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Morphine and dynorphin(1–13) microinjected into the medial preoptic area and nucleus accumbens: effects on sexual behavior in male rats

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The effects on sexual behavior of opiate receptor stimulation within A10 and A14 terminal areas were examined in the following experiments. Morphine (0.01–6 nmol) and dynorphin(1–13) (0.01–3 pmol) were microinjected into the medial preoptic area (MPOA). Morphine (10–100 pmol) and dynorphin (10–100 fmol) injected into the MPOA reduced both the latency to ejaculate and the number of intromissions triggering ejaculation. Morphine (6 nmol) produced a failure to resume copulating following the second ejaculation. Morphine (1–10 nmol) injected into the nucleus accumbens (ACC) shortened the latency to the first intromission and lengthened the second postejaculatory interval. Naloxone (3 mg/kg i.p.) reversed the effects of morphine on intromission latency and attenuated the lowering of ejaculatory threshold.

INTRODUCTION

The impairment of male reproductive behavior by opiates has long been recognized. Documentation has included clinical reports of sexual dysfunction occurring among chronic heroin users[11,51] and has been further substantiated by animal research. Copulation in male rats is inhibited by systemic administration of morphine[33,47], microinjection of morphine, D-Ala^2-Met-enkephalinamide[43], and β-endorphin[33,35] into the ventricles[33], and by β-endorphin microinjected into the medial preoptic area (MPOA)[23]. Early reports suggested a facilitation of sexual behavior in males by the opiate antagonist naloxone[17,33]. That the primary function of opioid peptides in reproductive behavior is inhibitory continues to be postulated[15]. A suggestion that the role played by opioids may be more complex has emerged from reports of facilitation as well as inhibition of sexual behavior by both opioids and the opiate antagonist naloxone. Evidence for opioid facilitation of male sexual behavior includes naloxone-induced reduction of sexual motivation[36] and slowing of copulatory rate[32,50]. Conversely, ejaculatory threshold has been lowered following systemic administration of low morphine doses or following ventricular injections of enkephalin[1,47].

Two areas of research have provided direction for determining the anatomical specificity of opiate effects on sexual behavior. Previous work has established the MPOA as important to masculine sexual behavior. This line of evidence includes the discovery that lesions of the MPOA impaired[21], whereas electrical stimulation[58] or androgen implanted into the structure[12] facilitated mating. More recently, a pharmacological profile of the MPOA in relation to male sexual behavior has been derived through microinjection of agonists and antagonists[4]. These studies suggest that the MPOA is important in regulating copulatory rate, efficiency, and penile erection[25,41]. Injection of β-endorphin into the MPOA produces cessation of copulation[23]. The second area of investigation is an extension of attempts to identify anatomical substrates of reward. The mesolimbic dopamine system has been strongly implicated in reward, particularly A10 neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (ACC)[6,16,59]. The activity of this pathway has been associated with human drug abuse[13,60], hypermotility[9,22,27,29,46], enhancements of self-administration in animals[5,52], electrical self-stimulation of the brain[7,30,38,48,53], and feeding[8,19,31,39].

The relative roles of the VTA and ACC in opiate reward have generated controversy. Evidence has been presented that rats will acquire self-administration of morphine delivery to the VTA, but not the ACC[5,6]. Amphetamine, but not morphine, delivered to the ACC reinstates intravenous self-administration of morphine[54]. However, opioids microinjected into the ACC increase motor activity, increase feeding[31,39], and sustain self-administration[18,40]. Furthermore, blockade of accumbens opioid receptors attenuates self-administration of heroin[10,57].

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Preliminary reports suggest that A10 neurons also mediate sexual arousal. The latency to begin copulating has become accepted as an index of the arousal component of sexual behavior, first described by Beach[3] and later refined as the 'initiation factor' emerging from factorial analysis of copulatory measures in male rats[49]. Injection of morphine and dynorphin A(1–13) into the VTA of castrated male rats given testosterone replacement facilitates initiation of copulation and increases dopamine release in the ACC[37]. However, facilitation of sexual behavior is marginal in intact males[2]. Higher morphine doses inhibit initiation in castrated males and preclude copulation in intact animals[2,37].

The following experiments assess the effects of stimulating opioid receptors within the ACC and the MPOA on male sexual arousal and performance, respectively. It is hypothesized that the lowering of ejaculatory threshold by low doses of morphine may be mediated by the MPOA. Therefore, microinjection of opiates and opioids should reduce ejaculatory threshold. In addition, it is hypothesized that an opioid system within the ACC may mediate sexual arousal. Microinjections of opiates should therefore shorten the latency to initiate copulation.

MATERIALS AND METHODS

Animals

Adult male Long–Evans rats, 300–350 g, purchased from Blue Spruce Farms (Altamont, NY), were housed singly in plastic cages (20 × 20 × 40 cm); cage floors were covered with wood shavings. Ovariectomized stimulus females of the same strain and source were housed in a separate room. Food and water were available ad libitum. Lights were off between 11.00 and 21.00 h.

Stimulus females. Females were injected with 20 μg estradiol benzoate 48 h before behavioral tests. Shortly before microinjection of male rats, female rats were placed with non-experimental males and screened for sexual receptivity; only females displaying lordosis during a minimum of 3 intromissions were used as stimulus females.

Baseline sexual performance. Males were placed with stimulus females for two 30-min intervals, one interval per week. If copulation did not occur, the interval was lengthened. One week after the second interval, males were tested for copulatory behavior. Only males that achieved one or more ejaculations were cannulated. Postoperative tests were conducted prior to the beginning of experiments; animals failing to ejaculate were retested.

Surgery

Animals were anesthetized with sodium pentobarbital (55 mg/kg i.p.) or with ketamine hydrochloride (50 mg/kg i.m.) combined with xylazine hydrochloride (4 mg/kg i.m.). The animal was then placed in a Kopf stereotaxic apparatus with the incisor bar positioned 5 mm above the interaural line. Animals were then implanted unilaterally with guide cannulae positioned 1.0 mm above the left MPOA (AP +2.4 from bregma, ML +0.2, DV −6.6; incisor bar +5)[44] or bilaterally with guide cannulae positioned at a 5° angle in relation to the midsagittal plane and aimed at the medial region of the ACC (AP +3.4 from bregma, ML ±2.8, DV −7.4)[44]. Surgical procedures and cannula construction were as previously described[25].

Microinjection

Each weekly behavioral test was preceded by an injection of a single drug dose into the guide cannula(e). Drug doses were delivered in a counterbalanced order. An injection cannula made from 27-gauge hypodermic tubing was inserted in the guide cannula; injection cannulae were 17 mm (ACC) or 18 mm (MPOA) in length and therefore extended 1 mm below the guide cannula. The injection cannula was inserted into a polyethylene delivery tube attached to a hypodermic syringe (1 ml) placed in a Harvard microinjection pump. Before microinjection, the obturator and polyethylene sheath were removed from the guide cannula and replaced by the injection cannula while the animal was gently held. The animal was then returned to his home cage and allowed to move freely during microinjection. Injection took place over 30 s. Following injection, drugs were allowed to diffuse for 30–60 s before the injection cannula was removed from the guide.

Drugs

Morphine sulfate, naloxone hydrochloride, and dynorphin A(1–13), obtained from Sigma Chemical Company, were dissolved in

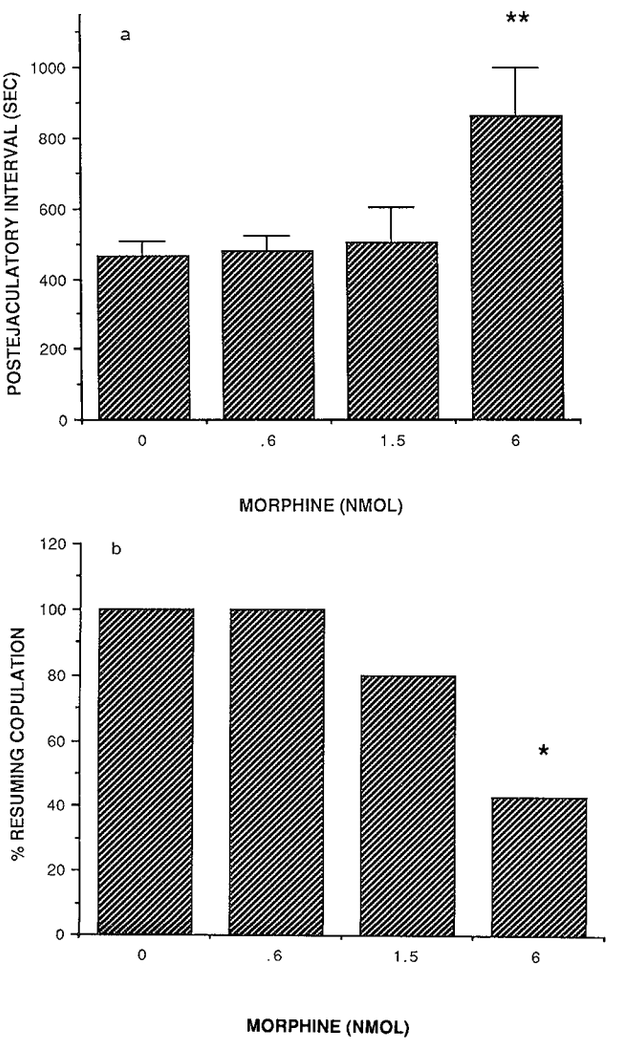

Fig. 1. a: the effects of morphine microinjected into the MPOA on the second postejaculatory interval (in s). Values are means ± S.E.M. The 6 nmol dose delayed the occurrence of intromission following the second ejaculation, compared to control (**$P < 0.01$). b: the effects of morphine microinjected into the MPOA on the resumption of mating following ejaculation. The 6 nmol dose reduced the percentage of animals intromitting within 20 min after the second ejaculation, compared to control (*$P < 0.05$).

TABLE I

Copulatory measures affected by morphine and dynorphin(1–13) microinjected into the MPOA, Expt. 2, 2a

Values are means ± S.E.M. EL_2, ejaculation latency in the second ejaculatory series; IF_1, number of intromissions in the first copulatory series; IF_2, number of intromissions in the second ejaculatory series; NAL, naloxone (3 mg/kg i.p.).

	Morphine (pmol)				
	0	*10*		*100*	*100 + NAL*
EL_2	276.6 ± 28.9	199.2 ± 24.7**		192.6 ± 15.4**	258.3 ± 31.4
IF_1	14.6 ± 0.49	12.0 ± 0.83*		10.7 ± 0.57**	11.3 ± 0.98**
IF_2	7.02 ± 0.82	6.03 ± 0.54		5.00 ± 0.38*	5.20 ± 0.72
	Dynorphin (pmol)				
	0	*0.01*	*0.1*	*3*	*0.01 + NAL*
EL_2	260.9 ± 25.9	143.0 ± 16.8*	204.1 ± 31.4	234.2 ± 33.0	191.5 ± 23.9
IF_2	7.05 ± 0.66	5.03 ± 0.35*	5.83 ± 0.57	6.00 ± 0.65	3.7 ± 0.47*

*$P < 0.05$ compared to control; **$P < 0.01$ compared to control.

0.9% sodium chloride solution (saline). Injection volume was 0.5 μl per cannula.

Behavioral testing

Sexual behavior. Five min following microinjection, a stimulus female was introduced. Behavior was observed from the point of introduction of the female until 30 min following the first intromission, or for 30 min if no intromission occurred. The time of occurrence for each mount, intromission, and ejaculation was recorded. Intromissions were distinguished from mounts by the presence of a deep thrust followed by a springing dismount. A deep thrust of longer duration, slow dismount, and subsequent period of rest were characteristic of ejaculation.

From these data, the following measures were derived: (1) latency to the first mount; (2) latency to the first intromission; (3) number of mounts preceding ejaculation; (4) number of intromissions preceding ejaculation; (5) ratio of intromissions to mounts plus intromissions leading to ejaculation (intromission ratio); (6) latency to ejaculate following first intromission in each ejaculatory series (ejaculation latency); (7) latency from ejaculation to next intromission (postejaculatory interval); (8) ejaculation frequency.

Motor activity. Three types of motor activity were scored: (1) moving from within 1 inch of one cage wall to within the same distance from another (crossing); (2) raising the body from having all 4 paws to only the hindpaws in contact with the cage floor (rearing); (3) cleaning the fur or genitalia (grooming). The number of instances of each behavior occurring during a 5-min interval immediately following microinjection was recorded for each animal in Expt. 4.

Experimenter bias. Although drug treatment was not concealed, behavioral observation was generally done by persons other than those performing microinjection. Observers were not directly informed concerning agents administered or specific behavioral hypotheses prior to behavioral testing. In most instances, observers tested two animals simultaneously. Some effort would therefore have been required to identify each animal as having received a specific treatment.

Histology

After behavioral testing was completed, animals were decapitated. Brains were mounted in a cryostat (American Optical). Coronal sections 40 μm in thickness were cut and stained with Cresyl violet. Using a projector magnifier, sections were then examined for accuracy of placement. Only data from animals whose cannulae were positioned within 0.5 mm of their intended sites were included in statistical analyses.

Statistical analysis

One-way analysis of variance with repeated measures was applied to dose–response data. Specific dose levels producing behavioral change were then identified using the Newman–Keuls procedure for multiple comparisons. Paired observation *t*-tests were used to determine the blockade or attenuation of opioid effects by an antagonist. The χ^2-test was applied to ordinal data. Logarithmic transformations were performed on latency scores prior to analysis in instances where treatment condition produced differences in variability.

Expt. 1. Higher morphine doses microinjected into the MPOA

Morphine doses of 0.0, 0.6, 1.5 and 6.0 nmol were microinjected into the MPOA to determine the effects of opiates within this structure on sexual performance.

Expt. 2. Lower morphine and dynorphin doses microinjected into the MPOA

The possibility that lower doses of opioids microinjected into the medial preoptic area may be excitatory was examined. Doses were 0, 10, and 100 pmol of morphine and 0.01, 0.1, and 3 pmol dynorphin A(1–13), resulting in 6 counterbalanced injections.

Expt. 2a. Receptor specificity of MPOA opioid effects

An opiate antagonist was administered in order to specify the involvement of opioid receptors in the facilitation of sexual behavior by opiates. Animals in Expt. 2 received two additional treatments. Naloxone hydrochloride (3 mg/kg i.p.) was injected 5 min prior to microinjecting an effective dose of dynorphin or morphine into the MPOA.

Expt. 3. Morphine microinjected into the ACC

The effect of morphine injected into the ACC on sexual arousal was examined. Morphine doses were 0, 0.1, 1, and 10 nmol per cannula.

Expt. 3a. Receptor specificity of ACC morphine effects

An effective morphine dose injected into the accumbens was preceded by naloxone.

RESULTS

Expt. 1

Following histological verification of cannula placements, data from all subjects were included in statistical tests. Morphine increased the second postejaculatory interval ($F_{3,21} = 5.21$, $P < 0.01$; Fig. 1a). The effective

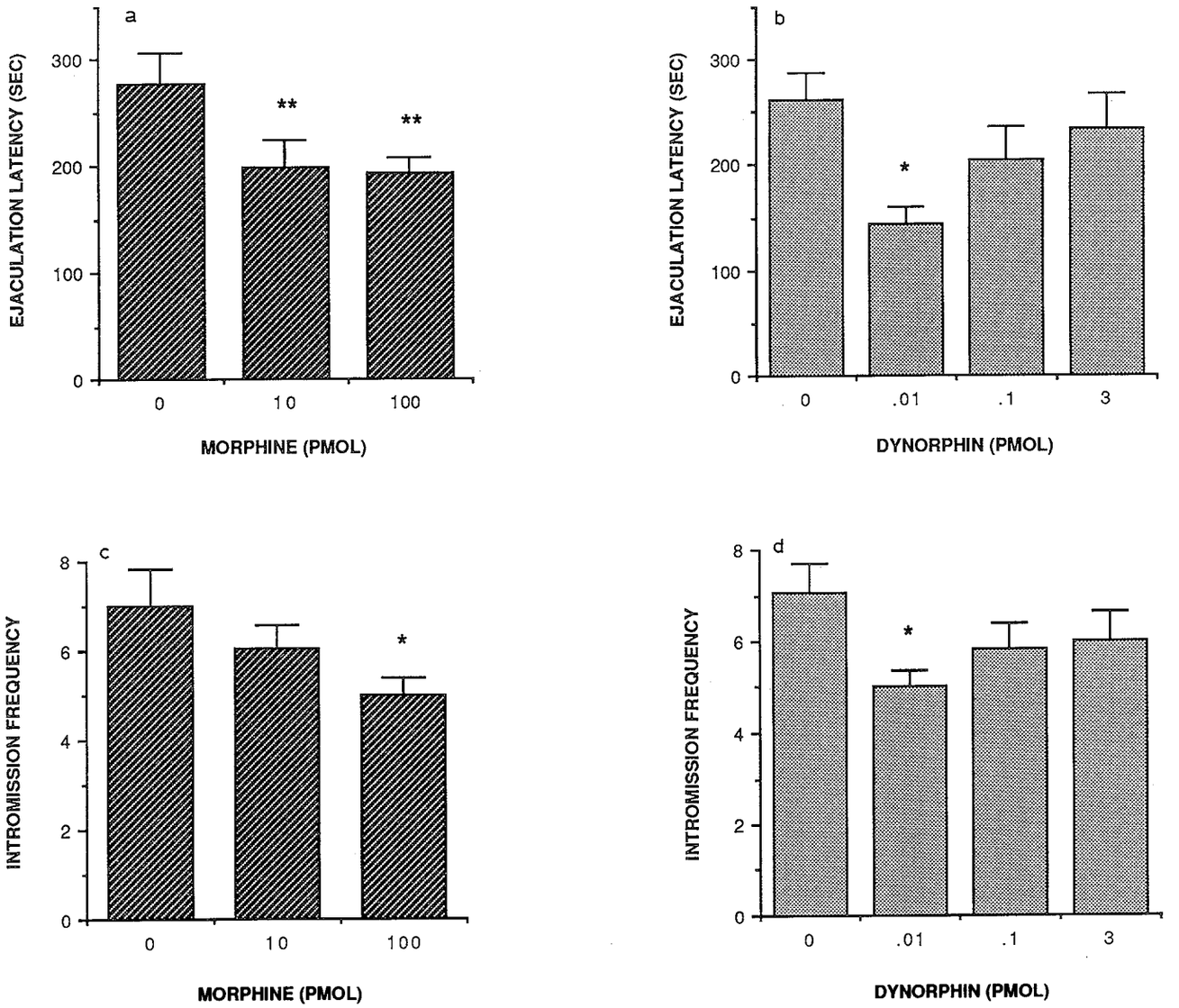

Fig. 2. a and b: the effects of morphine (a) and dynorphin(1–13) (b) microinjected into the MPOA on the mean latency (in s, ± S.E.M.) from the first intromission to ejaculation in the second ejaculatory series. The 10 and 100 pmol doses of morphine (**$P < 0.01$) and 0.01 pmol of dynorphin (*$P < 0.05$) reduced the latency to ejaculate, compared to control. c and d: the effects of morphine (c) and dynorphin (d) microinjected into the MPOA on intromission frequency in the second ejaculatory series. Morphine (100 pmol) and dynorphin (0.01 pmol) decreased the mean number of intromissions (± S.E.M.) leading to ejaculation, compared to control (*$P < 0.05$).

dose, 6 nmol ($P < 0.01$), produced a failure to intromit within 20 min after the occurrence of the second ejaculation ($\chi^2_1 = 6.25$, $P < 0.025$; Fig. 1b). No differences in duration of the second postejaculatory interval were revealed when animals failing to resume copulation were excluded from statistical analysis. Morphine also tended to reduce ejaculation frequency, ($F_{3,36} = 2.53$, $P < 0.07$).

Expt. 2.

Both morphine ($F_{2,20} = 12.07$, $P < 0.001$) and dynorphin ($F_{3,33} = 4.16$, $P < 0.03$) shortened the interval required to achieve the second ejaculation (Table I; Fig. 2a,b); 10 and 100 pmol morphine and 0.01 pmol dynorphin were effective ($P < 0.01$). Morphine ($F_{2,20} = 3.78$, $P < 0.05$) and dynorphin ($F_{3,33} = 3.06$, $P < 0.05$) decreased the number of intromissions leading to the second ejaculation. Effective doses for slowing ejaculation rate also decreased intromission frequency in the second ejaculatory series (Fig. 2c,d). In addition, morphine reduced the number of intromissions in the first ejaculatory series ($F_{2,32} = 8.19$, $P < 0.005$; Table I).

Expt. 2a

Pretreatment with naloxone (3 mg/kg) blocked the effects of both 100 pmol morphine and 0.01 pmol dynorphin on ejaculation latency (Table I). Naloxone eliminated the effects of morphine on intromission

TABLE II

Copulatory measures affected by morphine microinjected into the nucleus accumbens, Expt. 3, 3a

Values are means ± S.E.M. IL, latency to the first intromission in sexual behavior tests; PEI_2, second postejaculatory interval; NAL, naloxone (3 mg/kg i.p.).

	Morphine (nmol)				
	0	*0.1*	*1*	*10*	*1 + NAL*
IL	269.9 ± 172.4	111.4 ± 35.6	73.6 ± 38.5*	40.1 ± 38.5*	225.3 ± 120.6***
PEI_2	523.2 ± 34.7	525.7 ± 31.9	533.6 ± 27.1	760.9 ± 90.1**	598.5 ± 80.9

*$P < 0.05$ compared to control; **$P < 0.01$ compared to control; ***$P < 0.05$ compared to 1 nmol morphine.

frequency in the first and second ejaculatory series. However, naloxone pretreated animals receiving dynorphin (0.01 pmol) intromitted fewer times than saline controls in the second ejaculatory series ($t_9 = 3.1$, $P < 0.02$).

Expt. 3

Morphine injected into the ACC reduced the latency to the first intromission ($F_{3,27} = 5.06$, $P < 0.009$) and lengthened the second postejaculatory interval ($F_{3,24} = 5.83$, $P < 0.005$) (Table II; Fig. 3). The highest dose (10 nmol) was responsible for the observed increase ($P < 0.01$). However, differences in length of the interval were eliminated when animals failing to resume copulation ($n = 2$) were excluded from statistical analysis. Morphine produced no changes in any measure of motor activity.

Expt. 3a

Naloxone pretreatment reversed the morphine-induced reduction of intromission latency (Table II).

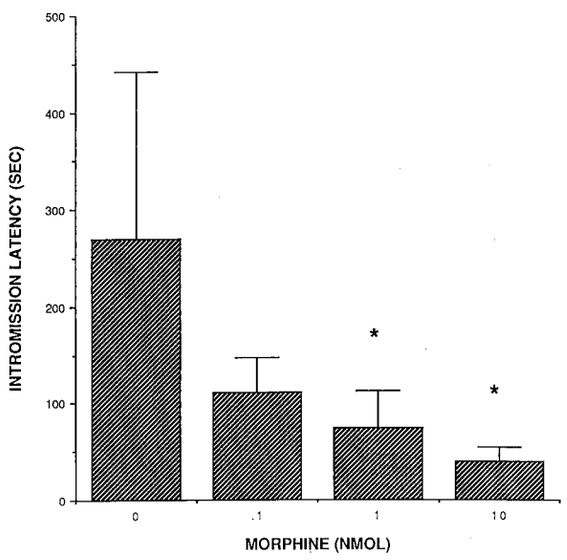

Fig. 3. The effects of morphine microinjected into the ACC on the mean latency (in s, ± S.E.M.) to the first intromission in sexual behavior tests. Both 1 and 10 nmol morphine enhanced initiation of copulation, compared to control (*$P < 0.05$).

Latencies of animals pretreated with naloxone were longer than those of animals receiving no pretreatment before central injection of morphine ($t_{10} = 2.7$, $P < 0.03$). Naloxone pretreatment in animals receiving morphine eliminated the difference between these animals and non-pretreated animals receiving saline injected into the ACC.

DISCUSSION

The highest morphine dose injected into the MPOA produced a premature cessation of copulation. The lack of an inhibitory influence appearing prior to the second ejaculation is intriguing and suggests that the failure to resume intromission may be a function of the occurrence of ejaculation. In this case, either the intensity of reward or physiological consequences of ejaculation may delay the resumption of mating. Dopamine agonists injected into the MPOA facilitate erection in rats[42], suggesting that dopaminergic neurons within this structure regulate erection. This finding may have implications for the inhibition of mating seen following injection of higher morphine doses into the MPOA. A possible underlying mechanism might be that stimulation of certain opioid receptors attenuates dopaminergic activity within the MPOA, resulting in suppression of erection. The observation that no effect on sexual behavior was seen until the second ejaculatory series suggests that the combined effects of higher morphine doses and endogenous opioids released during copulation may have precluded further mating[1]. A second possibility is that the delay may be the result of diffusion of morphine to another brain site.

Morphine microinjected into the ACC produced an increase in the second postejaculatory interval attributable to the failure of a small number of animals to resume copulating. Increases are also seen in animals injected systemically with naloxone[32,50]. The consistent lengthening of the postejaculatory interval resulting from manipulation of opioid receptors suggests either non-specific effects or that mechanisms involved are anatomically distinct. In the latter case, reduced capacity for erection

may account for the delay in mating seen following microinjections of opiates into the MPOA; whereas, opiates introduced into the ACC may induce a competing reward state that interferes with copulatory behavior.

The facilitative effects produced by lower doses of opioids injected into the MPOA are consistent with a dose-dependent regulation of copulatory rate. In particular, ejaculatory threshold appears to have been lowered, reflected both by a shortened latency and a reduced number of intromissions leading to ejaculation. A similar effect has been reported for enkephalin injected intraventricularly, suggesting that the site of action for these effects is the MPOA[1]. It is interesting to note that the dopamine agonist apomorphine injected into the MPOA produces an increase in copulatory rate which includes shortened ejaculation latencies[25]. Lowered intromission count has been reported following microinjection of the muscarinic agonist oxotremorine into the MPOA[24,26]. These findings suggest opioid modulation of preoptic dopaminergic and cholinergic substrates regulating ejaculation.

Morphine microinjected into the ACC decreased the latency to intromit. The effect of morphine on initiation was observed within 2 min after the introduction of the female, occurring in close temporal proximity to the measurement of motor activity, which was unaffected. It is therefore unlikely that the reduced intromission latency is solely the result of increased motor output. These results are somewhat surprising given that morphine and enkephalin analogs injected into the ACC have produced hypermotility[20,28,45]. However, the excitatory effects of intra-accumbens morphine have been shown to have a gradual onset[45]. In the present studies activity may have increased after the period of observation ended. Pretreatment with naloxone returned intromission latencies to control levels, evidence that morphine facilitation of sexual arousal is mediated by opioid receptors.

Injection of morphine into the ACC increases dopamine release measured by microdialysis procedures[34], suggesting the possibility that increased dopaminergic output underlies the reduced latency to initiate copulation. However, the exact relationship between opioid and dopaminergic systems within the ACC is not clear. Certain lines of evidence indicate that the two systems produce similar behavioral effects independently. Previous work has shown that the increase in spontaneous motor activity induced by intra-accumbens morphine injection is antagonized by naloxone, but not by the dopaminergic antagonist haloperidol. Conversely, haloperidol antagonized the excitatory effects of the dopaminergic agonist apomorphine but not morphine[45]. However, chronic treatment with neuroleptics and 6-OHDA lesions of A10 neurons have been found to enhance the motor activation induced by intra-accumbens morphine[55,56]. These results indicate that interruption of dopaminergic transmission alters the accumbens opioid system[56].

Further studies are needed to clarify the relationship between accumbens opioid and dopaminergic activity in regulating sexual behavior. Increased dopamine release following injection of morphine into the ACC is only partially blocked by naloxone and is not enantioselective[34]. The complete blockade of morphine effects on intromission latency is therefore compatible with some degree of dopamine-independence. Furthermore, dopamine agonists injected into the ACC produce only a marginal reduction in intromission latency[25]. These results are consistent with the induction of reward through stimulation of opioid receptors on accumbens efferents[14].

These experiments reveal no unitary opiate or opioid effect on masculine sexual behavior; rather the aspect of sexual behavior affected is anatomically specific and related to dose. Morphine introduced into the MPOA produced changes in ejaculatory threshold; morphine injected into the ACC reduced intromission latency, considered a measure of sexual arousal. In the case of the MPOA, lower morphine doses increased copulatory rate, whereas higher doses produced premature cessation, suggesting the possible involvement of multiple receptor subtypes. Although no direct statistical comparisons were made between effects of opioids injected into the two structures, it may be noted that a morphine dose that facilitated sexual behavior when injected into the ACC produced premature cessation when injected into the MPOA. The relative sensitivity of the MPOA in producing an opiate-induced inhibition suggests that this structure may be involved in sexual dysfunctions consistently seen among heroin users.

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