights on at 8:30 a.m., a procedure which synchronizes deliveries so that nearly all occur between 9 a.m. and 4 p.m. on the 22nd day after mating [43]. Except as noted, females were housed in groups of 2–6 in suspended metal cages; water and Purina rat chow were available at all times.

Procedures

On Day 20 of pregnancy primigravid females were placed individually in 15 gal glass aquaria (76×29×30.5 cm) containing pine shavings and equipped with water bottles and feeders. One day prior to parturition 9 females were implanted SC in the scapular region with 2 pellets each containing 30 mg of naltrexone (kindly provided by A. Gintzler); 13 females were sham-implanted. Both procedures were performed under light Metofane anesthesia (Pitman-Moore).

On the day of parturition each female was observed at frequent intervals until regular abdominal contractions indicated that labor had begun. Thereafter observations were more frequent until delivery commenced, and nearly continuous until no pups had been delivered for 30 min, when it was assmumed that parturition was complete. The female then was removed to a holding cage while the litter and nest site were inspected, after which she was returned. The pups were reinspected the following day and the litter culled (or increased) to 10. The naltrexone pellets were then removed and the female and young were rehoused in a translucent maternity cage (24×45×15 cm) with wire top holding a water bottle and food pellets where they remained undisturbed for 6 days when a final inspection was made.

A 30 min interval between delivery of the (assumed) last pup and inspection of the litter was selected for the following reasons: first, since a female that already has delivered several pups rarely delivers additional pups after a delay of 30 min, it can be assumed that parturition is complete. Second, rats tend to cease active parturitional behavior within a few min following the last delivery [16]; they then assume a crouched posture over the pups and remain quietly in this position for some hours, occasionally briefly regathering and licking pups or pulling in nest material.

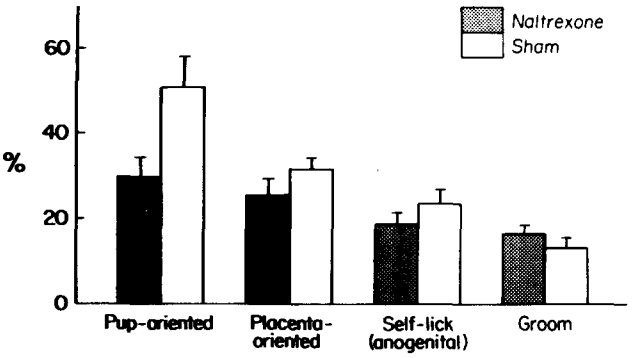

FIG. 1. Mean scores (SEM) obtained by naltrexone- and sham-implanted females (n=6 per group) on various categories of parturitional behavior. Observations were begun with delivery of the first pup and terminated 30 min following delivery of the last. Using a checklist of behavior items (see text), all items observed during the first 15 sec of each min were recorded. For each item, frequency was divided by the number of observation periods and multiplied by 100. To obtain Pup-oriented, Placenta-oriented, and Self-groom scores, which were based on more than one item, scores for the relevant items were summed.

Responses were compared between naltrexone- and sham-implanted groups in the following parameters: (a) duration of parturition, measured from delivery of the first pup to delivery of the last pup; to control for variation in litter size, duration was divided by number of pups; (b) condition of the litter 30 min after delivery—number of dead pups, number of live pups with placentas attached, number of free placentas at the nest site; (c) condition of the litter on Day 1—percentage of pups surviving, pup weights; and (d) condition of the litter on Day 7—pups surviving, pup weights. Litter size was similar in the naltrexone and control groups.

Additionally, observations of behavior during parturition were recorded in detail for 6 naltrexone- and 6 sham-implanted females (of the above groups), using a time-sampling technique and an exhaustive checklist of behavior items seen during parturition (modified from [16]): Pup-oriented behavior—Sniff, Lick, Manipulate with forepaws, Mouth, Gather, Crouch over; Placenta-oriented behavior—Lick-bite, Lick-bite while holding in forepaws, Eat; Anogenital self-lick; Self-groom—Lick paws, Groom face, Groom other; Twitch (brief raising of ventrum), Locomote, Bite wood shavings, Move wood shavings, Drop placenta. Also recorded were Posture—Stand, Sit on haunches, Head-between-legs, Lie extended, Lie on back, Turn; Abdominal contractions—"Lordosis" type, vertical, and intermediate; and Deliveries of pups and placentas. All items observed during the first 15 sec of each min were checked.

Statistical Analysis

Raw data in this and subsequent experiments included counts of pups and placentas at the 30 min inspections. From these we estimated for each female the number of placentas delivered (one per pup) and the percentage eaten. (The occasional stillborn pup and its attached placenta were not counted for this statistic.) Since 75% or more of the control

TABLE 1

LITTERS OF NALTREXONE- AND SHAM-IMPLANTED FEMALES 30 MINUTES FOLLOWING PARTURITION

Treatment	n	Percent of litters containing: Pups with placentas	Detached placentas	Dead pups	Mean litter size	Mean delivery time (min/pup)
Naltrexone	9	78*	88†	0	12.1	8.4 ± 1.05‡
Sham	13	23	23	8	12.8	5.8 ± 0.45

*Compared to Shams, $\chi^2=6.4$, $p<0.05$.
†Compared to Shams, $\chi^2=9.2$, $p<0.01$.
‡Compared to Shams, $t(16)=2.35$, $p<0.05$; data from 8 naltrexone- and 10 sham-treated females.

TABLE 2

LITTERS OF NALTREXONE- AND SHAM-IMPLANTED FEMALES 1 AND 7 DAYS FOLLOWING PARTURITION

		Day 1		Day 7	
Treatment	n	Mean pup weights (g)	Percent containing fewer pups than at previous inspection	Mean pup weights (g)	Percent containing fewer pups than at previous inspection
Naltrexone	9	6.0 ± 0.16*	33.3	13.6 ± 0.82	22.2
Sham	11	6.5 ± 0.11	27.3	12.8 ± 0.63	27.3

*Compared to Shams, $t(18)=3.08$, $p<0.01$.

females ate 100% of the placentas they delivered, groups were compared by dichotomizing the subjects into those consuming 100% and those consuming fewer than 100%; χ^2 tests of independence or Fisher Exact Probability tests were applied to these data.

The total number of occurrences per female of each item of behavior recorded during time-sampling observations was divided by the number of observations for that female. Since items in many cases were observed at rates too low for meaningful group comparisons, mean frequencies per observation of related items were added, i.e., Pup-oriented, Placenta-oriented, and Self-groom. Naltrexone- and sham-treated groups were compared by means of Student's t-test.

RESULTS AND DISCUSSION

Behavior During Parturition

All females regardless of treatment group showed the general pattern of parturitional behavior described by Dollinger [16] who observed females giving birth under relatively undisturbed conditions. With few exceptions the females remained at a previously established nest site; adopting a head-between-legs posture, they licked the pups as they emerged, and licked their own anogenital regions. Within 10 min or so of the final delivery they had gathered the pups and assumed a crouched posture over them. Continuous recordings did not reveal significant differences between naltrexone- and sham-implanted females in the incidence of pup-oriented, placenta-oriented or grooming behavior (Fig. 1).

Duration of Parturition

Naltrexone-treated females required 45% more time to deliver than did sham-treated females (8.4 vs. 5.8 min/pup; Table 1). However, subsequent experiments using other methods of opiate blockade did not replicate this prolongation of labor.

Condition of the Litter 30 Min After Parturition

Consumption of placentas was significantly reduced among naltrexone-treated females relative to controls (Table 1); 89% of naltrexone-treated but only 23% of sham-treated females failed to consume all placentas, $\chi^2=9.2$, $p<0.01$. The 8 (of 9) naltrexone-treated females that did not eat all placentas consumed an average of 33.5%. The 2 (of 13) saline-treated females that did not eat all placentas also showed a considerable reduction in percent eaten, averaging 45.2%.

Only one litter contained a dead pup. All live pups appeared active and well oxygenated.

Condition of the Litter on Days 1 and 7

All females were observed to nurse and care for their pups following delivery and through the ensuing week. The body weights of pups of naltrexone-treated mothers one day postpartum were 8% lower than those of sham-treated females (Table 2), but were not significantly different on Day 7. (Naltrexone pellets were removed on Day 1.) The relatively small weight gain by pups of naltrexone-implanted females probably can be attributed to a partial inhibition of prolactin release by this drug [45] rather than to deficiencies in maternal care. No other differences between naltrexone- and control-groups were observed (Table 2).

Placentas were not found at any nest site, either attached to pups or in the bedding, on the first postpartum morning, although naltrexone pellets were not removed until after inspection. Although we continued to observe many of the naltrexone-implanted females for some hours postpartum, nevertheless we rarely saw them eat placentas after the 30 min inspection despite the fact that all had disappeared by the next day. We suspect that many placentas were not consumed until the dark phase of the diurnal cycle.

Summary

Placentophagia during parturition was significantly reduced among naltrexone-treated females while other aspects of parturitional behavior appeared unaffected. We next asked whether the reduction in placentophagia was related, directly or indirectly, to interference with endogenous opiate systems or to some other effect of naltrexone. In the following experiment we attempted to replicate the reduction in placentophagia using a different opiate antagonist.

EXPERIMENT 2. EFFECTS OF NALOXONE ON PARTURITIONAL BEHAVIOR

METHOD

Two days before parturition primigravid females were housed individually in clear plastic cages (24×45×23 cm

TABLE 3

LITTERS OF NALOXONE- AND SALINE-INJECTED FEMALES 30 MINUTES FOLLOWING PARTURITION

Treatment	n	Percent of litters containing: Pups with placentas	Detached placentas	Dead pups	Mean litter size	Mean delivery time (min/pup)
Naloxone	8	88*	62	25	11.8	7.8 ± 1.2
Saline	8	12	25	0	13.1	7.5 ± 0.71

*Compared to saline-injected, Fisher's exact test, $p=0.0005$.

with wire tops holding water bottles and food pellets). Pine shavings were provided for litter and bedding. On the day of parturition they were watched closely; when repeated abdominal contractions indicated that labor had begun, the females were briefly removed from their cages and injected SC; alternate females received 10 mg/kg of naloxone (hydrochloride; Endo) in isotonic saline (10 mg/ml) or an equivalent volume of saline only. They were returned to the nest site and observed continuously during parturition. When no pup had been delivered for 30 min, the females were removed to permit inspection of the pups and cage as in Experiment 1. After inspection the females and their pups were reunited and left undisturbed until the following morning when the pups again were inspected. The experiment then was terminated.

The moderately high dose of naloxone was felt necessary because it has been reported that low doses may be effective for only 1–2 hr [5,31] which would not have been sufficient for this experiment.

RESULTS AND DISCUSSION

The only significant difference between naloxone- and saline-injected females or their pups, on the day of parturition or the following day, was in placentophagia as observed 30 min after the last pups had been delivered (Table 3). At that time 8 of 8 naloxone-injected, but only 2 of 8 saline-injected females had failed to consume all placentas, $p=0.003$, Fisher Exact Probability Test. The mean percent of placentas eaten by the naloxone-treated females was 44.5; the two saline-injected females leaving placentas had consumed 85.7 and 57.1%.

The finding that naloxone had the same effect on placentophagia as naltrexone, and the same lack of effect on other variables, suggested that the reduction in placenta-eating was related to opiate blockade rather than to nonspecific effects of the drugs. However, opiate receptor blockers have been reported to interact with other neurotransmitter systems [50]. Therefore, we next investigated whether an induced tolerance to opiates would be as effective as opiate antagonists in reducing placentophagia at parturition.

EXPERIMENT 3: MORPHINE TOLERANCE

Primigravid females, beginning on Day 9 of pregnancy, received daily injections of morphine sulfate (Lilly) to induce tolerance. Injections were given 3 times per day (at 10 a.m., 3 p.m. and 8 p.m.) in accord with the following dosage schedule: 1.5, 3.0, 5.0 and 7.5 mg/kg on 4 consecutive days,

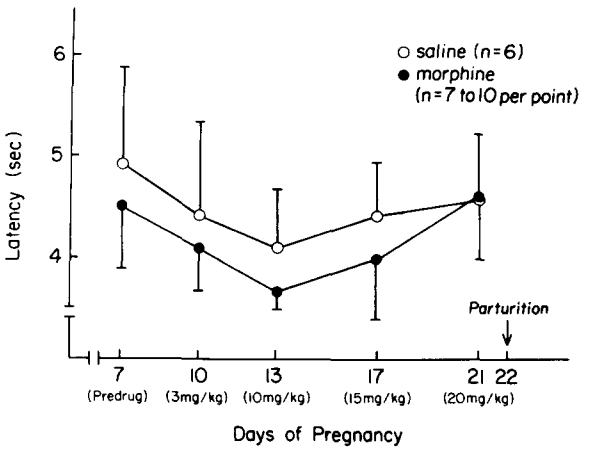

FIG. 2. Mean tailflick latencies (SEM) to warm water (see text) of morphine-injected and saline-injected females over the 15 days prior to parturition. Injections were given three times per day starting on the 8th day of pregnancy; morphine doses (in parentheses) were increased gradually. Latencies were measured 30 min after the third daily injection.

then 10 mg/kg for 2 days, 15 mg/kg for 4 days, and finally 20 mg/kg for the remaining 3 days prior to and through the day of parturition (dosages expressed in terms of the morphine salt). The concentration in the injection vehicle was adjusted at each dose level except the highest to yield an injection volume of 1 ml/kg; at the 20 mg/kg level the volume was 1.3 ml/mg. Control females were injected with equivalent volumes of saline. To determine the onset of tolerance to the analgesic effects of morphine, responsiveness to a painful stimulus was measured by the tailflick to warm (55°C) water test [32] at 3–4 day intervals. Except on the first occasion when pre-drug values were obtained, measurements were made 30 min after the 3rd daily morphine or saline injection. Females were weighed on Day 9 of pregnancy and every 2–3 days thereafter.

One day prior to parturition the females were moved to the clear plastic cages described in Experiment 2. They were observed prior to and during parturition and their litters were inspected 30 min and 1 day after parturition in the same manner as in previous experiments. Injections were continued until termination of the experiment; however when parturition was in progress, scheduled injections were postponed until 30 min following delivery of the last pup.

Several females were eliminated from both groups because they were found not to be pregnant, they appeared unhealthy, or their deliveries were abnormal. Final groups consisted of 10 morphine- and 6 saline-injected females. However, 4 morphine-injected females gave birth unusually early before observers had arrived; therefore data pertaining to placentophagia and delivery durations were obtained on only 6.

RESULTS AND DISCUSSION

Confirmation of Tolerance to Morphine

Tailflick latencies measured 30 min following injection of morphine or saline on Days 10, 13, 17 and 21 of pregnancy did not differ between morphine- and saline-injected groups (Fig. 2). Since doses of 10 mg/kg of morphine (or less) signif-

TABLE 4

LITTERS OF MORPHINE-TOLERANT AND SALINE-INJECTED FEMALES 30 MINUTES FOLLOWING PARTURITION

Treatment	n	Percent of litters containing: Pups with placentas	Detached placentas	Dead pups	Mean litter size	Mean delivery time (SEM) (min/pup)	Mean pup weight (SEM) (g)
Morphine-tolerant	6	67*	67*	50	13.0†	5.8 ± 0.81	5.5 ± 0.17†
Saline-injected	6	0	0	0	12.7	5.2 ± 0.36	5.9 ± 0.07

*Compared to saline-injected, Fisher's exact test, $p=0.03$.
†n=9.

icantly elevate tailflick latencies in non-tolerant females, these data indicate that the treated females were indeed tolerant to the analgesic effects of morphine well before the end of pregnancy. The gradual induction of tolerance produced few symptoms of distress. The appearance and behavior of the morphine-injected females were not noticeably different from those of the control females, except that some females showed a transient increase in activity, particularly gnawing, shortly after each injection. Weight gain during the last days of pregnancy was not less in the morphine-injected group (Fig. 3).

Placentophagia was significantly reduced in the morphine-tolerant group relative to control females (Table 4). Whereas saline-injected females consumed all placentas by 30 min after the final delivery, only 1 morphine-tolerant female did so; the remaining 5 females consumed an average of 33.3% of the placentas delivered (excluding those attached to dead pups). No other differences between these groups were significant.

Effects of Morphine Tolerance on Pup Survival

Although the pups delivered by morphine-tolerant females appeared normal at birth, most did not survive to Day 1; of the first 6 litters born to this group, only 27% of the pups were alive the following day. These survivors had decreased in weight from birth by an average of 3.6%. In contrast, all of the pups born to 5 saline-injected females survived and *gained* 13.6% of birth weight. Subsequently we fostered 6 newborn pups from non-treated females to each of 2 morphine-tolerant females 30 min after parturition; all of these pups survived to Day 1. We also fostered 6 newborn pups from morphine-tolerant females to each of 2 saline-injected females; in one case all died and in the other only 4 survived. These observations, while based on small n's, suggest that morphine-tolerant females are behaviorally competent to care for healthy newborn pups. Their own pups, for whom birth involves opiate withdrawal, have reduced chances of surviving their first 24 hours, presumably as a result of withdrawal.

EXPERIMENT 4: PLACENTOPHAGIA BY NONPREGNANT FEMALES: EFFECTS OF NALOXONE

Reductions in placentophagia by morphine tolerance as well as by opiate receptor antagonists suggest that placentophagia at parturition can be facilitated by endogenous opiate systems. Many *nonpregnant* rats eat placenta avidly if it is available [36]. We next investigated whether placentophagia is reduced in nonpregnant females by an opiate antagonist and by morphine tolerance in order to address the question of whether the reduction is related to the conditions of parturition.

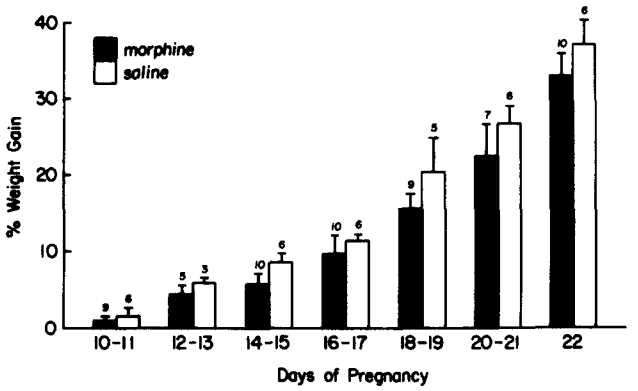

FIG. 3. Mean weight increase (SEM) of morphine- and saline-injected females over the last 12 days of parturition, during which they were receiving SC injections three times per day. Morphine doses were increased gradually, the final dose being 20 mg/kg. Weight gain is expressed as percent of weight on Day 8 of pregnancy, prior to the beginning of the injection schedule. Numbers indicate n's per group.

METHOD

Nonpregnant primiparous females were housed in individual observation cages (Plexiglas, 15×43×24 cm, with hinged front openings and wire mesh floors). The following day they were pretested for placentophagia by presenting each with one placenta on a glass slide; all ate the placentas, as do nearly all primiparous females of our strain. Several days later the females were injected SC either with naloxone (10 mg/kg) or with saline (n=8 per group). After an interval of 40 min, placentophagia tests were conducted in a manner intended to simulate the number and pacing of placentas encountered during parturition—a glass slide holding one placenta was placed in each cage, and thereafter additional

placentas were added every 8 min until 8 had been introduced. The number of placentas present (i.e., uneaten) was recorded each time a new placenta was added and 30 min following the 8th. Placentas for this (and the following) were obtained from females that were decapitated on the morning of the expected day of parturition; the placentas were frozen and held at −20°C until shortly before needed, when they were warmed to approximately 28°C [36].

RESULTS AND DISCUSSION

Within 30 min after the 8th placenta had been introduced, uneaten placentas were present in none of the cages of saline-injected females but in 5 cages of naloxone-injected females, $p=0.013$, Fisher Exact Probability Test. Thus there was a significant reduction in placentophagia by nonpregnant females subsequent to treatment with naloxone. Every naloxone-treated female, however, had eaten at least 6 placentas. The mean number consumed was 7 (87.5%). In contrast, consumption of placentas during parturition had been reduced by naloxone-injection from an average of 12.1 placentas per control female to 4.8 per naloxone-injected female (92.8 vs. 44.5% of placentas delivered).

EXPERIMENT 5: PLACENTOPHAGIA BY NONPREGNANT FEMALES: EFFECTS OF MORPHINE TOLERANCE

METHOD

Nulliparous females were housed in individual observation cages as in the previous experiment, and were injected SC 3 times per day with increasing doses of morphine sulfate (n=9) or with saline (n=10) over 14 days, following the schedule described in Experiment 3. On the 14th day they were tested for placentophagia by introducing 8 placentas at 8 min intervals as in Experiment 4. Finally, tolerance to the analgesic effects of morphine was confirmed 2–3 hr following the placentophagia test by measuring tailflick latencies (to warm water; see Experiment 3) before and 30 min after SC injection of females in both morphine-treated and control groups with 15 mg/kg of morphine.

RESULTS AND DISCUSSION

Morphine-tolerant and saline-injected groups did not differ in percentages eating all 8 placentas (66.7% vs. 60% respectively). However, 1 morphine- and 3 saline-injected females failed to eat placentas at any presentation; a 4th saline-injected female ate 1 only. Nevertheless, even when these 5 nonplacentophages were omitted from the experimental groups no difference emerged between morphine-tolerant and control females; 6 of 8 morphine-tolerant females ate all placentas, and the remaining 2 ate all but 1, while 6 of 6 saline-injected females also ate all placentas.

Tolerance to the analgesic effects of morphine was confirmed by the fact that the tailflick latencies of females chronically treated with morphine were only slightly elevated above baseline 30 min following administration of 15 mg/kg of morphine (8.57±1.37 sec post-drug vs. 5.32±0.47 sec pre-drug), whereas following morphine injection latencies in the nontolerant saline-treated group rose from a pre-drug level (6.12±0.59 sec) not significantly different from that of the morphine-tolerant females to more than 30 sec (cut-off point employed to avoid tissue damage).

These data suggest that morphine tolerance as induced in these experiments does not reduce placentophagia by nonpregnant females.

EXPERIMENT 6: PLACENTOPHAGIA AND PUP-CLEANING

We next asked whether parturient females treated with opiate antagonists avoid placenta exclusively, or also show a reduced consumption of the other afterbirth products—membranes, umbilical cords, and fluids, The former hypothesis might imply that the reduction in placentophagia represented a specific taste aversion; Engwall and Kristal [17] have reported that an aversion to placenta can be induced by pairing placentophagia with illness (lithium chloride injections), and that females treated in this manner show normal parturitional behavior with the exception that placentas are seldom consumed. The latter hypothesis, that *all* afterbirth products are consumed in lesser quantity, would suggest that opiate antagonists disrupt the female's first behavior vis-a-vis their newborn pups. In Experiment 1, the incidence of pup-oriented items of behavior among naltrexone-treated females was only 60% that of sham-treated females (Fig. 1), a suggestive difference although not statistically significant (with n's of 6 per group).

When deliveries occur on a substrate of wood shavings as in prior experiments, pups can be dried and cleaned as they roll about on the absorbent material. To better observe the extent to which the females themselves cleaned their pups, we forced them to give birth without absorbent nest material and inspected the litters 30 min later.

METHOD

Two days before parturition primigravid females were placed in the wire-mesh-bottomed Plexiglas cages described in Experiment 4. They were given narrow strips of black plastic film (from trash bags) which they readily used to construct nests. The following day alternate females were implanted SC with 2 naltrexone pellets (n=7), or sham-implanted (n=6), as in Experiment 1. The females were observed during parturition and the litters and cages were inspected 30 min after the last pups had been born. In addition to recording the number of pups with attached placentas and the number of detached placentas in the cages, pups that were free of placentas were examined for the presence of umbilical cords longer than 1 cm. Finally each pup was examined for wetness and rated on a 3-point scale: 0=clean or retaining only traces of blood or fluids; 2=fluids or blood over more than half the body surface; 1=all others. Dried amniotic fluid was apparent on the skin as a clear, shiny, crinkled coating.

Additional primigravid females were preadapted to clear plastic cages with wire tops, containing wood shavings, and when abdominal contractions indicated that labor had begun, were injected SC with 10 mg/kg of naloxone (n=4) or with saline (n=4) and were placed in similar cages which, however, contained no shavings or other absorbent material. Their pups were examined in the same manner as those of the naltrexone- and sham-implanted females.

RESULTS AND DISCUSSION

Treatment with either naloxone or naltrexone significantly reduced removal of umbilical cords and cleaning of birth fluids and blood from the pups, and inhibited consumption of placentas (Table 5). It appears, therefore, that opiate blockade not only reduces placentophagia per se, but also

TABLE 5

LITTERS OF NALOXONE-INJECTED, NALTREXONE-IMPLANTED AND SHAM-TREATED FEMALES 30 MINUTES FOLLOWING PARTURITION WITHOUT ABSORBENT NEST MATERIAL

		Percent of litters containing:						
Treatment	n	Pups with placentas	Detached placentas	Pups with cords >1 cm	Wet pups	Mean cleanliness rating	Mean litter size	Mean delivery time (min/pup)
Naloxone	4	100*	75*	75*	75*	1.63 ± 0.30†	12.0	6.3 ± 0.8
Naltrexone	7	57*	86*	43*	57*		12.8	7.1 ± 0.6
Saline	4	0	0	25	25	0.94 ± 0.22	12.5	10.4 ± 4.1
Sham-implant	6	0	0	0	17		12.7	6.2 ± 0.7

*Comparisons between Naloxone-injected and Naltrexone-implanted females combined vs. all sham-treated females, Fisher's exact test: Pups with placentas, $p=0.0008$; detached placentas, $p=0.0002$; pups with cords >1 cm, $p=0.04$; wet pups, $p=0.056$.

†Pups were rated individually on a 3-pt scale according to how much body surface remained coated with birth fluids; 0: traces only; 1: <1/2 coated, 2: >1/2 coated. Comparing naloxone-injected and naltrexone-implanted females combined vs. all sham-treated females, $t(19)=2.15$, $p<0.05$.

adversely affects the extent to which the pups are licked and cleaned.

EXPERIMENT 7: A LOW DOSE OF OPIATE ANTAGONIST

The doses of naltrexone and naloxone used in the previous experiments have been reported to suppress feeding and drinking in both deprived [3, 5, 6, 23, 31, 42, 47, 52] and non-deprived rats [3, 8, 10, 37, 41, 49], and thus may have reduced placentophagia and pup cleaning through a general suppression of ingestive behavior. The fact that morphine tolerant rats consumed less placenta than control females during parturition but did not consume less when nonpregnant argues against attributing the reduction in placentophagia by opiate blockade solely to effects on ingestion, independent of the conditions of parturition. Moreover, the morphine tolerant pregnant females that consumed less placenta cannot have been experiencing a generalized diminution of appetite, since their weight gain over the last days of pregnancy equaled that of saline-injected females (Fig. 2). Nevertheless, relatively high doses of opiate antagonists, and perhaps also the state of morphine tolerance, may produce malaise or dysphoria. Indeed it has been shown that 5 mg/kg or more of naloxone injected after ingestion of a novel flavor will support a conditioned taste aversion [23,55]. Moreover, naloxone at 10 mg/kg and above has been reported to decrease spontaneous activity [15] and interactions with the environment [1]. Thus the possibility remained that the reduced placentophagia and pup cleaning by the three groups subjected to blockade of endogenous opiate systems was a pharmacologic effect, without implications regarding a natural role of endogenous opiates at parturition. Therefore in a final experiment parturient females were treated with 0.1 mg/kg of naloxone. This dose will block the analgesic effects of endogenous opiates [9] or 10 mg/kg of morphine [58]. In most paradigms, however, it is at or below the threshold for effects on feeding or drinking [48]; it will not support a conditioned taste aversion [23], and is below the threshold for effects on general activity [1,15]. In fact Arnsten and Segal [1], found that 0.5 mg/kg of naloxone enhanced interaction with environmental stimuli. Naloxone-injected females again were compared to saline-injected controls for placentophagia and pup cleaning.

METHOD

Procedures were as in Experiment 2 in which females were treated with 10 mg/kg of naloxone or saline, with the following changes; (1) in each group of 10 females, 6 had previously borne litters; (2) rather than injecting the females when parturition was judged to be imminent, injections (0.1 mg/kg naloxone in saline or saline only) were given SC after delivery of the first pup. This was done in anticipation that the period of action of the reduced dose would be relatively brief [5,31]. (Intervals between injection and delivery of the last pup in Experiment 2 averaged 136 min); (3) 30 min following the final delivery the pups were inspected not only for attached placentas but also, as in Experiment 6, for umbilical cords longer than 1 cm.

RESULTS AND DISCUSSION

The percentage of pups that were not completely cleaned of placentas and umbilical cords (cord stumps longer than 1 cm) was significantly higher in the naloxone-treated than in the saline-treated group; naloxone-treated mothers failed to completely clean 28% of their pups, whereas saline-injected mothers failed to clean 11.5%, $U=12$, $p<0.02$. The effects of naloxone treatment on the consumption of placentas, per se, was less clear. The naloxone-treated females overall consumed an average of 87.5% of the placentas delivered, whereas the saline group consumed 94.9%. Half of the naloxone-treated females had not eaten all placentas 30 min following parturition, but 2 (of 10) saline-treated females also had not consumed all placentas. This difference was not statistically significant. On the other hand, if we consider all the sham-treated parturient females tested during these experiments (i.e., the control groups of Experiments 1, 2, 3, 6 and 7), only 15% (40/47) failed to eat all placentas. The difference between this group and the naloxone-treated females of Experiment 7 is significant; $\chi^2=6.1$, $p<0.02$. Hence the hypothesis that naloxone at 0.1 mg/kg reduces placenta-eating remains tenable.

Prior parturitions did not reduce the effect of naloxone on pup cleaning; comparing experienced females only (6 per group), those receiving naloxone did not completely remove placentas and cords from an average of 26.5% of their pups whereas experienced females receiving saline failed to completely clean only 5.4%, $U=2$, $p<0.005$.

The conditions of this experiment provide a more rigorous test of the hypothesis that opiate antagonists reduce pup cleaning at parturition, and also of the hypothesis that this effect is mediated by blockade of endogenous opiates which otherwise support the behavior. The subjects included experienced females, whose maternal behavior in other respects has been found to be less easily disrupted than that of females delivering for the first time [2, 19, 44, 51]; the dose of naloxone was low; and the drug (or saline) was injected after one delivery so that at least the first pups were born before the naloxone had diffused to the relevant tissues. The results confirm both hypotheses. They do not, however, differentiate between the possibilities that the relevant action of the antagonist is blockade of opiate mediated analgesia or interference in an opiate-mediated system supporting ingestive behavior.

GENERAL DISCUSSION

In summary, naloxone (at even a low dose), naltrexone, and tolerance to the analgesic effects of morphine all reliably reduced placentophagia and/or pup cleaning by parturient rats. Thus the blockade of endogenous opiate systems appears to have a relatively specific, disruptive impact on maternal behavior during delivery: pups receive less licking and cleaning and fewer placentas are removed and eaten. Females, nevertheless, are responsive to pups: they gather them at the nest site and crouch over them to allow suckling. Under the conditions of these experiments, which favored the maintenance of maternal behavior, temporary opiate blockade lasting only through parturition had no effects on pup survival or weight gain over the first 24 hours that were attributable to the quality of maternal care. However, since Denenberg, Holloway and Dollinger [14] have shown that the quality of care received by rat pups during the first 12 hours influences their subsequent development, the possibility that opiate blockade during parturition can have lasting effects on the offspring deserves further study.

Bridges and Grimm [4,29] have shown that moderate doses of morphine delay the onset of maternal behavior and disrupt ongoing maternal behavior displayed by pregnancy-terminated rats toward test pups. Maternal responsiveness was restored to the levels of control females by naloxone, indicating that the morphine-induced disruption was mediated by an opiate receptor mechanism. They have proposed that "lower opiate concentrations at the time of parturition are essential for maternal behavior" [4]. Though not intended to test this hypothesis, our results with respect to pup-oriented activities are contrary to those it would predict: Females giving birth while opiate systems were antagonized were not more responsive to their pups but engaged in *less* pup licking and cleaning, indicating that opiate systems play a positive rather than negative role in at least these aspects of maternal behavior.

While the data indicate that endogenous opiate systems play a significant role in supporting pup cleaning and consumption of placentas at parturition, it is not clear that the reduction in these activities by opiate blockade is exclusively a reduction in an aspect of maternal behavior; it may also constitute an instance of the known involvement of opiate systems in ingestive behavior (for a recent review see [48]). It is clear that there are close links between ingestive and maternal behavior at parturition: In the course of eating the afterbirth the parturient female severs the umbilical cord, clears material from the pup's face that might obstruct breathing, stimulates respiration by body licking, cleans itself and the nest site, and so forth. Kristal [36] has shown that placentophagia under some conditions is influenced by factors that affect other ingestive behavior, while under other conditions it responds to factors that influence maternal behavior. A possibly similar intertwining of ingestive and maternal behavior occurs during the period of lactation. Rat pups urinate reflexively when stimulated by the mother's licking of their anogenital areas, during which she consumes their urine [22]; her anogenital licking of the pups has been found to be supported by an enhanced salt appetite [30]. Thus it may be that endogenous opiates support pup cleaning and placentophagia during parturition by generally stimulating appetite, or perhaps by altering taste perception to increase the attractiveness of afterbirth products. Mediation of opioid effects on ingestion by alteration in taste or taste preferences has been implicated in several studies [38, 39, 47].

Under some conditions opiate antagonists and tolerance to morphine produce hyperalgesia in animals subjected to pain [9,53]. Therefore it is possible also that the reduction in pup cleaning and placentophagia by opiate blockade is mediated by stress. It has been suggested repeatedly that some degree of analgesia normally accompanies parturition [12, 13, 18, 20]. The experimental evidence for this appealing assumption, however, as yet is limited to reports in the rat that flinch-jump thresholds (to footshock) rise prepartum ([25,26]. Fletcher *et al.* [21], however, found that flinch-jump thresholds remained constant during late pregnancy as did tail-flick in the present study (Experiment 3, Fig. 2). The mechanisms modulating nociception are complex, and it is not uncommon for analgesia or effects of specific opiate antagonists to be documented by one test but not by another [11, 32, 57]. Also, there is no reason to assume that pain thresholds during the last days of pregnancy necessarily predict pain thresholds during parturition. Nevertheless, the evidence now at hand does not warrant concluding that all rats experience significant analgesia during parturition.

The young rats used in the reported experiments appeared to give birth easily; behavior indicative of stress was seldom observed whether or not they had been subjected to opiate blockade. When such females commence parturition, pain-modifying mechanisms may be activated minimally or not at all [40]. On the other hand, during pilot work we implanted several older "retired breeders" with naltrexone pellets and gained the impression that these females experienced significant stress during labor; they showed considerable piloerection, kept their eyes closed for prolonged periods, intermittently showed quivering of the jaw muscles, and often left the nest site in the midst of delivery, trailing masses of uncleaned pups and placentas behind them. It seems possible, therefore, that endogenous opiate systems play a particularly important role in reducing stress during difficult deliveries, stress which if not reduced could lead to behavioral abnormalities. In this regard, the analgesia produced by vaginal stimulation [35] may contribute an opiate-mediated [32,54] stress-reducing component to parturition.

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