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Opioids and Sexual Behavior in the Male Rat

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AGMO, A. AND R. PAREDES. *Opioids and sexual behavior in the male rat.* PHARMACOL BIOCHEM BEHAV **30**(4) 1021–1034, 1988.—Naloxone in the doses of 4 or 16 mg/kg failed to affect copulatory behavior of testosterone-treated castrated male rats. Morphine 10 mg/kg, administered 60 min before behavioral observation, reduced the proportion of animals displaying sexual behavior. Doses of 2.5 or 5 mg/kg reduced the latency to the second ejaculation, whereas the few animals still copulating after morphine 10 mg/kg showed a reduced latency to the first ejaculation. The same doses of morphine administered 5 min before behavioral observation produced a dose-dependent reduction of mount, intromission and ejaculation percentages. However, those animals that did copulate showed a normal copulatory behavior. D-Ala^2-Met^5 enkephalinamide (DALA) infused into the left cerebral ventricle in a dose of 5 μg 5 or 60 min before tests had no effect. When the peptide was infused 30 sec after the first intromission, the number of intromissions as well as the latency to ejaculation were reduced. Opioids may facilitate ejaculatory mechanisms, perhaps as a consequence of their rewarding properties. Moreover, in animals treated with DALA after the first intromission, the number of intromissions and the latency to ejaculation were similar for the first and second copulatory series, while these parameters were much reduced upon the second ejaculation for control animals. It is possible that liberation of endogenous opioids is the cause of ejaculation-induced facilitation of subsequent sexual behavior.

Sexual behavior Naloxone Morphine Enkephalin

SEVERAL lines of evidence suggest a role for endogenous opioids in the regulation of sexual behavior in the male rat. Early studies, employing intraventricular administration of beta-endorphin or d-Ala^2-Met^5 enkephalinamide (DALA) showed that sexual behavior was completely inhibited by doses that had no effect on locomotor activity [42,53]. A more comprehensive study showed that morphine in a subcutaneous dose of 6 mg/kg, as well as β-endorphin in an intraventricular dose of 6 μg, completely abolished sexual behavior [37]. Moreover, methadone has been found to inhibit sexual activity in male hamsters [47]. The observation that the opiate antagonist naloxone induced sexual behavior in sexually inactive rats [23] added support to the hypothesis that endogenous opioids are involved in inhibitory mechanisms related to this behavior. Data showing that opioids inhibit gonadotropin secretion (for a review see [40]) also supported the hypothesis of a role for endogenous opioids in the control of reproductive functions. The finding that sexual activity produces analgesia and reductions in midbrain endorphin levels [66] is also concordant with such an hypothesis.

However, several studies with naloxone have yielded conflicting results. Some authors have reported reductions in the number of intromissions necessary to achieve ejaculation as well as in ejaculation latency [37, 48, 54]. Others have found that naloxone increases the postejaculatory interval without affecting other aspects of sexual behavior [59,66]. In all these studies intact rats were used. Considering the stimulatory effects of naloxone on gonadotropin secretion [13], it is possible that some of the diverse effects observed after administration of this drug were due to endocrine actions of naloxone, or to an increased release of cerebral LH-RH. It has been found, actually, that subcutaneous LH-RH reduces ejaculation latencies [50]. In the same study it was found that naloxone was without effect on sexual behavior in castrated, testosterone-implanted males. The authors concluded that a drug-induced release of LH-RH is at least partly responsible for the effects of naloxone on masculine sexual behavior. In a later study none of these results was replicated. The only effect obtained with naloxone, in intact as well as in castrated testosterone-implanted rats, was a lengthening of the postejaculatory interval. This effect was apparently unrelated to naloxone-induced stimulation of LH release [36].

A recent article [35] adds further confusion to the field of opioids and sexual behavior. It was reported that both naloxone and morphine in low doses inhibited sexual behavior in castrated rats, whereas these drugs were without effect in intact animals. The authors suggested that opioids normally have minimal effects on sexual behavior, but become critical under androgen deprivation, i.e., after castration. It could be argued that such an effect must be of slight physiological importance. Moreover, the fact that agonist and antagonist have the same effect is difficult to interpret.

Despite a considerable number of studies, the effects of opioids on sexual behavior are far from clear. It was, there-

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fore, decided to more carefully investigate the effects of naloxone, morphine and DALA on sexual behavior in the castrated, testosterone-treated male rat. Three specific questions were addressed, each in a separate experiment. First, the effects of naloxone were studied in castrated animals bearing Silastic capsules, showing a sexual activity indistinguishable from that of intact animals, and castrated animals given weekly injections of a low dose of testosterone propionate, displaying a subnormal sexual activity. This would allow us to evaluate a possible tonic inhibition of sexual behavior exerted by naloxone-blockable opioid systems. Second, a dose-effect curve was obtained for morphine administered at two different intervals before behavioral observation in castrated animals bearing Silastic implants. In this way, possible phasic, mainly μ receptor-mediated, opioid influences on sexual behavior would be detected. Third, the effects of DALA were investigated with the peptide being administered at different intervals before behavioral observation, and finally DALA was infused after that sexual behavior had been initiated. Here, the role of phasic, mainly δ opioid receptor-mediated influences on sexual behavior could be evaluated. The detailed rationale for these experiments will be discussed below. Taken together, it was expected that the data obtained would allow for the formulation of more definite hypotheses as to the role of opioids in the control of sexual behavior. It should be noted that the drugs employed in the present studies were not selected because of receptor specificity. Rather it was considered convenient to use drugs with which extensive behavioral data have been obtained, in order to facilitate the interpretation of the results.

GENERAL METHOD

SUBJECTS

Male Wistar rats (300–400 g) from a local colony were kept two to a cage under a controlled light/dark cycle (12:12 hr) and given commercial rat pellets and tap water ad lib. Those males ejaculating at least once in three 30-min mating tests with receptive females were used in the experiments. They were castrated under Brevital anesthesia (40 mg/kg) and immediately thereafter implanted with a 20 mm long Silastic capsule (0.062 in. i.d.; 0.125 in o.d.; Dow-Corning Corporation) filled with testosterone (Sigma). The animals were allowed 7 days to recover.

Females (Wistar, 200–300 g) used in tests for sexual behavior were ovariectomized at least two weeks before use. They were injected with 25 μg estradiol benzoate (Sigma) 53–55 hr prior to mating tests, and with 1 mg progesterone (Aldrich) 4–6 hr before. The steroids were dissolved in olive oil and injected subcutaneously in a volume of 0.2 ml.

BEHAVIORAL TESTING PROCEDURE

All tests were performed between the 3rd and 7th hr of the dark period under dim white light, using rectangular observation cages (40×60×40 cm) with acrylic fronts. Unlike most studies, the males were not allowed to adapt to the observation cage. A cage-adapted, experienced male has usually very short mount and intromission latencies, making it difficult to observe stimulatory effects of drugs. In the present experiments, therefore, the male was introduced into the observation cage 5 min after the female.

The following parameters of sexual behavior were recorded: (a) number of mounts and intromissions before ejaculation; (b) mount and intromission latencies (time from introduction of the male until the first mount with pelvic thrusting or until the first vaginal penetration, respectively); (c) ejaculation latency (time from the first intromission in a series until ejaculation); (d) postejaculatory interval (time from ejaculation until the next intromission).

EXPERIMENT 1

The purpose of this experiment was to reinvestigate the effects of naloxone on sexual behavior in castrated, testosterone-implanted animals. This seems warranted in view of the many contradictions reported with intact animals and because of the difficulty in interpreting results obtained with drugs having strong endocrine actions, such as naloxone, when adequate controls are lacking. Although castration and testosterone-replacement do not eliminate endocrine effects of a drug, the effects should at least be substantially reduced. Moreover, since the only effect obtained with naloxone which has not been challenged (possibly because no replication has been attempted) was induction of sexual behavior in inactive rats [23], a group of castrated animals treated with a very low dose of testosterone propionate and, therefore, showing low sexual activity, was included in the present study.

It has been found that castration is followed by an increase in specific naltrexone binding in rat brain homogenates [26,27]. This increase could be prevented by a high dose of testosterone propionate (2.5 mg/kg per day) [26]. It is possible that the decline in sexual behavior following castration is related to the observed increase in opioid receptors. An additional purpose of this experiment was to investigate this hypothesis. An increase in sexual behavior after naloxone treatment would be expected in the group treated with a low dose of testosterone propionate, whereas the antagonist would be without effect in animals bearing Silastic implants causing plasma testosterone concentration to be close to that found in intact animals [16].

Since naloxone reaches its maximal brain concentrations within a few minutes of administration [67] and has a relatively short half-life (0.4 hr [43]), only one ejaculatory series was observed in this experiment.

METHOD

Subjects

In addition to animals implanted with a Silastic capsule, groups of males displaying a low level of sexual activity were used. Animals belonging to these latter groups were subjected to the typical selection procedure and then castrated and left three weeks. Thereafter, they were injected with 0.4 mg/kg of testosterone propionate (TP; Sigma) in 1 mg/kg olive oil. Two days later, weekly mating tests were begun. The TP injection was repeated once a week during the entire experiment, always 48 hr before behavioral observation. Drug treatment was initiated 3 weeks after the first postcastrational mating test. Sexual behavior had then reached a stable, low level.

Procedure

Naloxone HCl (du Pont) was dissolved in distilled water and administered intraperitoneally (IP) in a volume of 1 ml/kg b.wt. 15 min before behavioral observation. A counterbalanced design was used, in such a way that at the first test half of the animals were injected with drug and half with

TABLE 1

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR IN MALE RATS TREATED WITH NALOXONE (N=16 IN ALL GROUPS)

	Animals With Normal Sexual Activity				Animals With Low Sexual Activity			
Behavior Parameter	Water	Naloxone 4 mg/kg	Water	Naloxone 16 mg/kg	Water	Naloxone 4 mg/kg	Water	Naloxone 16 mg/kg
Mount percentage	94	94	100	91	63	50	56	50
Intromission percentage	81	94	100	91	38	38	25	31
Ejaculation percentage	38	50	64	55	0	13	0	6
Number of mounts	6.9±1.41	7.1±1.43	5.6±1.33	8.7±2.31	5.3±1.5	2.8±0.74	3.6±1.08	2.0±0.83
Number of intromissions	7.0±1.09	6.1±0.92	8.4±1.04	5.9±1.04	1.7±0.70	2.4±1.32	2.5±1.30	1.4±0.62
Mount latency*†	1.3±0.36	1.3±0.39	2.2±1.16	0.9±0.27	5.8±0.97	3.4±0.90	5.5±1.40	5.4±0.62
Intromission latency*†	1.7±0.42	2.5±0.74	3.0±1.26	1.9±0.83	7.2±1.12	6.25±1.95	4.5±2.36	6.1±1.65
Ejaculation latency*†	6.4±0.81	6.9±1.12	7.6±0.76	6.8±1.85				
Postejaculatory interval*†	7.1±0.72	6.6±0.66	5.9±0.27	5.9±0.39				

*Only animals for which these parameters actually were registered are included.
†Min.

vehicle. At the second test, one week later, the treatments were reversed. Separate groups were used for the different doses of naloxone.

Mating tests ended 15 min after introduction of the male. In case that ejaculation occurred within this time, the test was finished at the end of the postejaculatory interval.

Statistical Analysis

The proportion of animals showing mounts, intromissions or ejaculation were evaluated with McNemar's test for the significance of changes, or the binomial test when appropriate. Mount and intromission latencies as well as postejaculatory intervals were evaluated with the *t*-test for independent samples. Although the same animals were used under both control and experimental treatments, tests for independent groups had to be used, since these parameters were not always registered for all animals. This, however, was considered more adequate than assigning latencies to those animals from which they could not be registered. The number of mounts and intromissions were analyzed with the *t*-test for repeated measures. In these analyses all animals were included. Probabilities given are two-tailed.

RESULTS AND DISCUSSION

Naloxone, doses of 4 or 16 mg/kg, administered 15 min before behavioral observation, had no effect on sexual behavior, neither in testosterone-implanted nor in TP-injected animals (Table 1). These data confirm the observation by Myers and Baum [50]. The lack of effect in animals with low or no sexual activity (only about half the animals in the TP-treated group displayed mounts) suggests that the ability of naloxone to induce sexual behavior in inactive animals [23] is due to its endocrine actions. A similar argument could be advanced to account for the effects of naloxone in intact animals with a normal sexual activity. Moreover, no evidence could be found supporting the hypothesis that a castration-induced increase in opioid receptors is related to the decline of sexual behavior following castration.

The absence of an effect of naloxone in castrated, testosterone-implanted animals does not necessarily mean that opioids are not involved in the control of sexual behavior. According to biochemical studies, naloxone binds preferentially to the morphine (μ) receptor, whereas the endogenous opioid peptides Leu^5-enkephalin and Met^5-enkephalin preferentially bind to the peptide (δ) receptor [58]. It is, therefore, possible that naloxone administration is not an adequate way for blocking physiological effects of endogenous opioid peptides. Some evidence supports such a contention. In a recent study it was found that the δ opioid receptor antagonist ICI 154,129 altered social behavior in mice, whereas naloxone was largely without effect [8]. In addition, ICI 154,129 has been found to stimulate vertical climbing activity in mice, while naloxone was inactive by itself [20]. Surprisingly, naloxone enhanced the effects of ICI 154,129. These observations show that delta and μ antagonists may have different behavioral effects, and may suggest that naloxone does not block all actions of endogenous opioids.

Moreover, naloxone may act as an agonist when administered in high doses (reviewed in [61]). In the studies where naloxone was found to reduce the number of intromissions before ejaculation and ejaculation latency the doses (10–45 mg/kg) were within the range where agonist activity can become evident.

We are thus faced with at least two possibilities to explain the lack of effect of naloxone in this study, as well as the previously obtained positive results. The possible impor-

TABLE 2

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR IN MALE RATS TREATED WITH MORPHINE 60 MIN BEFORE BEHAVIORAL OBSERVATION [N=72 (SALINE); 18 (MORPHINE 2.5 AND 5 mg/kg); 36 (MORPHINE 10 mg/kg)]

	First Ejaculatory Series				Second Ejaculatory Series			
Behavior Parameter	Water[a]	Morphine 2.5 mg/kg	Morphine 5 mg/kg	Morphine 10 mg/kg	Water[a]	Morphine 2.5 mg/kg	Morphine 5 mg/kg	Morphine 10 mg/kg
Mount percentage	83	72	67	24*	67	67	67	22*
Intromission percentage	72	67	67	22*	67	67	67	22*
Ejaculation percentage	67	67	67	22*	61	67	67	19*
Number of mounts	3.8±0.1	4.8±1.27	4.0±1.06	1.2±0.39*	1.9±0.60	2.2±0.59	1.8±0.47	0.7±0.28*
Number of intromissions	8.4±1.45	8.2±1.53	7.0±1.28	2.1±0.69*	4.5±0.87	4.7±0.87	4.3±0.78	1.6±0.58*
Mount latency[b,c]	5.3±1.97	1.1±0.27	4.0±1.06	8.4±2.86				
Intromission latency[b,c]	5.6±2.12	3.1±1.08	5.9±1.98	8.5±2.08				
Ejaculation latency[b,c]	14.9±2.17	15.7±2.05	12.9±1.75	7.2±0.94*	10.9±1.01	8.0±1.69	6.8±1.14	6.5±2.35
Postejaculatory interval[b,c]	8.5±0.73	9.3±1.13	7.2±0.44	8.9±1.56	9.2±0.80	11.9±1.47	9.1±0.50	13.9±2.05

[a]Since the three water-treated groups did not differ significantly on any parameter, they have been pooled for presentation. Statistical comparisons were, however, always made between the experimental treatment and the respective control.

[b]Only animals for which these parameters actually were registered are included.

[c]Min.

*$p<0.05$.

tance of endocrine effects have already been discussed. Interestingly, the actions of opioids on the release of LH have been shown to be mediated by the μ receptor [12]. This lends additional support to the hypothesis that the effects of naloxone on sexual behavior are dependent upon its actions on the endocrine system. However, it is also feasible that the effects of naloxone are due to its agonist activity, especially in view of the high doses used in previous studies. These are far above those necessary for antagonism of morphine-induced analgesia, or for antagonism of the effects of morphine on sexual behavior in the female [52]. In the present study, however, the doses used were lower, and agonist activity should be less evident or absent, at least with the lowest dose. If this argument is correct, then morphine should have effects similar to those of high doses of naloxone. In the following experiment, this possibility was investigated.

EXPERIMENT 2

In this experiment, the effects of morphine on sexual behavior were studied. It is well known that morphine administration has a biphasic effect on spontaneous behaviors such as locomotor activity, initially producing an inhibition and later stimulation [51, 62, 63]. Such an effect is particularly evident with high doses, and the initial inhibitory effect may be absent with low doses. It has similarly been found that morphine has a biphasic effect on body temperature when given in intermediate doses [39,68]. Here the initial effect is hypothermia, followed by hyperthermia. The duration of the inhibitory effect depends on the dose. For moderate doses it is less than 1 hr when locomotor activity is measured [63], and even after a high dose (10 mg/kg) stimulation appears after about 60 min [28]. In order to cover the initial, usually inhibitory phase of the action of morphine, as well as the later, usually stimulatory phase, morphine was administered 5 min before behavioral observation in one part of the experiment and 60 min before in another.

METHOD

Subjects

All animals used were implanted with a Silastic capsule as described earlier.

Procedure

Morphine HCl (Ministry of Health, Mexico) was dissolved in distilled water and administered IP 5 or 60 min before observation in doses of 2.5, 5 or 10 mg/kg.

A counter-balanced design was used with an interval of 7 days between experimental sessions. No animal received more than one dose of the drug. Mating tests were ended when one of the following criteria was met: End of the second postejaculatory interval; 30 min without intromission; ejaculation had not occurred within 30 min of the first intromission in a series.

Motor deficiencies, such as catalepsy, are frequently reported after morphine treatment. To evaluate their importance for the effects of morphine on sexual behavior, rats were injected with morphine 10 mg/kg 5 or 60 min before being placed on a treadmill (rotarod), and their motor execu-

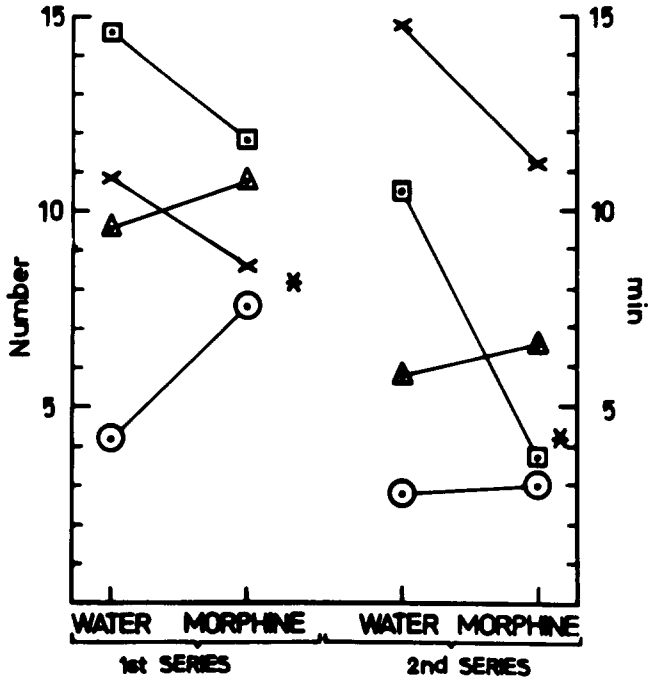

FIG. 1. Mean number of mounts and intromissions, ejaculation latency and postejaculatory interval in animals treated with water or morphine 2.5 mg/kg 60 min before behavioral observation. Only animals displaying two ejaculations under both treatments are included. Although 12 animals ejaculated after morphine 2.5 mg/kg, only 9 of these did ejaculate when treated with saline. Therefore, N=9. ○, mounts; △, intromissions; □, ejaculation latency; ×, postejaculatory interval. *Different from saline in the same ejaculatory series, $p<0.05$.

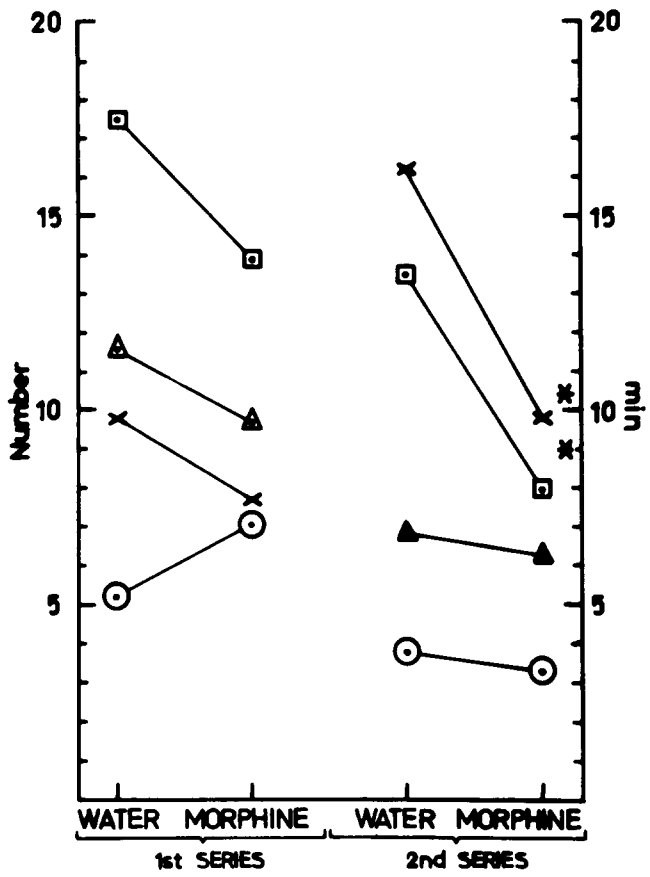

FIG. 2. Mean number of mounts and intromissions, ejaculation latency and postejaculatory interval in animals treated with water or morphine 5 mg/kg 60 min before behavioral observation. Only animals displaying two ejaculations under both treatments are included. Although 12 animals ejaculated twice after morphine 5 mg/kg, only 9 of these did ejaculate twice when treated with saline. Therefore, N=9. Symbols as in Fig. 1.

tion compared to that of saline-treated animals. This procedure has been found very useful, and a high correlation between motor impairment thus established and reductions in sexual behavior has been reported [3].

Statistical Analysis

Each copulatory series was first analyzed for overall effects in the same way as described in Experiment 1.

A 2×2 analysis of variance for repeated measures on both factors was then performed for those animals that ejaculated twice under both control and experimental treatments, the factors being treatment (control vs. drug) and ejaculation (1st vs. 2nd). Simple main effects were analyzed rather than principal main effects in order to avoid confounding interactions. The number of mounts and intromissions, ejaculation latency and postejaculatory intervals were evaluated in this way. A computer program generously provided by Dr. D. Coulombe, University of Ottawa, Canada [14] was used for this analysis.

It was considered of importance to perform this rather extensive analysis for several reasons: (a) Limiting the analysis only to animals showing the complete copulatory pattern twice, as was done with the ANOVA, would have meant that a large proportion of the sample must have been discarded, and valuable information probably lost. (b) Making only overall comparisons could be misleading, since the same animals would not always be included in experimental and control treatments. (c) A reliable effect would be one that is large enough to be manifest in the entire sample (overall comparisons) *and* in the animals that displayed the complete copulatory pattern under both control and experimental treatments (repeated measures design, here evaluated with ANOVA).

RESULTS AND DISCUSSION

Morphine, at a dose of 10 mg/kg, had no effect on motor execution neither when administered 60 min before behavioral observation nor when administered 5 min before (data not shown).

Morphine 2.5 or 5 mg/kg had no overall effect on sexual behavior when administered 60 min before observation, whereas a dose of 10 mg/kg reduced the mount-, intromission and ejaculation percent as well as the number of mounts and intromissions in both copulatory series (Table 2). However, the ejaculation latency was reduced for the few animals that did ejaculate at this dose.

When data were analyzed only for animals ejaculating twice under both control and experimental treatments, a slightly different picture emerged. Morphine 2.5 mg/kg significantly increased the number of mounts before the first ejaculation, $F(1,8)=33.37$, $p<0.001$. The ejaculation latency

TABLE 3

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR IN MALE RATS TREATED WITH MORPHINE 5 MIN BEFORE BEHAVIORAL OBSERVATION [N=54 (SALINE); 18 (ALL OTHER GROUPS)]

	First Ejaculatory Series				Second Ejaculatory Series			
Behavior Parameter	Water[a]	Morphine 2.5 mg/kg	Morphine 5 mg/kg	Morphine 10 mg/kg	Water[a]	Morphine 2.5 mg/kg	Morphine 5 mg/kg	Morphine 10 mg/kg
Mount percentage	83	72	61	44	72	33	39	33
Intromission percentage	78	61	56	39	72	33*	39*	28*
Ejaculation percentage	72	39*	39*	39*	72	28*	39*	22*
Number of mounts	3.6±0.96	4.5±1.37	4.1±1.49	1.3±0.56	1.2±0.30	0.7±0.31	0.6±0.26	0.5±0.25
Number of intromissions	6.4±0.91	5.1±1.10	4.6±1.14	3.0±0.95	3.6±0.58	2.1±0.71	2.4±0.93	1.9±0.79
Mount latency[b]	2.9±0.75	2.8±0.77	4.0±1.30	2.0±0.74				
Intromission latency[b,c]	4.9±1.27	6.1±1.37	7.1±2.21	4.4±1.76				
Ejaculation latency[b,c]	14.5±2.16	12.9±2.39	15.1±3.93	11.5±3.88	6.5±0.83	8.5±1.63	7.2±1.46	9.8±6.39
Postejaculatory interval[b,c]	7.6±0.41	8.1±0.85	8.4±0.78	10.5±3.61	8.7±0.78	8.6±0.83	11.7±2.01	9.7±0.60

[a]Since the three water-treated groups did not differ significantly on any parameter, they have been pooled for presentation. Statistical comparisons were, however, always made between the experimental treatment and the respective control.
[b]Only animals for which these parameters actually were registered are included.
[c]Min.
*$p<0.05$.

for the second ejaculation was reduced, $F(1,8)=11.36$, $p=0.009$ (Fig. 1). Morphine 5 mg/kg further reduced the ejaculation latency for the second ejaculation, $F(1,8)=7.08$, $p=0.02$, as well as the postejaculatory interval, $F(1,8)=6.95$, $p=0.02$ (Fig. 2). Data from animals treated with morphine 10 mg/kg 60 min before observation were not analyzed in this way, since only 4 animals ejaculated twice under both control and experimental treatments.

When morphine was administered 5 min before behavioral observation, ejaculation percentage was reduced after all three doses for the first copulatory series, whereas both intromission and ejaculation percentage were reduced in the second copulatory series (Table 3). When data were analyzed only for those animals that did ejaculate twice under both treatments, no significant differences were obtained between saline and morphine treatment (data not shown).

The short interval, 5 min, between drug administration and behavioral observation could have been too short to allow morphine to be adequately distributed within the brain. However, the relatively strong inhibitory effects obtained, as well as previous observations showing that morphine 10 mg/kg reduces locomotor activity 10 min after administration (Agmo and Patargo, unpublished observations), indicate that the drug acts quite rapidly.

It thus appears that morphine, regardless of whether administered 5 or 60 min before behavioral observation, has a generally inhibitory effect on sexual behavior. This inhibition consists of reduction of the probability that an animal will engage in copulatory behavior (reduced mount, intromission and ejaculation percentage). However, those animals that did copulate when treated with morphine 5 min before observation showed a normal copulatory behavior, while those animals that copulated when treated with morphine 60 min before behavioral observation showed a facilitated copulatory behavior. There was an overall reduction in latency to the first ejaculation, without any significant reduction in the number of intromissions preceding ejaculation after morphine 10 mg/kg (control 11.14±0.79; morphine 8.5±1.05, $t=1.82$, $p=0.07$). A dose of 5 mg/kg reduced both the ejaculation latency to the second ejaculation and the postejaculatory interval after it, while morphine 2.5 mg/kg reduced ejaculation latency without affecting postejaculatory interval. In sum, these data clearly suggest a facilitation of sexual behavior in those animals still ejaculating after morphine treatment. A reduced ejaculation latency after morphine 6 mg/kg, administered 45 min before behavioral observation, has recently been reported [55]. Moreover, the proportion of animals copulating was found to be much reduced, as found in the present studies.

The fact that many animals ceased to copulate after morphine administration cannot be explained by possible physical discomfort produced by the drug. Morphine and endogenous opioid peptides are known to induce a reward state by stimulation of opiate receptors ([24, 25, 38, 45] and references therein), and such a state would not be easily compatible with physical ill-being. This reward state could reduce the drive level of the animal, making the initiation of copulatory behavior less probable. Observations from human opiate addicts seem to support such an argument. Intravenous heroin is reported to produce and orgasm-like sensation, and this may be related to the loss of interest in sexual activites reported by such subjects [55]. The inhibition of feeding and

drinking in animals deprived of food and water produced by low doses of morphine [21] as well as the reduction in sexual behavior in castrated male rats treated with this drug [35] has been explained in terms of drive reduction, consequent to a general reward state.

The effects of large doses of morphine on sexual behavior seem to be similar to its effect on other behaviors in the way that the drug is inhibitory shortly after administration and stimulatory after a longer interval between administration and behavioral observation. In the present experiments, only inhibitory effects were obtained when morphine was administered 5 min before behavioral observation, whereas both inhibitory and stimulatory effects were observed when the drug was administered 60 min before observation. It must be noted, however, that the stimulatory effect is limited to the small number of animals still displaying sexual behavior. It could be imagined that a still longer interval between drug administration and behavioral observation would produce only stimulatory effects. This does not seem to be the case, however, since morphine in a dose of 20 mg/kg reduced mounting 2.5 hr after administration. Lower doses had no effects [46]. Unfortunately, no other parameters of sexual behavior were reported. The stimulatory effects of morphine found in the present studies are similar to those reported after naloxone administration (see Introduction). It is therefore suggested that the hypothesis presented in Experiment 1, that the doses of naloxone used in previous studies were such that agonist activity had become evident, has been confirmed. Interestingly, naloxone has never been found to inhibit sexual behavior, even in very high doses, nor has the antagonist been reported to have rewarding properties. This might support the hypothesis presented above that the inhibitory effects of morphine are due to drive reduction through its rewarding properties.

As already mentioned, both morphine and naloxone act preferentially at the μ receptor, whereas some endogenous opioids, such as the enkephalins, act preferentially at the δ receptor. It has also been mentioned that specific δ antagonists can have behavioral effects different from those obtained with naloxone, indicating that μ and δ receptors may subserve different behavioral functions. It was therefore considered of interest to investigate the effects of an enkephalin on sexual behavior.

EXPERIMENT 3

The previous experiment showed that opioids can influence male sexual behavior. However, it would be ventured to affirm that endogenous opioid peptides participate in the control of that behavior only because morphine has effect. Actually, the support for a role of endogenous opioid peptides in sexual behavior is extremely weak. Studies with β-endorphin [37, 41, 42] and the synthetic enkephalin analogue DALA [53] have only shown that sexual behavior can be reduced or abolished with these peptides at moderate doses. The obvious conclusion was that opioids are engaged in inhibitory processes related to sexual behavior. However, the fact that a drug can inhibit or abolish this behavior does not necessarily mean that it is acting upon mechanisms controlling that behavior. For example, it has recently been shown that GABAergic drugs can inhibit sexual activity independently from their effects on locomotor activity [2], but no evidence has been found for a participation of GABA in the normal expression of sexual behavior [3]. The same could be true for the opioid peptides.

Other evidence for a role of opioid peptides in sexual behavior is equally shaky. It has been observed that copulation induces analgesia [66]. Shock-induced vocalizations were readily reduced during copulation, but hind-limb withdrawal in response to paw pinch was reduced only after the second intromission of the fourth copulatory series. Even if analgesia did occur, it is not all clear whether it was due to release of opioid peptides or of other transmitters, since no opioid antagonist was used to block the effect. A host of transmitters have been found to produce analgesia, among them GABA, acetylcholine and serotonin [17, 18, 70].

The observation that β-endorphin-like immunoreactivity is elevated 86-fold in plasma of male hamsters following ejaculation [48] is not more conclusive. Plasma endorphin is probably of pituitary origin, or endorphins may be released from the autonomic nervous system. Moreover, a large variety of situations cause increase in plasma β-endorphin levels (for a review see [5]). Again, it seemed useful to systematically reinvestigate the effects of opioid peptides on sexual behavior. Since the peptides have the same biphasic effect as morphine [28, 29, 31], regardless of whether infused into the cerebral ventricles or in localized brain structures, it was decided to administer DALA at the same intervals before observation as morphine. In addition, it was thought of interest to administer DALA to animals that had already initiated copulation. If endogenous opioids participate in the control of sexual behavior, it is possible that they are released during the course of that behavior. An intraventricular infusion of DALA should then reinforce the effects of endogenously released opioids.

METHOD

Subjects

In this experiment, males bearing Silastic capsules were implanted with a 21 gauge guide cannula in the left cerebral ventricle using standard stereotaxic techniques (coordinates: 0.1 mm anterior to bregma, 1.5 mm lateral to the midline and 4.0 mm below the *dura mater*. The head was fixed in such a way that lambda was 1 mm lower than bregma). Brevital anesthesia (40 mg/kg) was used. The animals were then subjected to two weekly mating tests, and experiments began one week after the second test.

At the end of the experiments, males with intraventricular cannulas were injected with 5 μl methylene blue through the cannula and killed by an overdose of anesthesia. The brain was removed, cut and examined under a dissection microscope for correct implantation. In that way, a small number of animals were discarded, and data from them were not used.

Procedure

d-Ala^2-Met^5 enkephalinamide, acetate salt (Sigma) was dissolved in physiological saline and infused into the left cerebral ventricle in a volume of 5 μl during 2 min. The injection cannula (27 gauge) protruded 0.5 mm from the guide cannula and was left in place for 1 min after the end of infusion, and was then replaced by a dummy cannula.

DALA, in a dose of 5 μg, was administered 5 or 60 min before behavioral observation. In addition, DALA in a dose of 5 or 10 μg was administered 30 sec after the first intromission of the first copulatory series to other groups of animals. In the latter case, the male was withdrawn from the observation cage immediately after intromission, and placed on an adjacent table where drug infusion was performed. Four min after withdrawal, the male was reintroduced into the obser-

TABLE 4

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR AFTER ADMINISTRATION OF DALA 5 μg 60 MIN BEFORE BEHAVIORAL OBSERVATION (N=15)

Behavior Parameter	First Ejaculatory Series		Second Ejaculatory Series	
	Saline	DALA	Saline	DALA
Mount percentage	80	80	47	40
Intromission percentage	67	73	47	40
Ejaculation percentage	47	40	40	40
Number of mounts	9.9±3.93	8.9±2.21	3.6±1.68	1.9±0.88
Number of intromissions	7.1±1.53	7.0±1.28	3.4±1.09	2.7±0.91
Mount latency*†	1.4±0.40	5.6±2.40		
Intromission latency*†	3.1±1.12	7.7±3.00		
Ejaculation latency*†	14.0±2.22	15.1±1.64	8.7±2.76	10.2±2.54
Postejaculatory interval*†	9.3±1.62	11.6±2.45	9.9±1.28	9.2±0.80

*Only animals for which these parameters actually were registered are included.
†Min.

TABLE 5

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR AFTER ADMINISTRATION OF DALA 5 μg 5 MIN BEFORE BEHAVIORAL OBSERVATION (N=20)

Behavior Parameter	First Ejaculatory Series		Second Ejaculatory Series	
	Saline	DALA	Saline	DALA
Mount percentage	55	65	40	30
Intromission percentage	55	60	45	30
Ejaculation percentage	45	35	45	30
Number of mounts	6.6±0.18	8.3±3.06	2.4±1.07	1.8±0.66
Number of intromissions	5.0±0.78	5.1±1.26	3.2±0.91	1.7±0.64
Mount latency*†	3.5±1.47	4.4±1.65		
Intromission latency*†	8.4±3.49	2.8±0.69		
Ejaculation latency*†	10.8±2.22	12.4±2.71	10.0±2.15	12.7±3.81
Postejaculatory interval*†	8.7±0.75	11.1±3.55	10.6±0.49	10.5±1.01

*Only animals for which these parameters actually were registered are included.
†Min.

TABLE 6

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR AFTER ADMINISTRATION OF DALA 5 μg 30 SEC AFTER THE FIRST INTROMISSION (N=32)

Behavior Parameter	First Ejaculatory Series		Second Ejaculatory Series	
	Saline	DALA	Saline	DALA
Mount percentage	91	72	69	69
Intromission percentage	91	72	69	63
Ejaculation percentage	69	69	63	63
Number of mounts	6.0±1.20	4.2±0.86	2.2±0.49	3.2±0.65
Number of intromissions	10.3±1.05	6.8±0.98*	5.2±0.94	4.8±0.64
Mount latency[a,b]	2.7±0.67	2.3±0.62		
Resumption latency[a,b]	3.5±0.83	3.7±1.10		
Ejaculation latency[a,b]	13.4±1.94	10.5±1.91	5.7±0.78	8.1±1.26
Postejaculatory interval[a,b]	7.1±0.38	7.8±0.96	9.6±0.77	8.8±0.80

[a]Only animals for which these parameters actually were registered are included.
[b]Min.
*$p<0.05$.

vation cage. Besides the usual record of behavior, the resumption latency (time from reintroduction into the observation cage until the following intromission) was registered. Control animals were treated in exactly the same way, except that saline was infused instead of DALA. If intromission did not occur within 30 min of reintroduction of the male, the test was ended. With these exceptions, mating tests were performed in the same way as in Experiment 2.

The animals were treated with DALA or vehicle according to a counterbalanced design, i.e., each animal served as its own control. No animal was treated more than once with the peptide. Accordingly, separate groups were used for each interval between peptide infusion and behavioral observation, and for each dose when the peptide was infused after intromission.

Statistical analysis was performed as in Experiment 2.

RESULTS AND DISCUSSION

DALA 5 μg administered 5 or 60 min before observation had no effect on sexual behavior, neither when analyzed for overall effects (Tables 4 and 5) nor when data from animals ejaculating twice under both control and experimental treatments were analyzed (data not shown).

DALA in a dose of 5 μg, administered after the first intromission, produced an overall reduction in the number of intromissions in the first ejaculatory series (Table 6).

When only animals ejaculating twice under both experimental and control treatments were included in the analysis, ANOVA showed a significant reduction in the number of intromissions before the first ejaculation, $F(1,12)=11.96$, $p=0.004$, and of the ejaculation latency, $F(1,12)=4.78$, $p=0.049$ (Fig. 3). When DALA was administered in a dose of 10 μg after the first intromission, an overall reduction in the number of intromissions before both the first and second ejaculation, and an overall reduction in latency to the first ejaculation, were observed (Table 7). When only animals showing two ejaculations under both treatments were included in the analysis, ANOVA showed a significant reduction in the number of intromissions before the first ejaculation, $F(1,4)=8.25$, $p=0.04$, as well as of the ejaculation latency, $F(1,4)=7.78$, $p=0.048$ (Fig. 4).

The results obtained with DALA administered 5 or 60 min before behavioral observation are at variance with earlier studies [23,53] where DALA in a dose of 6 μg completely inhibited sexual behavior, and a dose of 3 μg prolonged mount and intromission latencies. No convincing explanation can be offered for the discrepancy. It should be noted, however, that for some unknown reason, conflicting results are common with the opioid peptides. For example, some authors found a stimulation of locomotor activity after intracerebroventricular administration of DALA in doses ranging from 3 to 100 μg [9,28], whereas others found doses within this range to be ineffective [1,31]. Some found that DALA induced wet-dog shakes in a dose-dependent way, the optimal dose being 5 μg [19], while others have failed to find a direct dose-effect relation, and estimated the optimal dose to 20 μg [15].

β-Endorphin has also been found to inhibit copulatory behavior in doses ranging from 1 μg [42] to 6 μg [37]. It must be noted, though, that the enkephalins and β-endorphin are quite different with regard to precursors, distribution and receptor specificity [5].

An interesting observation in the present experiments

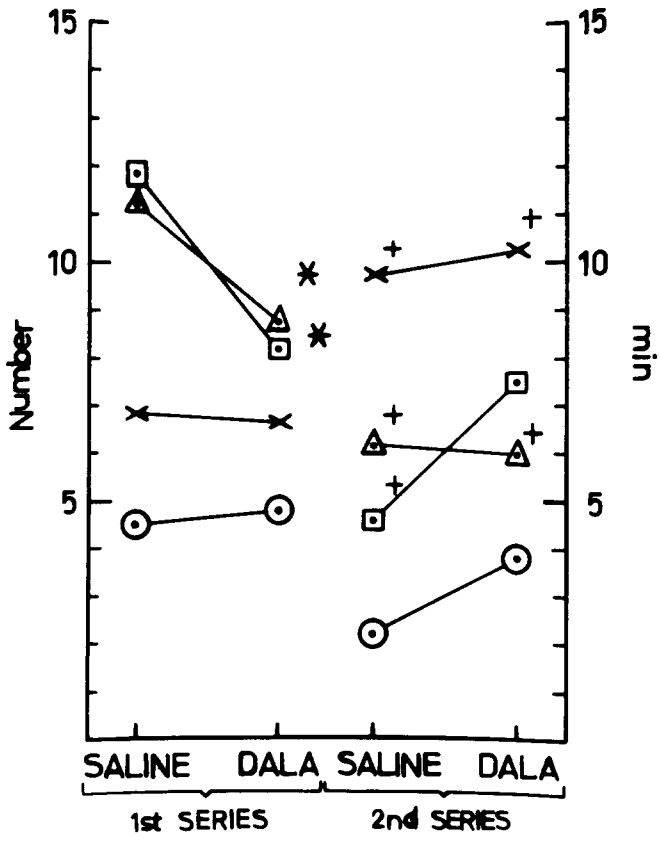

FIG. 3. Mean number of mounts and intromissions, ejaculation latency and postejaculatory interval in animals treated with saline or DALA 5 μg 30 sec after the first intromission. Only animals displaying two ejaculations under both treatments are included. Of the 20 animals ejaculating twice after DALA 5 μg, only 13 ejaculated twice when treated with saline. Therefore, N=13. Symbols as in Fig. 1. *Different from saline in the same ejaculatory series, $p<0.05$; †different from the same treatment in the first ejaculatory series, $p<0.05$.

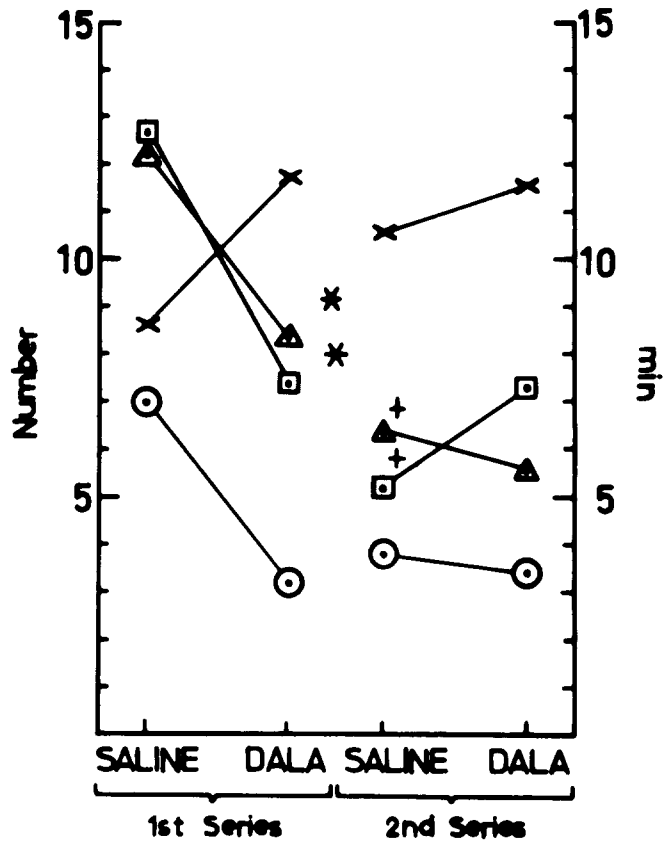

FIG. 4. Mean number of mounts and intromissions, ejaculation latency and postejaculatory interval in animals treated with saline or DALA 10 μg 30 sec after the first intromission. Only animals displaying two ejaculations under both treatments are included. Of the 6 animals ejaculating twice when infused with DALA 10 μg, 5 ejaculated twice after saline treatment. Therefore, N=5. Symbols as in Fig. 1. *Different from saline in the same ejaculatory series, $p<0.05$; †different from the same treatment in the first ejaculatory series, $p<0.05$.

was that the number of intromissions and the ejaculation latency were similar for the first and second ejaculation in animals treated with DALA. It has been known for many years that the number of intromissions necessary to achieve ejaculation as well as the ejaculation latency are reduced on the second ejaculation [32, 34, 64, 65]. This was regularly observed in saline-treated animals [controls for DALA 5 μg: Intromissions to 1st and 2nd ejaculation, $F(1,12)=9.81$, $p=0.008$; ejaculation latency, $F(1,12)=9.81$, $p=0.008$. Controls for DALA 10 μg: Intromissions, $F(1,4)=29.77$, $p=0.006$; ejaculation latency, $F(1,4)=278.68$, $p=0.0005$]. However, animals treated with DALA 10 μg after the first intromission required about the same number of intromissions to achieve the first and second ejaculation, $F(1,4)=6.93$, NS, and had the same ejaculation latency, $F(1,4)=0.78$, NS. Animals treated with DALA 5 μg after the first intromission required fewer intromissions to achieve the second ejaculation than the first, $F(1,12)=6.64$, $p=0.02$, but had the same ejaculation latency, $F(1,12)=1.13$, NS. It is therefore possible that the behavioral changes produced by ejaculation (facilitation of subsequent sexual behavior) are due to liberation of endogenous opioids. Indeed, a high dose of naloxone (30 mg/kg), administered to male rats 30 min before an exhaustion test, significantly increased ejaculation latencies in all but the first copulatory series. No other effects were reported [55]. This coincides with the hypothesis presented here.

Another consequence of ejaculation is an increase in postejaculatory interval. This increase is quite small between the first and second ejaculation [32,64], and was not always evident in the present experiments. Nevertheless, it was found that the increase in postejaculatory interval between the first and second ejaculation was significant for animals treated with DALA 5 μg after the first intromission, $F(1,12)=11.75$, $p=0.005$, as well as for the respective controls, $F(1,12)=12.13$, $p=0.004$. It thus appears that the increase in postejaculatory interval was not affected by DALA. Interestingly, it has been proposed that a common mechanism controls the reduction of both the number of intromissions and the ejaculation latency, whereas the increase in postejaculatory interval depends on an entirely different mechanism [32]. The present results coincide with this hypothesis.

GENERAL DISCUSSION

Successful copulation in the rat depends on a two-stage

TABLE 7

MEAN (±SE) OF PARAMETERS OF SEXUAL BEHAVIOR AFTER ADMINISTRATION OF DALA 10 μg 30 SEC AFTER THE FIRST INTROMISSION (N=10)

Behavior Parameter	First Ejaculatory Series		Second Ejaculatory Series	
	Saline	DALA	Saline	DALA
Mount percentage	90	80	90	60
Intromission percentage	90	70	90	60
Ejaculation percentage	90	60	70	60
Number of mounts	8.9±2.36	5.1±2.17	5.5±1.79	2.7±0.99
Number of intromissions	10.7±1.50	6.2±1.48*	6.9±1.12	3.6±1.15*
Mount latency[a,b]	3.5±2.20	3.6±1.84		
Resumption latency[a,b]	3.9±2.17	4.6±2.07		
Ejaculation latency[a,b]	13.8±1.66	8.0±1.97*	4.9±0.47	7.8±2.10
Postejaculatory interval[a,b]	8.9±0.79	10.9±3.45	10.0±1.37	11.6±1.55

[a]Only animals for which these parameters actually were registered are included.
[b]Min.
*$p<0.05$.

process. First, the initiation of sexual contact with a female (sexual motivation), and second the deposition of spermatozoa in the vagina by ejaculation (sexual performance). Such a two-stage process was originally proposed by Beach [6], and has thereafter been found useful by most researchers in the field. Our data indicate that sexual motivation, reflected as the latency to initiate sexual activity and the postejaculatory interval, again according to Beach [6], is not affected by opioids. In no case could an increase in mount latency or postejaculatory interval be registered. This is in agreement with other studies (see Introduction and Discussion sections. Experiment 2). The absence of an effect of DALA when administered before the behavioral observation further supports the notion that opioid peptides do not participate in the mechanisms of initiation of sexual behavior, i.e., sexual motivation. The effects of opioids on motivational processes have been little studied. It has been found, however, that opioid agonists can increase feeding behavior, whereas naloxone has the opposite effect [44,60]. Similar results have been obtained with regard to drinking [11]. Since the latency to initiate feeding or drinking is not affected by opioid agonists or antagonists it has been suggested that their effect is primarily on reward mechanisms determining maintenance or termination of behavior [57].

Sexual behavior is qualitatively different from ingestion behaviors, which is why results obtained from the latter cannot be completely generalized to the former. Nevertheless, it could be assumed that also with regard to sexual behavior, reward mechanisms are those preferentially affected by opioids. It is generally accepted that sexual behavior is rewarding [69]. This could be due to liberation of opioid peptides in the course of copulatory behavior. In the present experiments, the rewarding properties of exogenous opioids would thus add to the reward state induced by liberation of endogenous opioids. If ejaculation is achieved when a threshold "reward level" is reached, it is not surprising that the number of intromissions and the ejaculation latency are reduced when DALA is administered. Since the processes involved in ejaculation are not well known, this is of course entirely speculative. However, a study of Herberg [30] showed that intracranial self-stimulation produced seminal emission when the stimulation electrode was placed within the medial fibers of the medial forebrain bundle, a site where electrical stimulation is known to be highly rewarding. This might indicate that a central ejaculatory mechanism actually is activated through a reward-related process, and that the rewarding properties of ejaculation are not a secondary feature, but part of the ejaculatory mechanism. It should be observed that ejaculation also can be of entirely spinal origin, since it can easily be provoked in animals and men with spinal transection [7,71]. Moreover, spontaneous ejaculation in the rat, probably of spinal origin, persists after lesions in the preoptic area, abolishing all sexual behavior [4], suggesting that spinally-induced ejaculation is independent from copulation-induced ejaculation. In the latter process, reward mechanisms may be of fundamental importance.

If DALA administration produced a reward state facilitating ejaculatory mechanisms, it is difficult to understand why administration of the peptide 5 min before behavioral observation was ineffective. However, studies using the conditioned place preference paradigm have shown that the minimal effective dose of DALA administered into the ventral tegmental area varies between 100 ng [56] and 8 μg [24]. Intracerebroventricular administration would most probably require higher doses to be effective. It is therefore possible that the dose of 5 μg used in the present studies was unable to induce

a reward state by itself. When the peptide was administered after the first intromission, endogenous liberation may have occurred, and the dose could thus become effective. The lack of effect of the peptide when administered 5 or 60 min before observation, in contrast to its clear effects when administered during observation, can indeed be regarded as supporting the hypothesis that endogenous opioids are liberated during sexual activity.

The fact that morphine did reduce the proportion of animals copulating, both in the present and in other studies, might appear contradictory to the suggestion that opioids do not directly affect sexual motivation. If motor and perceptual disturbances are excluded, such a reduction would logically be due to reduced sexual motivation. No effects of morphine were found on motor execution. It is, therefore, concluded that the reduction in the proportion of animals copulating after morphine is not due to motor disturbances. Neither is it likely that perceptual deficiencies were so severe as to impede copulatory behavior.

Inhibitory effects similar to those of morphine have been obtained with a variety of GABAergic drugs, and a careful analysis of their behavioral actions led to the conclusion that their inhibitory effects on the initiation of sexual behavior are independent of that behavior itself [2,3]. Indeed, it has been suggested that any drug may inhibit any behavior, if the dose is sufficiently high [33]. The morphine doses used in the present and other studies can be regarded as high. The ED_{50} of subcutaneous morphine in the tail-flick procedure for analgesia has been reported to be 2.5 mg/kg [10] and in the NaCl writhing test the ED_{50} has been found to be as low as 0.12 mg/kg [22]. On the other hand, the ED_{50} for DALA infused in the cerebral ventricles has been found to be 8.7 μg in the tail-flick procedure [10], i.e., above the lowest dose used in the present studies.

Sexual performance was affected by both morphine and DALA. The facilitatory effects of morphine were limited to experiments where the drug was administered 60 min before observation, and with the lower doses, to the second copulatory series. DALA had to be administered after the first intromission to be effective, and then mainly affected the first copulatory series. Independently of that, the effects were quite consistent. Ejaculation latency alone was reduced by morphine, whereas DALA reduced both the ejaculation latency and the number of intromissions necessary to reach ejaculation. Moreover, DALA made the first ejaculation almost indistinguishable from the second with regard to the number of intromissions and ejaculation latency. Morphine had no such effects. It seems therefore reasonable to suggest that morphine was far less effective than DALA. This can be due to the fact that morphine acts mainly at μ receptors, or that the time of administration is critical. The lack of effects of naloxone would support the former possibility. However, the present data do not allow for any definite conclusions as to the receptors involved in the effects obtained.

To summarize, the present studies have shown that opioids can facilitate sexual performance without having direct effects on sexual motivation. The facilitation of sexual peformance seems to be due to facilitation of ejaculatory mechanisms. It was proposed that ejaculation is achieved when a reward threshold is reached, and that opioid peptides can contribute to the attainment of this threshold. This might indicate that opioid peptides are released during sexual activity, as previously suggested by Szechtman *et al.* [66]. Release of opioid peptides could be responsible for the facilitation of subsequent sexual behavior, reflected in shortened latency to ejaculation and reduced number of intromissions in the second copulatory series. In order to confirm these hypotheses, it is imperative to administer enkephalinase inhibitors before initation of sexual activity, to see if the effects of DALA can be mimicked. It would also be useful to administer specific δ receptor antagonists, expecting them to produce increased ejaculation latency and number of intromissions before ejaculation.

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