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Inhibition of Mating by Naloxone or Morphine in Recently Castrated, but Not Intact Male Rats

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LIEBLICH, I., M. J. BAUM, P. DIAMOND, N. GOLDBLUM, C. ISER AND C. G. PICK. *Inhibition of mating by naloxone or morphine in recently castrated, but not intact male rats.* PHARMACOL BIOCHEM BEHAV 22(3) 361–364, 1985.—Administration of naloxone (SC 5 mg/kg) significantly reduced ejaculation and mounting in male rats in the weeks following castration. A similar effect was obtained by injecting morphine (SC 1 or 5 mg/kg). In contrast, the same dosages of naloxone or morphine did not affect the sexual performance of gonadally intact males. Opioid peptides may contribute to the temporary persistence of sexual behavior in testosterone-deficient male mammals, in which incentive qualities of the female partner are an important determinant of sexual arousal.

Rat sexual behavior Naloxone Morphine Endorphins

SINCE the discovery of opiate receptors [26, 29, 32] and of enkephalin and endorphin-containing neurons [10] in the brain, much attention has been given to the possibility that these systems are involved in the control of masculine sexual behavior in vertebrates. It has been known for some time that chronic injection of opiate drugs by men reduces the testicular secretion of testosterone [1] and causes a loss of libido and sexual potency [3]. Moreover, acute administration of morphine [19,20], beta-endorphin [19,21] or methadone [23] to male rats or hamsters caused dose-dependent reductions in masculine sexual performance at dosages and times which would not suppress plasma testosterone or alter animals' general level of activity. These latter findings raise the possibility that endogenous opioid peptides may normally act at central opioid receptors to inhibit masculine sexual arousal. Indeed, in some instances administration of opiate receptor antagonists facilitated the occurrence of ejaculation in male rats tested with females [7, 19, 24, 25] as well as in a single human male subject during masturbation [8]. In other experiments, however, [18, 28, 30] administration of opiate receptor antagonists failed to influence the pre-ejaculatory performance of male rats.

All of the above studies were conducted using males which were either gonadally intact or castrated and maintained on testosterone. It is well established in nearly all mammalian species studied that masculine coital performance gradually declines after castration [4]. In the rat this decline extends over 3–6 weeks even though plasma testosterone concentrations drop within hours after castration. Little is known about the cause of this temporary persistence of what is otherwise a hormone-dependent behavior. However, one important determinant of the rate at which mating declines after castration is the stimulus quality of the estrous females used to test the male. Thus, male rats placed with females which displayed high levels of solicitational behavior continued to mount and ejaculate longer after castration than males tested with less proceptive females, i.e., females which displayed fewer sexual solicitations [17]. In a related study these same workers [16] found that gonadally intact males which lacked previous mating experience were more likely to initiate copulation with highly proceptive females than with females which displayed lordosis in response to mounting but failed to display precoital solicitational behavior.

Several lines of evidence [12, 13, 14, 15, 27] suggest that endogenous opioids are especially involved in promoting the consumption of both nutritive and non-nutritive sweet substances in water replete rats. In other words, opioid peptides may contribute importantly to the regulation of drinking when the taste of a particular compound is the primary determinant of whether or not it is ingested. We hypothesized that an endorphinergic mechanism may also become critically important for the facilitation of masculine coital behavior during the postcastration period, when sexual arousal is especially dependent on the incentive qualities of the female partner. We initially tested this hypothesis by comparing the effects of an opiate receptor antagonist, naloxone, on the sexual behavior of male rats which had recently been castrated or left gonadally intact. After finding that naloxone significantly inhibited coital performance in

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castrated but not gonadally intact animals, we conducted a second experiment to compare the effects of administering low dosages of an opiate receptor agonist, morphine, on the sexual behavior of males maintained under these two different endocrine conditions.

GENERAL METHOD

Subjects

Adult male and female rats of the Hebrew University Colony (Sabra) strain were used in these experiments. They were housed in unisexed groups of 2–4 with water and food available ad lib. The colony lights were off each day for 12 hours beginning at 11:00. Prior to being used in either experiment, all males were observed ejaculating with estrous females during each of 3 tests given on separate days. The females were ovariectomized using ether anesthesia and were made sexually receptive and proceptive for tests with males by SC injection of 20 μg estradiol benzoate in oil 1–3 days prior to testing; they also received progesterone (1 mg SC in oil) 4–6 hr prior to testing.

Behavioral Testing and Data Analysis

All behavioral tests were conducted when the colony lights were off. Observations of sexual behavior were performed in a red-lighted room. The rats were tested in 4 semicircular (60 cm diam) Plexiglas cages with floors covered with sawdust bedding. The following masculine behaviors were recorded using an Esterline-Angus event recorder: Mounts, intromissions, ejaculations, as well as the first intromission following an ejaculation. If no mount was observed within 15 min after the beginning of a test or if no intromission occurred within 30 min, the session was terminated. Otherwise, tests lasted until the first intromission after an ejaculation was observed (defining the post-ejaculatory interval, PEI), or until 60 min elapsed from the beginning of a test, whichever occurred earlier. Mount rates (number of mounts + number of intromissions/time in minutes from beginning of test until ejaculation or test termination) were calculated for each test. Mount rate data were subjected to analysis of variance; between group comparisons of the number of tests with ejaculation were made using 2-tailed Mann-Whitney U tests.

EXPERIMENT 1: EFFECT OF NALOXONE IN CASTRATED VERSUS GONADALLY INTACT MALE RATS

Method

After one baseline test, two groups of males were castrated using ether anesthesia and two additional groups of males were left gonadally intact. Beginning 4 days later one group of castrates (n=7) and one group of intact males (n=7) received SC injections of 5 mg/kg naloxone hydrochloride in 0.9% saline (1 ml/kg); additional groups of castrated (n=8) and gonadally intact (n=7) males received only saline injections. This particular dosage of naloxone was chosen because it fell in the middle of a range of dosages of this drug which effectively inhibited the intake of sucrose solution by rats with open gastric fistulas [27]. Immediately after the injections males were placed into the testing cages for 5 min, whereupon an estrous female was introduced into each cage. Mating usually lasted 10–15 min after the introduction of the female. Previous research showed that a 2–5-fold increase in plasma luteinizing hormone was induced by naloxone in male rats within 15 min after SC or IP injection in saline, suggesting that this opiate receptor antagonist exerts its central action rapidly. Each male was tested for sexual behavior at subsequent intervals of 3–4 days, for a total of 6 tests.

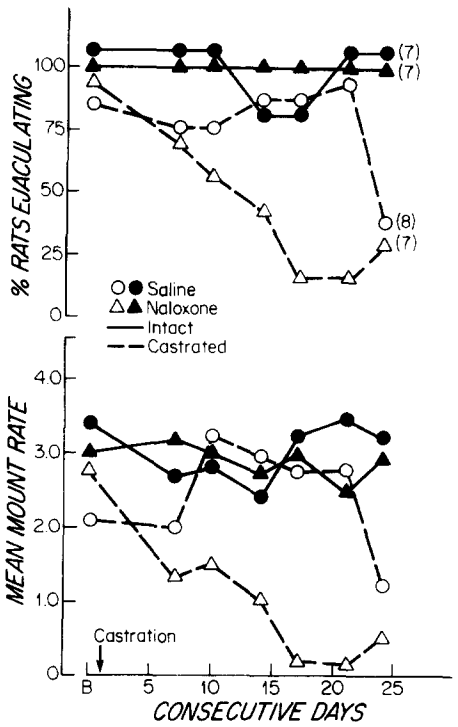

FIG. 1. Effect of naloxone hydrochloride (5 mg/kg) or saline on ejaculation and mean mount rate in sexually experienced, adult male rats which were either castrated or left gonadally intact. The number of males in each group is given in parentheses. B=baseline test

Results

Administration of naloxone to castrated males caused significant reductions in mounting rates and the incidence of ejaculation whereas no such changes occurred in gonadally intact males given this drug (Fig. 1). An ANOVA performed on mount rates revealed a significant gonadal status × drug treatment interaction, $F(1,22)=8.38$, $p<0.05$, and castrated males given naloxone ejaculated during significantly fewer tests ($p<0.05$) than each of the other 3 groups of males. Naloxone also lengthened mount, intromission, and ejaculation latencies as well as PEI's (data not shown) in castrated males, whereas administration of this dosage of naloxone failed to affect any parameter of sexual behavior in gonadally intact rats.

EXPERIMENT 2: EFFECT OF MORPHINE IN CASTRATED VERSUS GONADALLY INTACT MALE RATS

Method

New groups of male rats were given ejaculatory experience with sexually receptive females in three tests. Following one additional baseline test, two groups of males were bilaterally castrated while two other groups were left gonadally intact. One group of castrates (n=8) and one group of gonadally intact (n=7) males received SC injections of mor-

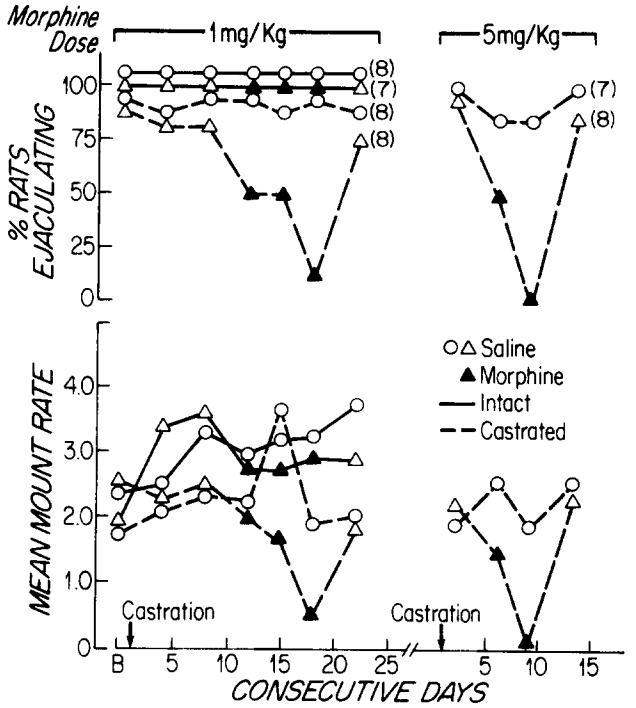

FIG. 2. Effect of morphine hydrochloride (A: 1 mg/kg; B: 5 mg/kg) or saline on ejaculation and mean mount rate in sexually experienced, adult male rats which were either castrated or left gonadally intact. The number of males in each group is given in parentheses. B=baseline test

phine hydrochloride (1 mg/kg) in 0.9% saline (1 ml/kg) prior to tests given on days 11, 14, and 17 after the precastration baseline test; these same rats received SC injections of saline vehicle prior to tests given 3, 7, and 21 days after the baseline test. Additional groups of castrated (n=8) and gonadally intact (n=8) males received saline prior to all tests. After the completion of these tests, those males which were still gonadally intact were castrated and divided into two groups. Both groups were tested 1 day later after SC injection of saline. One group continued to receive saline prior to additional tests given 5, 8, and 12 days after castration. The second group received morphine hydrochloride (5 mg/kg) SC prior to tests given 5 and 8 days postcastration, and again received saline prior to a final test given 12 days postcastration. In both phases of Experiment 2 injections of morphine or saline vehicle were given SC to males 5 min prior to the introduction of an estrous female into the test cage. Tests of sexual behavior were conducted using the procedure described for Experiment 1.

Results

Administration of morphine (either 1 or 5 mg/kg) mimicked the effect of naloxone by significantly reducing the coital performance of castrated males, whereas at these dosages morphine had no effect on gonadally intact males (Fig. 2). As shown in Fig. 2A, castrated males given morphine (1 mg/kg) ejaculated during significantly fewer tests ($p<0.05$) than gonadally intact males given morphine, or either group of saline-treated controls. Mount rates were also significantly lower in castrated males given morphine than in other groups: the ANOVA revealed a significant gonadal status × drug treatment interaction, $F(1,27)=6.03$, $p<0.05$. Likewise, administering a higher dosage of morphine (5 mg/kg) reduced the incidence of ejaculation (Fig. 2B), and mount rates were significantly lower in castrated males given this dosage of morphine as opposed to saline, $F(1,13)=5.60$, $p<0.05$. In castrated rats both dosages of morphine lengthened mount, intromission and ejaculation latencies as well as the PEI (data not shown). However, no significant effects of morphine (1 mg/kg) on these parameters of mating were detected in gonadally intact males.

GENERAL DISCUSSION

Administration of naloxone or morphine to male rats during the weeks following castration markedly inhibited masculine sexual performance, whereas no such effect was obtained in gonadally intact males given the same dosages of these drugs. In previous studies administration of either naloxone or naltrexone to gonadally intact male rats or hamsters caused a variety of behavioral changes, ranging from reductions in ejaculation latency [19,24] to lengthening of the postejaculatory interval [18, 28, 30] to the initiation of mounting in sexually unresponsive males [7]. In no instance, however, has an inhibitory effect of opiate receptor antagonists on mounting or ejaculation been reported for any rodent species. In previous studies administration of morphine to gonadally intact male rats caused a dose-dependent inhibition of masculine sexual behavior, with more than 5 mg/kg being a minimally effective dosage [19,22]. In those studies opiate agonists' inhibitory effects on sexual behavior were rapid and occurred without reducing males' general locomotor activity, at least at the lower dosages used. In Experiment 2 giving as little as 1 mg/kg morphine inhibited the sexual performance of castrated males, whereas this dose failed to affect coital behavior in gonadally intact animals. Thus, at the dosages used, the behavioral effects of naloxone and morphine were similar in the present experiments. They had no influence on the coital behavior of intact, sexually-experienced males, whereas they markedly inhibited mating in males after castration.

It seems unlikely that the inhibitory effects of either naloxone or morphine on the sexual behavior of castrates was due to some non-specific debilitating action of these treatments, since these agents failed to influence the behavior of gonadally intact animals studied at the same time. It also seems unlikely that the inhibitory effect of naloxone on mating (Experiment 1) depended on the repeated administration of this drug. In a recent study (I. Lieblich, unpublished results), a single SC injection of 5 mg/kg naloxone given to males approximately 4 weeks following castration strongly inhibited mounting rate and ejaculation. The coital performance of these males recovered in a subsequent test prior to which saline was injected instead of naloxone. It has been suggested that castration augments the number of binding sites for naloxone in the rat brain [9,11]. However, this observation was not replicated in more extensive experiments [2, 5, 32]. Thus it seems unlikely that potent inhibitory effects of naloxone and morphine on the sexual behavior of castrated animals can be explained by steroid-dependent changes in brain opiate receptor concentrations.

We have no simple explanation for the fact that at the dosages used, both an opiate receptor agonist and an antagonist inhibited the sexual behavior of castrated male

rats. However, it is worth noting that in a previous study [6] administering low dosages of either naloxone or morphine both significantly inhibited feeding and drinking behavior in male rats which had been deprived of food and water for 24 hr. These workers suggested that naloxone attenuated the endorphin-mediated reward which normally results from the ingestion of food or water whereas morphine induced reward by activating opiate receptors, thereby reducing the drive state which normally promotes feeding and drinking. As pointed out in the introduction, endogenous opiates may contribute most to regulating the drinking of those substances for which the incentive taste value is the primary determinant of whether or not ingestion will occur. The present results suggest that endorphins may influence masculine sexual arousal in an analogous fashion. Their contribution normally may be minimal. However, they may become of critical importance for the facilitation of sexual arousal under conditions of androgen deprivation (present study), after prolonged bouts of copulation leading to sexual exhaustion [31], or in sexually inexperienced animals [16]. Under each of these circumstances the incentive qualities of the female partner are a primary determinant of whether masculine sexual activity will occur.

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