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Morphine and Naloxone's Effects on Sexual Behavior of the Female Golden Hamster[1]

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OSTROWSKI, N.L., J.M. STAPLETON, R.G. NOBLE AND L.D. REID. *Morphine and naloxone's effects on sexual behavior of the female golden hamster.* PHARMAC. BIOCHEM. BEHAV. 11(6)673–681, 1979.—The effects of morphine and naloxone were observed after administration to female golden hamsters (*Mesocricetus auratus*). Large doses of morphine, 80 mg/kg, consistently produced sedation and behavioral depression of responses to nociceptive stimuli. Smaller doses of morphine (e.g., 10 mg/kg), that produced few other behavioral changes, suppressed a measure of female sexual responding. The suppressive effects on sexual behavior were reversed by 4 mg/kg of naloxone. Morphine administered intracerebroventricularly had little effect on sexual responding, even at doses which produced other side effects. Doses of 4 and 8 mg/kg of naloxone in opioid-naive subjects did not reliably alter sexual responding up to 2 hr after administration. These observations lead to the suggestion that morphine produces effects which are incompatible with full sexual functioning in female hamsters.

Sexual behavior Hamsters Morphine Naloxone Opioids

A GROWING body of evidence indicates that opioids modify reproductive functioning. Acute or chronic administration of narcotic agonists decreases serum luteinizing hormone (LH) and testosterone levels in male rats [3, 9, 10, 11, 12] and in human beings [21,22]. Chronic morphine administration results in the atrophy of accessory reproductive organs in male rats [8, 12, 13, 38]. The detrimental effects of narcotics on reproductive functions of males are thought to result in part from the critical actions of the opioids at the level of the hypothalamus [4, 9, 11, 12].

Behavioral changes in copulatory performance have also been demonstrated in male rats after chronic administration of morphine [38], and after acute administration of morphine [16], β-endorphin [23] and *d*-ala²-metenkephalinamide [34]. The administration of opioid agonists has been reported to produce behavioral deficits in copulatory performance at doses which do not produce apparent decreases in motor activity [16, 23, 34]. It has also been suggested that the opioid antagonist, naloxone, may improve copulatory functions in male rats [14,16].

The effects of morphine on female reproductive functioning have not been studied as extensively. Decreases in LH levels have been reported after morphine administration, but larger doses of morphine are required to produce this effect in female rats [12,31]. There is also evidence to suggest that a small dose of morphine may stimulate LH release in female rats while the same dose inhibits LH release in males [12, 32, 33]. Other effects of opiates, including inhibition of ovulation, have been reported [2, 3, 31], but there has been little experimental investigation of the effects of acute or chronic opioid administration on sexual performance in females. Given the profound effects seen in male's copulatory behavior after administration of morphine, we thought it of interest to observe female sexual behavior after morphine using a sensitive measurement system, to index sexual responsivity, in addition to measures of lordosis.

The golden hamster, because of the consistency of its copulatory episodes, is a particulary attractive rodent to use in studies of female sexual behavior [5, 6, 37]. A system for obtaining measures of major dimensions of sexual behavior in the hamster has been developed [28,29]. While Houchin [17] reported that doses of morphine up to 150 mg/kg produced no discernible effects in hamsters regardless or age, weight or sex, our pilot data indicated that much smaller doses of morphine did produce a variety of effects. Preliminary observations, for example, suggested that doses of morphine which did not produce gross motor impairment in female hamsters were adequate to produce alterations in sexual responses.

The first experiment was conducted to assess the effects of morphine in the female hamster. Motor functions and responses to nociceptive stimuli were observed in addition to sexual behavior. The second experiment investigated, in more detail, the effects of several doses of morphine on female sexual behavior and determined whether the putative morphine-effects were reversed with naloxone. The effects

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of intraventricularly administered morphine were assessed in an attempt to localize the site of morphine's action on sexual behavior. Finally, experiments were conducted to determine whether naloxone, in opioid-naive subjects, produced effects on measures of female sexual functioning.

EXPERIMENT 1

Because previous work [7,17] and our own pilot data suggested that hamsters might be refractory in their responses to morphine, we administered a range of doses. The purpose of this experiment was to determine whether morphine produces measurable behavioral effects in hamsters including effects on sexual responding.

METHOD

Subjects

Twenty-five, sexually experienced female hamsters (*Mesocricetus auratus*) were used. The hamsters were purchased from Engle's Laboratory Animals, Inc. (Farmersburg, Indiana) at 90 days of age, and housed in groups of four per cage in solid-bottom, plastic cages (38×33×17 cm) on corn-cob bedding (Sanicel). Cages were in a large colony room maintained on a reverse light-dark cycle (LD 14:10) and this room was kept at 22° C. Sexually active male hamsters, housed in the same room, were used as stimulus males in tests of sexual behavior. Standard laboratory chow and tap water were available continuously.

Females were ovariectomized under sodium pentobarbital anesthesia at least 2 mo prior to morphine tests. At the time of surgery each subject was implanted, subcutaneously (SC), with a Silastic-capped metal tube (1 cm length) containing 17-β-estradiol in crystalline form. Similar implants have been calibrated to provide constant circulating levels of estradiol which approximate those observed in proestrous females [27]. Each female was then injected with progesterone (0.5 mg in 0.1 ml of corn oil, SC) 4 hr before testing to induce behavioral sexual receptivity.

Procedure

Testing began at 1900 hr and was conducted in three well-illuminated rooms. Subjects were randomly assigned to groups. Thirty min before a series of tests, each subject received an intraperitoneal (IP) injection of morphine. The doses used were 0, 10, 20 or 40 mg/kg dissolved in 1 ml/kg of physiological saline, or 80 mg/kg of morphine dissolved in 2 ml/kg of saline. Five subjects were tested at each dose. The experimenters conducting the tests were unaware of the group assignments.

Each hamster was tested for righting reflex, leg withdrawal reflex, immobility, exploratory activity, general motor activity, sexual behavior and responses to nociceptive stimuli in that order. Righting reflex was judged impaired if the subject failed to right within 0.5 sec after being placed on its back. The test for leg withdrawal reflex was modeled after tests of waxy flexibility in rats [18]. The hamster's hind paws were extended and if the animal failed to retract them within 30 sec, leg withdrawal reflex was judged impaired. To test for immobility (catatonia/catalepsy) the hamster's front paws were placed over a narrow rod suspended 7 cm from the surface of the table and the time that the animal remained in this position was recorded. If the animal did not retract its forepaws within 60 sec, immobility was scored as present. Exploratory activity was tested by placing the animal in a C-shaped alley (280×12.5×26 cm) for 2 min. The farthest forward distance that the animal travelled was recorded to the nearest 5 cm. General motor activity was tested in a standard shuttle box (48×21×23 cm). The number of shuttles in a 2-min period was recorded.

To test for sexual behavior, the female was placed in a 5.5 gal glass aquarium and exposed to a sexually active male hamster until the female assumed lordosis or failed to assume lordosis within 3 min. Males were not permitted to mount the female during this time. The glass aquarium provided consistent olfactory stimulation.

If the female assumed a lordotic posture, the experimenter maintained this position by manually stimulating the female's back and flanks. Lateral displacement was measured by applying standardized tactile stimulation to the subject's perineal region and measuring the magnitude of pelvic adjustment. Prior to time of testing, hair of the perineal region was shaved off to allow localized application of stimuli and unhampered observation.

Four calibrated Semmes-Weinstein aesthesiometric probes (pressures ranging from 20.7 to 1.83 g of pressure) calibrated in equal log-unit steps were used as the stimulators. Pulsatile applications of these instruments to the female's perineal region (outer zone = ±0.7 cm from midline, inner zone = ±0.2 cm from midline) typically produces a lateral movement of the subject's hindquarters toward the stimuli. The pelvic adjustment facilitates insertion by the male during coitus [28]. The magnitude of this response is measured by aligning a ruler under the perineal region and using the ano-vaginal midline as the reference point.

The magnitude of this response has been demonstrated to be a good index of the female's state of sexual responsivity. For example, even though a female may assume lordosis under many circumstances, there is little information inherent within this behavior to predict how responsive the female is to the male, or, how long she will continue to be responsive. Pregnant females, females which have just been mated, and, females close to the termination of their estrous period may assume lordosis, but the magnitude of lateral displacement is severely depressed or absent. This apparently functions to reduce the probability of non-fertile copulation. Conditions under which lateral displacement is depressed are associated with termination of estrus, sudden aggression toward the male and less solicitation behavior [40]. Proceptivity fluctuates in a manner similar to lateral displacement; increasing about mid-estrus and gradually declining toward the end of the estrous period in unmated females.

This measure, of an active contribution of the female to insertion by the male during coitus, can be used to discriminate among apparently receptive females. Lateral displacement, then, is a sensitive index of female sexual responsivity after the female demonstrates lordosis behavior [28,29].

To obtain measures of lateral displacement, each of 4 aesthesiometric probes was applied twice to each of the following regions: The left outer zone, the right outer zone, left inner zone and right inner zone. Means were calculated for the responses to each, which yielded 4 scores for the outer zone and 4 scores for the inner zone for each animal. These scores were summed to index outer zone responsivity and inner zone responsivity. The maximum response given by an animal was also recorded. The response threshold was also obtained and was defined as the least pressure to which an animal gave a response greater than 0. These measures of lateral displacement, (inner and outer zone sums, maximum

response, and, thresholds) are highly correlated with each other (rho approximates 0.90). Inner zone sums of lateral displacement are reported in this paper to summarize results.

If an animal failed to assume lordosis and lateral displacement measures could not be obtained during a given test, the animal was assigned a score of 0 for purposes of statistical analysis. The number of subjects failing to assume lordosis is also reported.

After measures of sexual responding were obtained, a test was conducted to determine the animal's responses to nociceptive stimuli. This was done by placing the female on a warm plate maintained at 43°–44°C which was surrounded by a wire mesh (1 × 1 cm mesh, 20 cm high). The latencies to (a) a clear, characteristic paw shake, and, (b) an escape from the heated surface by climbing onto the wire mesh were recorded. If no attempt was made to escape, testing was terminated after 180 sec. No swelling or tissue damage has been observed with this procedure.

To summarize, 30 min after injection with morphine or placebo, each female was tested for righting reflex, leg withdrawal reflex, immobility, exploratory activity (C-shaped alley), shuttle-box activity, sexual behavior and responses to nociceptive stimuli. The series of tests was completed in under 15 min for each animal.

Fischer's exact probability test was used to assess scores of subjects receiving morphine compared to those receiving placebo when test results were scored as dichotomous variables. Otherwise, analyses of variance (ANOVAs) were used. When an ANOVA indicated that there was a drug effect, post-hoc comparisons were made using the Newman-Keuls procedure.

RESULTS AND DISCUSSION

Morphine, at least at some doses, produced impaired performance on all tests. One animal of the five tested at 20 mg/kg of morphine demonstrated an impaired righting reflex. Four hamsters tested at 40 mg/kg ($p<0.03$) and all 5 hamsters tested at 80 mg/kg ($p<0.01$) also showed impaired righting. Animals receiving 0 or 10 mg/kg of morphine did not show any impairment of the righting reflex. The leg withdrawal reflex was also impaired in 2 animals tested at 40 mg/kg of morphine and in 4 animals receiving 80 mg/kg ($p<0.03$). Immobility was present in only 1 animal after 40 mg/kg of morphine, but was present in all 5 subjects given 80 mg/kg ($p<0.01$).

Morphine also decreased forward movement in the alley, $F(4,20)=7.45$, $p<0.001$, with post-hoc comparisons indicating that 40 and 80 mg/kg produced reliable decreases compared to the other groups ($p<0.05$) (Fig. 1, Panel A). The number of shuttles was decreased in a dose dependent fashion by morphine, $F(4,20)=4.93$, $p<0.005$, with the post-hoc comparisons indicating that the scores of the group receiving 80 mg/kg of morphine were reliably different from the scores of other groups ($p<0.05$) (Fig. 1, Panel B).

The latency to shake a paw on the warm plate increased with increasing doses of morphine, $F(4,20)=3.88$ $p<0.02$, (Fig. 1, Panel C). A comparison of group means indicated that the 80 mg/kg group differed from placebo group and from groups receiving 10 and 20 mg/kg of morphine ($p<0.05$). The latency to escape from the heated surface differed among groups, $F(4,20)=3.75$, $p<0.02$, (Fig. 1, Panel D). In this case, 40 and 80 mg/kg produced reliable increases in the latency to escape ($p<0.05$) compared to groups receiving other doses.

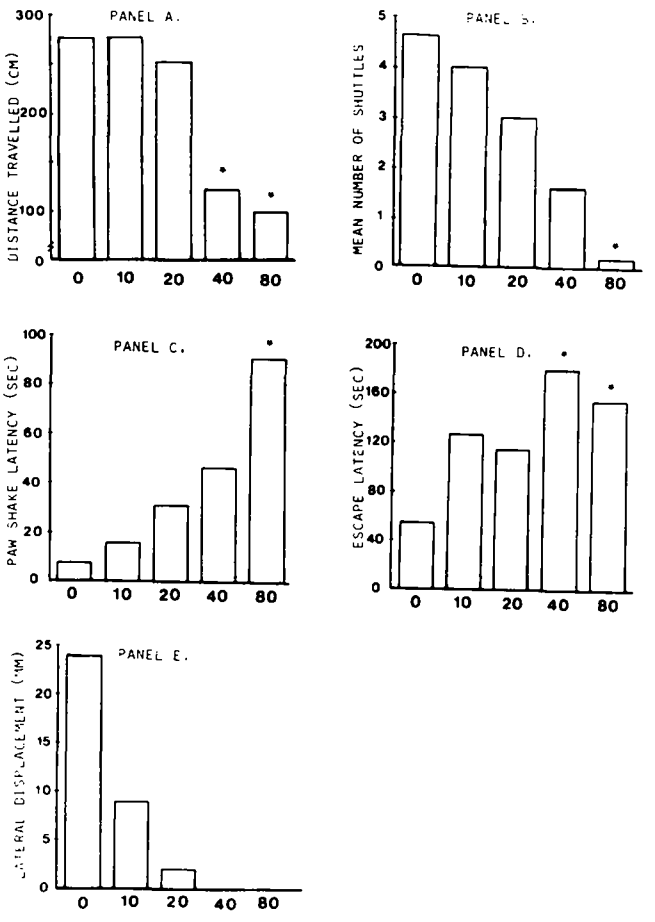

FIG. 1. Female hamsters show behavioral impairment with high doses of morphine. Depicted are means for subjects receiving various doses of morphine, as designated on the abscissa (mg/kg of morphine sulfate). Panels A–E represent data obtained from behavioral tests for motor responses, responses to nociceptive stimuli and sexual responding. Asterisks indicate group means are reliably different ($p<0.05$) from groups receiving 0 and 10 mg/kg of morphine.

Contrary to a previous report [17], this experiment demonstrates dose-dependent effects of morphine in the hamster. These data and pilot data comparing hamsters to rats on the same tests suggest, however, that motor impairments are seen in the hamster only with large doses of morphine. As can be seen in Fig. 1 (Panel E), however, doses of morphine produced profound effects on sexual behavior.

All animals receiving placebo maintained lordosis but 5 of the 20 subjects receiving morphine did not. When these 5 subjects were assigned scores of zero for lateral displacement, lateral displacement scores decreased with increasing doses of morphine. When these scores were submitted to an ANOVA, the resultant $F(4,20)=2.74$, $p<0.057$. Subjects receiving 40 mg/kg either would not maintain lordosis (3 of 5) or showed no lateral displacement. Even though all subjects receiving 80 mg/kg of morphine maintained lordosis, there was no displacement in response to tactile stimulation by any animal.

EXPERIMENT 2

This experiment was done to provide more information concerning morphine's effects on female sexual responding. We were particularly interested in testing whether morphine modified lordosis and produced dose-dependent reductions in lateral displacement and whether these putative effects could be reversed by naloxone.

METHOD

Subjects

Twenty-eight, sexually experienced, opioid-naive female hamsters were used as subjects. Subjects were ovariectomized about 60 days prior to testing. The conditions of housing and hormone administration were similar to those in Experiment 1. Progesterone (0.5 mg) was injected 4 hr before the beginning of testing.

Procedure

The subjects were randomly assigned to four groups (n=7) to receive 0, 10, 30 or 60 mg/kg of morphine sulfate in 1 ml/kg of physiological saline (SC). Only females in behavioral estrus were used. Each female was tested before and 15 min after injection with morphine or placebo. Immediately after the second test, each female was injected with 4 mg/kg of naloxone hydrochloride, SC, and tested again 15 min after naloxone injection. Consequently, each group was tested three times with 15 min separating each test.

Females were tested for lordosis duration and lateral displacement at each of the three test periods. Lordosis durations were obtained by exposing the female to a sexually active male hamster for up to 3 min with no mounts permitted during this time. If a subject assumed the lordosis posture, the experimenter applied manual stimulation to the animal's flanks for 10 sec. The time from termination of manual stimulation until the female moved a paw from the floor of the chamber was recorded as a measure of lordosis duration. This procedure was repeated 3 consecutive times to yield a mean score which was recorded as the duration of lordosis for a subject. Lateral displacement scores were obtained in the same manner as described in Experiment 1.

To determine if there were drug-effects, ANOVAs were used, with repeated measures where appropriate. When an ANOVA indicated that drug-effects were present across groups, Newman-Keuls tested were performed to compare specific groups' scores. As in Experiment 1, only data for inner zone sums are reported to index lateral displacement since the various indices are highly correlated and conclusions are similar regardless of which index is analyzed.

RESULTS AND DISCUSSION

All females displayed lordosis during baseline testing before injections. Following injections with morphine, one female receiving 30 mg/kg and one receiving 60 mg/kg failed to assume lordosis or failed to maintain lordosis during tests for lateral displacement. These subjects were assigned scores of zero for lateral displacement on the morphine-test for purposes of statistical analyses. After naloxone injections, all females again assumed lordosis.

The mean lordosis duration score was 44 sec across all subjects and tests. When scores were submitted to an ANOVA, there were no main effects or interactions that were statistically significant, leading to the conclusion that morphine has little or no effect on lordosis duration, one of the typical measures of female sexual behavior.

On tests of lateral displacement, morphine decreased responding, with larger doses producing dramatic decreases, and these effects were reversed by naloxone. The scores obtained for inner zone sums are summarized in Fig. 2. With the scores used to derive the means of Figure 2, a 4 × 3 ANOVA having repeated measures indicated that there were no reliable group differences, $F(3,24)=2.48$, $p=0.085$. There was a reliable test-effect (baseline, after morphine, after naloxone), $F(2,48)=48.9$, $p<0.001$, and a group × test interaction, $F(6,48)=5.09$, $p<0.001$. Tests for simple main effects indicated that inner zone displacement scores did not differ significantly among groups at baseline, $F(3,24)=0.87$, $p>0.5$, or at tests after naloxone injections, $F(3,24)=0.96$, $p>0.5$, but did differ when groups were under morphine, $F(3,24)=17.8$, $p<0.001$. Further comparisons indicated that the scores of all groups receiving morphine (10, 30 and 60 mg/kg) reliably differed from the scores of the control group ($ps<0.05$).

As can be seen from Fig. 2, mean scores at baseline are not reliably different from mean scores after naloxone injections. Furthermore, there was a high correlation ($r=.91$, $p<0.005$) between scores of baseline and scores taken after naloxone injections, suggesting that regardless of the animal's baseline level of responding and the amount of morphine administered, naloxone was effective in reinstating the relative ranking of a subject.

These data indicate that lateral displacement is suppressed by morphine but that morphine does not have dramatic effects on lordosis duration at the doses used. Furthermore, morphine's effects are reversed by naloxone. A dose of morphine (10 mg/kg) that was effective in depressing lateral displacement was not effective in altering behavioral responses on a variety of other tests (Experiment 1).

It is possible that hamsters require higher doses of morphine because they dispose of opioids quickly or in a manner dissimilar to other rodents. In this experiment, tests were made of lateral displacement at 30 min after morphine (SC), and all subjects received naloxine prior to this test. These measures, after morphine and naloxone, were very similar to baseline responding. It can be asked whether the subjects are merely recovering from the effects of the drug and, thus, not demonstrating naloxone reversal of the drug effect. It should be noted, however, that in Experiment 1 lateral displacement was measured about 40 min after morphine administration (IP), and, at this time, subjects were still profoundly affected by morphine. Other data [in prep.] supports the contention that morphine is still exerting behavioral effects in hamsters at times extending well past times when tests were made in the experiments reported here. These results lead to the suggestion that lateral displacement (a contribution of the female hamster to mating) is particularly vulnerable to morphine administration and is an extremely sensitive index of drug-effects.

EXPERIMENT 3

It is evident that one component of the female hamster's sexual response, lateral displacement, is suppressed by doses of morphine which do not produce other motor impairments.

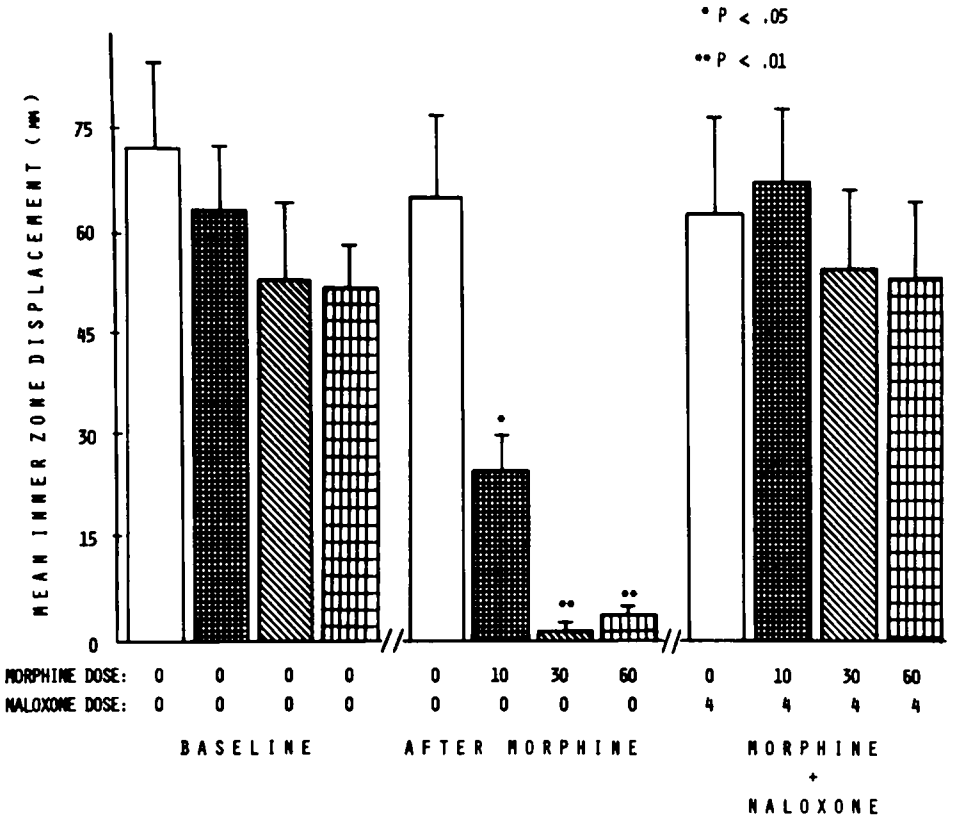

FIG. 2. Morphine, in doses of 10, 30 and 60 mg/kg, decreases lateral displacement in female hamsters. This decrease is reversed by injection with 4 mg/kg of naloxone. Depicted are means and standard errors of the means for groups before drug injection (on the left), after the appropriate dose of morphine (middle panel) and after injection with naloxone (on the right). Asterisks denote scores reliably differ from baseline scores.

It was reasoned that if the morphine-produced attenuation of sexual responding reflected a central nervous system site of action, then intracerebroventricular (ICV) administration of morphine should produce similar, if not more dramatic reductions in the magnitude of lateral displacement.

METHOD

Subjects

Subjects were 15, experimentally naive, female hamsters weighing about 120 g at the start of procedures. Animals were housed in groups prior to surgery and individually after surgery in the same colony room as subjects of the other experiments.

Each hamster was stereotaxically implanted with a 23-ga stainless steel guide cannula aimed so that infusion of fluids would reach the lateral ventricle (Coordinates: 1.0 mm anterior to bregma, 1.7 mm lateral to the midline, 2.1 mm below the surface of the dura, with bregma and lambda in the same horizontal plane) [20]. Skull screws and dental acrylic were used to hold the guide cannula in place. At the time of surgery, a 30-ga stainless steel stylet, 0.5 mm longer than the guide cannula was inserted into the cannula and left in place except during infusions.

At the time of the stereotaxic surgery, each animal was ovariectomized and implanted with 17-β-estradiol, as described in earlier experiments. Surgery was done using sodium pentobarbital anesthesia, with atropine sulfate as an adjunct and supplemental ether anesthesia when necessary.

After completion of behavioral testing, hamsters were sacrificed using an overdose of sodium pentobarbital and intracardially perfused with physiological saline followed by a 10% Formalin solution. For histological verification of cannula placements, 90-μ frozen sections of brain were treated as photographic negatives to produce an enlarged (10X) photograph of the section.

Procedure

Subjects were permitted at least 5 days recovery from surgery prior to testing. Progesterone (0.5 mg, SC, dissolved in 0.1 ml of corn oil) was injected 4 hr before the beginning of testing to induce behavioral receptivity.

Subjects received ICV infusions of 0, 1, 4 or 16 μg of morphine sulfate in 2-μl of 0.9% saline. Each subject was tested under all treatment conditions and received one dose per test session. Test sessions were at least 5 days apart with animals assigned to receive each dose in a manner which controlled for the order of treatments.

Infusions were done using a 10-μl syringe (Hamilton no.702) equipped with a 30-ga injection cannula, 1.0 mm longer than the guide cannula.

Tests were for latency to assume lordosis, duration of lordosis and lateral displacement. Measures of lordosis

latencies were obtained by placing a sexually active male on the female's back and permitting the male to investigate the female but not permitting the male to mount. The time from initial contact between the male and female to when the female assumed an immobile posture with her tail upright was measured. The mean of three consecutive trials, separated by about 15 sec, was used as the lordosis latency.

Measures were obtained as described, immediately before, and, at 5, 15, 30, 60 and 90 min after infusion.

RESULTS AND DISCUSSION

Difference scores were derived by subtracting the baseline scores prior to infusion for each hamster, from the corresponding scores after infusion. Data were collapsed across order of drug treatments and analyzed using repeated measures ANOVAs with factors for 4 drug treatment conditions (0, 1, 4 or 16 μg) and 5 measurements (5, 15, 30, 60 and 90 min after infusion). For lateral displacement scores, there was a third factor for the 2 zones (inner and outer).

One subject receiving saline and one subject receiving 4 μg of morphine on the first test failed to assume lordosis following the first infusion. Analyses with the data of these subjects deleted or with the missing data scored as 0 yielded similar results.

A 4 × 5 repeated measures ANOVA on the difference scores for latency to assume lordosis yielded no significant main effects or interactions, all Fs <1.30, ps >0.50.

Similar analyses of the difference scores for the duration of lordosis yielded no significant effect of drug treatment or drug × trial interaction, Fs <1.0, ps >50. There was a significant effect for the factor of measures after infusion, $F(4,48)=4.41$, $p<0.005$, indicating that the duration of lordosis increased with repeated testing, regardless of drug treatment.

Analyses of the difference scores for lateral displacement indicated that ICV morphine did not reliably modify this measure of female sexual responding. A 4 × 5 × 2 repeated measures ANOVA yielded a significant effect for the factor of zone, $F(1,12)=9.40$, $p<0.02$, indicating higher scores for the inner zone. No other main effects or interactions were reliable sources of variance.

Since there were no effects of 0, 1, 4 or 16 μg of morphine (ICV) on measures of female sexual responding, we tested 12 of the subjects at higher doses (0 or 64 μg and 0 or 128 μg). These tests yielded similar results, indicating no effect of ICV morphine on latency to assume lordosis, lordosis duration or lateral displacement even at doses which produced apparent side effects in some animals. The most pronounced side effect was a muscular twitch confined to one side of the body. Such twitching was never seen after administration of saline or the lower doses of morphine. At 64 or 128 μg, some animals also showed sedation and gave erratic lateral displacement scores. Data analyses done deleting animals showing such side effects yielded similar results.

Histological analysis revealed a good deal of necrotic tissue in and around the ventricle in most animals. Some animals sustained damage to the cortex, caudate nucleus or septal area. In four hamsters the guide cannula failed to reach the lateral ventricle with the tip lying dorsal to the corpus callosum. Data analyses done with these animals deleted yielded similar results leading to the same conclusions.

It is possible that later infusions might have failed to reach the ventricular system due to the buildup of necrotic tissue. For this reason, 10 experimentally naive female hamsters were tested with only one dose of morphine (32 μg) or saline. There was no effect of morphine on sexual responding in these animals whose histologies showed relatively little necrotic tissue.

Collectively, these data indicate no significant effect of these doses of morphine on sexual responding in female hamsters. Scores after morphine are consistently higher than preinfusion baseline scores. There is no evidence of the clear suppression of responding produced by systemic morphine in Experiment 2. Conclusions based on these data, however, must be considered tentative. There is little data on the effects of opioids in hamsters and little experience with ICV administration of drugs in this species. It is possible that insufficient amounts of morphine reached the appropriate brain sites with the doses and infusion technique used. With this reservation, these data suggest that morphine might be acting at a peripheral site, possibly in the spinal cord or in other tissues to produce the decrease in lateral displacement seen after systemic administration of morphine.

EXPERIMENT 4

Recently, there have been reports that naloxone, when administered to opioid naive subjects, affects reproductive functioning. Some of the most consistent results indicate that naloxone increases luteinizing hormone (LH) [2,3], presumably by increasing luteinizing-hormone releasing-hormone (LHRH) [2, 9, 11]. LHRH has been shown to facilitate sexual behavior in several species [24,25] but not in hamsters (Carter and Ramirez, personal communication). Chronic adminstration of naloxone to developing female mice has been reported to increase the weights of reproductive organs [41].

Several investigators have noted an apparent facilitation of selective components of copulatory behavior in male rodents after injections of opioid antagonists. Naloxone and naltrexone have been found to decrease the latency to ejaculate [1,16] and reduce the number of intromissions required for ejaculation in sexually experienced male rats [1]. Another study reported that naloxone was effective in inducing copulatory behavior in sexually dysfunctioning, noncopulating male rats [14]. Some pilot data collected in our laboratory suggested that female hamsters, likewise, may show increases in the magnitude of responding on some measures of sexual behavior after naloxone. Since there are no investigations of the effects of opioid antagonists on female sexual behavior, we designed the next experiment to determine whether naloxone alone, in opioid-naive females, produced effects which were opposite those observed after administration of morphine.

METHOD

Subjects

Subjects were 25 ovariectomized, drug-naive female hamsters, weighing approximately 115 g at the start of procedures. The animals were housed and prepared as previously described. Surgeries were done 60 days prior to testing.

Procedures

Subjects were randomly assigned to five groups

(n=5/dose), each subject to receive naloxone hydrochloride (0, 1, 2, 4, or 8 mg/kg, SC). All naloxone injections were given using a 0.4 mg/ml solution and the control goup received saline in a volume equivalent to the largest naloxone dose.

All animals were tested for lordosis duration and lateral displacement as previously described with the tester blind to drug dosage given. Testing began between 1400 and 1500 hr, during the active phase of the hamsters' cycles. Tests for sexual responding were conducted before and 5 min after injection with the doses of naloxone or placebo, and, at each 15 min interval post injection for 2 hr. All data were analyzed using ANOVAs for repeated measures.

RESULTS AND DISCUSSION

Statistical analysis of the scores for the duration of lordosis, using repeated measures ANOVAs with a factor for dose of drug (0 to 8 mg/kg) failed to reveal any reliable main effect or interaction. When scores were transformed to reduce the heterogeneity of variance, there was also no effect with regard to dose of drug.

ANOVAs having a factor for dose of drug and repeated measures on difference scores for inner zone lateral displacement also failed to reveal a main effect for dose and no dose × trials interaction. In general, subjects decreased responding to stimuli with repeated testing, $F(8,160)=4.42$, $p<0.001$. These analyses suggest that naloxone, at the doses tested, did not modify these measures of sexual responding.

Even though overall analyses failed to yield evidence of naloxone effects, there were some indications that naloxone might modify sexual responding. The variance of lateral displacement scores was increased among subjects receiving naloxone. Also, the dose of 4 mg/kg increased the magnitude of lateral displacement during the later tests (90, 105 and 120 min after injection). Statistical analysis of two groups (0 and 4 mg/kg) using a repeated measures ANOVA did yield statistically significant effects, and suggested that 4 mg/kg of naloxone resulted in animals maintaining high levels of responding across tests while the control group decreased responding with repeated testing.

In this experiment a small number of subjects were used in each group, thereby increasing the probability of a type II error. There was also a substantial amount of variance in the responses collected after administration of naloxone. The discrepancies between our pilot data and the results of this experiment, particularly in view of the reliable effects observed when two-group (4 mg/kg naloxone vs saline) comparisons were made, led us to approach the next experiment differently.

EXPERIMENT 5

In view of our results in Experiment 4, we attempted to minimize factors which could even potentially contribute to increased variance in the data. These procedural modifications included (a) extended (3-mo) habituation to the animal facility after purchase prior to testing, (b) use of intact, cycling, female hamsters, (c) daily handling of each subject for 3 weeks before the beginning of testing, (d) collection of all data by one, experienced experimenter who was unaware of the drug treatments of the subjects, and (e) matching subjects according to baseline response magnitude and then assigning animals to one of three groups. It was expected that these measures would decrease the variance seen in responding and, if naloxone was exerting effects on female behavior, make these effects apparent.

TABLE 1

MEANS AND STANDARD DEVIATIONS OF LATERAL DISPLACEMENT MEASURES

Naloxone Dose:	0 mg/kg	4 mg/kg	8 mg/kg
Baseline:	86 (20.9)	92 (23.9)	93 (24.9)
Post-Injection:			
5 min	88 (20.5)	93 (27.9)	100 (34.7)
30 min	87 (12.6)	91 (20.7)	95 (32.3)‡
60 min	89 (11.6)	91 (22.1)*	107 (33.5)‡
90 min	90 (12.9)	94 (23.2)*	97 (35.4)‡
120 min	89 (11.6)	91 (18.7)	90 (25.5)†
150 min	85 (10.0)	81 (22.7)†	91 (25.8)‡

Note: Standard Deviations are in parentheses. Asterisks refer to probability values from F-tests comparing a variance under naloxone to the comparable variance under saline (0 mg/kg): *=<0.05, †=<0.01, ‡=<0.001 [19]. Scores are sums of inner zone responses in mm.

METHOD

Subjects

Thirty, intact, cycling female hamsters were used. All subjects weighed about 110 g at testing and all were experimentally naive. Tests were conducted on the female's day of estrus during the first third of the activity cycle. Animals were housed 4 to a cage and under conditions similar to those described in Experiment 1.

Procedure

Tests for lateral displacement and lordosis duration were conducted in the manner previously described, prior to injections. After baseline measures were obtained, subjects were matched according to the magnitude of the sums of inner zone lateral displacement, and assigned to one of three groups (n=10/group). Subjects received SC injections of either saline, 4 mg/kg or 8 mg/kg of naloxone hydrochloride (2 mg/ml dissolved in physiological saline). The control group received injections of saline comparable in volume to the 8 mg/kg injections. Measures of lateral displacement and lordosis duration were obtained again at 5, 30, 60, 90, 120 and 150 min after injections.

RESULTS

ANOVAs, using difference scores for measures of lateral displacement and lordosis duration (score after injection minus score before injection), failed to yield values indicating a drug-effect. There were also no statistically significant drug × trials interactions. There were, as found in Experiment 3, a significant trials effects for both measures.

The data for lateral displacement, using sums of inner zone displacement across tests, are summarized in Table 1. The table presents mean inner zone sums and the standard deviations of the means. As can be seen, mean values for the groups under naloxone are slightly increased, but, this increase does not meet levels of statistical significance. The variances are reliably increased, however, in groups receiv-

ing naloxone as determined by F-tests [19]. Transforming the scores to reduce the heterogeneity of variance and then performing appropriate ANOVAs also fails to yield evidence of a naloxone-effect on mean responding.

DISCUSSION

Results of Experiments 4 and 5 lead to the conclusion that naloxone, in opioid naive female hamsters, does not facilitate sexual responding as measured here.

Similar experiments conducted in our laboratory, using doses of 50 and 100 mg/kg of naltrexone, the long-acting opioid antagonist, support the conclusion of no effect on sexual behavior. Also, when minimally receptive female hamsters (e.g., pregnant females which maintained lordosis, unpublished data; intact females near the end of their estrous period, in preparation) were tested after injections with naloxone or naltrexone, no reliable drug effects were found. While 4 mg/kg of naloxone does not produce alterations in measures of female sexual performance, this dose is adequate to facilitate responding on indices of male hamster copulatory performance (unpublished observations).

It cannot be concluded that naloxone is entirely without effect in female hamsters since there was a reliable increase in variance in female responding after injection. It is unclear whether this increase in variance reflects a drug-effect related to sexual performance or is merely a non-specific side effect of naloxone. It is possible that naloxone may have systematic effects on sexual behavior if other measures of sexual behavior are used (e.g., proceptivity). Since naloxone can sustain a conditioned taste aversion in rats, an index of the aversive properties of a drug [36,39], general behavioral arousal could account for the increase in variance which we observed.

It seems likely that our measure of lateral displacement is an adequately sensitive measure of the general reproductive status of the hamster to permit valid assessment of naloxone's effects on sexual behavior. Other manipulations which produce little effect on measures of lordosis behavior, such as running (in preparation), mating (in preparation) and drug treatment (Experiment 2) produce immediate alterations in measures of lateral displacement. These changes can be used to predict the female's state of responsivity.

Naloxone increases LH levels in several species [2,3], and this increase apparently results from increases in LHRH release [2, 9, 11]. LHRH has been shown to facilitate sexual behavior in several species [24,25], but not in hamsters (Carter and Ramirez, unpublished observations). Our finding, that naloxone does not produce effects on female sexual behavior is consistent with the inability of LHRH to produce effects in this species.

GENERAL DISCUSSION

Studies of female sexual behavior in rats are usually limited to lordosis behavior and solicitation [6]. While the hamster is an excellent subject for studying female sexual behavior, since several components of sexual behavior can be dissociated [29,30], hamsters have not been used for research with opioids. Our first experiment shows that hamsters, although requiring comparatively high doses of morphine, do respond to morphine in directions which would be expected given the responses observed in rats. Furthermore, since the measure of lateral displacement in hamsters is sensitive to morphine at doses which do not produce behavioral impairment, the hamster may be useful in studying sexual performance following administration of opioids.

In spite of anomalies which may exist in the hamster's response to opioids, we feel that a number of conclusions are supported by the data. In Experiment 1, we used a number of tests to assess narcotic effects in hamsters. Some tests may be less valid than others for use in this species, but it is apparent that, collectively, these data indicate unidirectional and systematic behavioral alterations after injections with morphine. Although, not reported here, informal observations indicate that these effects can be reversed with naloxone. These behavioral effects include motor impairment, sedation and increased latencies to respond to nociceptive stimuli. Hamsters do not show rigid immobility at any dose of morphine tested. Pilot observations indicate that the rigid immobility typically observed in rats does not occur in hamsters even when lethal doses (up to 880 mg/kg) of morphine are administered, which confirms a previous report [17].

Lateral displacement, an index of sexual responsivity in the female hamster [29,30], is sensitive to much smaller doses of morphine. This sensitivity appears to reflect specific effects of morphine at binding sites, since the effects are readily reversed with 4 mg/kg of naloxone. Although tentative, it is of special interest to note that morphine when administered ICV, a route of administration which is effective in producing profound effects in rats [18, 23, 34], does not affect lateral displacement in hamsters, even when doses of 128 μg were administered. Conclusions are similar when *d*-ala^2-enkephalinamide was administered into the lateral ventricle (unpublished observations). This finding suggests that there are sites which are not easily accessed by ICV drug administration which may play an important role in modulating reproductive behavior.

Since opioid receptors are known to be located in peripheral sites and in the spinal cord [35], morphine may be acting in areas outside of brain to produce effects which are incompatible with full sexual functioning in hamsters. Further, the effects on sexual behavior can apparently be produced with smaller doses than those which produce gross behavioral impairment. This suggests that the binding sites which mediate the inhibiton of sexual performance may not be identical to sites which produce other narcotic effects.

The recent finding that there is an 86-fold increase in beta-endorphin-like immunoreactivity in male hamster blood after the 5th ejaculation [26] implicates endogenous opioids in the modulation of reproductive functions. If endogenous opioids were tonically modulating reproductive responsivity, blocking opioid binding by the antagonist naloxone, could be expected to result in some alteration in sexual behavior. When doses of naloxone were tested in opioid naive subjects, there was not a consistent facilitation in scores of lateral displacement up to two hours after injection. The dose dependent increase in variance, however, suggests that naloxone was not entirely inert in this species.

These data indicate that a component of the female hamster's sexual response is relatively sensitive to disruption by morphine. It is possible that an opioid-like ligand modulates some aspect of sexual behavior in hamsters. The inability of naloxone to produce consistent effects on female sexual behavior argues against tonic modulation of lateral displacement by endogenous opioids. In view of the post-mating increase in beta-endorphin-like immunoreactivity found in males [26], it is possible that endogenous opioids function in

the context of coitus. Also, there may be substantial differences between males and females in sex-related functions of opioid-like ligands.

Because there are relatively few studies of the effects of opioids on female sexual performance, there are still many unanswered questions. Since we measured lateral displacement responses (an active contribution of the female to the sexual encounter) our conclusion is that morphine produces dramatic impairment in female sexual performance. This impairment was not found when indices of lordosis behavior were analyzed after morphine. It is important, therefore, to use multiple indices of sexual functioning since psychotropic drugs may affect various components of sexual functioning in different ways.

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