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Copulatory Behavior and Sexual Reflexes of Male Rats Treated With Naloxone[1]

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SACHS, B. D., R. J. VALCOURT AND H. C. FLAGG. *Copulatory behavior and sexual reflexes of male rats treated with naloxone*. PHARMAC. BIOCHEM. BEHAV. **14**(2)251–253, 1981.—Two experiments explored a potential role for endogenous opiates in the regulation of sexual behavior of male rats. Specifically, we questioned whether such opiates regulated the refractory period following ejaculation during copulation, or the latency period for the evocation of penile reflexes (erections, cups, and flips) from supine males. Animals were injected IP with 15–45 mg/kg naloxone hydrochloride 30 min prior to the start of reflex testing, and with 7.5–45 mg/kg naloxone hydrochloride 30 min before testing for copulation. Naloxone resulted in a small but reliable decrease in the number of penile flips. Reflex latency and other measures of penile reflexes were unaffected. At all doses used, naloxone significantly prolonged the postejaculatory refractory period, and there were no other effects on copulation.

Naloxone β-Endorphin Endogenous opiates Copulation Sexual reflexes Refractory period

SEVERAL recent publications have implicated endogenous opiates in the regulation of masculine sexual behavior [4, 10, 11, 18, 19, 20, 21, 22, 23, 24, 28]. Intuitively, opiates should be most closely associated with the postejaculatory refractory period, during which male rats exhibit reduced sexual excitability [2,3], reduced sensitivity to painful electric shocks [2], and, for most of the period, a high amplitude, slow wave, EEG [1,15]. These effects are consistent with the known analgesic and cataleptic consequences of exogenous β-endorphin [5, 14, 17], and may reflect the actions of endogenous opiates. Murphy, et al [19] found higher levels of serum β-endorphin in male golden hamsters immediately after ejaculation than after no copulation or non-ejaculatory intromission. They hypothesized that the release of β-endorphin was the basis for postejaculatory refractoriness in male hamsters. In the study reported here, naloxone, an opiate antagonist, reliably increased the PEI.

The sexual reflexes (penile erections, cups, and flips) characteristic of a copulating male rat can be evoked by restraining the male in a supine position with the penis extruded from within the penile sheath [12], and these reflexes are functionally related to the male's copulatory behavior [12, 26, 27]. The latency to the first reflex in intact animals is commonly 10 min or more [8,27], and appears to be associated with a general subsidence of the male's intermittent struggling against restraint. In many species restraint in the supine position induces tonic immobility or a similar state, which is potentiated by morphine in chickens [25,29], rats [16], and rabbits [7,9]. We questioned whether effects of the rat's endogenous endorphins regulated the duration of the penile reflex latency, perhaps by disinhibition of the spinal systems mediating the sexual reflexes.

METHOD

Twenty-two adult male Long-Evans rats from Charles River Breeding Laboratories were given 2 reflex screening tests spaced a week apart. The 11 animals that responded during both tests were included in the study, and the second test was used as a baseline. Animals were then given 3 weekly reflex tests following IP injections of naloxone hydrochloride in 0.9% saline. Three different dosages of naloxone were used: for Test 1, 15 mg/kg of body weight; for Test 2, 30 mg/kg; and for Test 3, 45 mg/kg. Animals were tested 30 min after injection [10].

The procedure for reflex testing and the characteristics of the penile reflexes have been described in detail elsewhere [26,27]. Briefly, the animal was restrained on its back and the penis was extruded from the penile sheath. Tests lasted for 15 min after the first reflex, or for 30 min if no reflexes occurred. Reflexes were scored on an Esterline Angus event recorder.

Three categories of penile reflexes were recorded. *Erection* is a swelling and extension of the glans penis, usually accompanied by a slight reddening. A *cup* was scored when the distal portion of the glans flared out during erection so that it was more bell-shaped than tubular. A cup was recorded either when it appeared immediately from the non-erect state or when it evolved out of an erection. A *flip* is a quick dorsal flexion of the penis.

The reflex measures used in this study were the latency (time from penis extrusion to first reflex), the number of erections, number of cups, and number of flips per testing session.

Ten days after the last reflex test, the 11 animals were

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TABLE 1

MEAN VALUES (± SEM) IN THE REFLEX TESTS (N=6)

Naloxone Dose	Reflex Latency (min)	Number of Erections	Number of Cups	Number of Flips
0 mg/kg*	12.1 ± 4.14	24.3 ± 2.94	1.0 ± .63	11.3 ± 4.35
15 mg/kg	5.9 ± 1.62	25.8 ± 4.24	2.7 ± 1.74	7.0 ± 2.44
30 mg/kg	7.1 ± 2.11	26.0 ± 3.01	2.7 ± 1.38	5.8 ± 2.59
45 mg/kg	9.9 ± 2.07	29.0 ± 5.13	2.7 ± 1.25	5.2 ± 1.76
F value (df=3,15)	1.95	0.35	0.48	3.30†

*Last baseline test. Animals were not actually injected with vehicle.
†$p<0.05$. No paired comparisons significant by Newman-Keuls test.

TABLE 2

MEAN VALUES (± SEM) IN THE COPULATION TESTS (N=8)

Naloxone Dose	Intromission Latency (sec)	Number of Intromissions	Ejaculation Latency (min)	Postejaculatory Interval (min)
0 mg/kg	14.6 ± 5.19	7.4 ± .74	2.9 ± .48	5.4 ± .38
7.5 mg/kg	17.4 ± 5.69	6.5 ± .53	3.3 ± .40	7.5 ± .85
15 mg/kg	21.2 ± 15.07	7.7 ± .69	3.7 ± .74	8.6 ± 1.73
30 mg/kg	21.4 ± 12.41	7.8 ± .84	3.2 ± .45	8.5 ± .76
45 mg/kg	18.9 ± 10.60	6.1 ± .61	3.3 ± .78	10.5 ± 2.70
F value (df=4,28)	0.21	2.36	0.97	3.55*

*$p<0.025$. By Newman-Keuls test, 0 mg/kg less than all other values, $p<0.01$; 7.5–30 mg/kg less than 45 mg/kg, $p<0.05$.

given 2 baseline copulatory tests spaced 5 days apart. One animal did not copulate to ejaculation on either occasion and was excluded from further testing. Ten days after the second baseline, copulation tests following naloxone treatment were begun. In this series of tests, the dosages of naloxone used were 45, 30, 15, 7.5, and 0 mg/kg of body weight, injected 30 min prior to the start of testing [10]. Animals were tested twice weekly and the testing order, in terms of naloxone dosage, was 30, 0, 45, 0, 15, 0, 7.5, 0, 0, 15, and 15 mg/kg.

Prior to each copulation test, males were given a 10-min adaptation period alone in the testing chamber (a 10 gal aquarium). The test began with the introduction of a female, made receptive by injections of estradiol benzoate and progesterone. If no intromission occurred after 15 min, a series of female changes occurred, each separated by 5 min. If an intromission still had not occurred 5 min after the third female change, the test was stopped. The tests of intromitting males were stopped following the first intromission after the first ejaculation, or after 30 min without an ejaculation.

The following copulation measures were used: intromission latency (IL), the time from introduction of the female to the first intromission; number of intromissions (NI) prior to an ejaculation; ejaculation latency (EL), the time from the first intromission to ejaculation; and postejaculatory interval (PEI), the time between an ejaculation and the next intromission.

RESULTS

Of the 11 animals tested for penile reflexes, 5 did not respond in all of the tests and were therefore excluded from statistical analysis. The data for the 6 consistent responders are summarized in Table 1. A one-factor repeated measures analysis of variance was used to determine if differences existed among the 4 drug conditions. Reflex latency, number of erections, and number of cups did not undergo significant changes. The decrease in the number of flips was of borderline significance by analysis of variance, $F(3,15)=3.30$, $p<0.05$, but none of the paired comparisons was significant when analyzed using a Newman-Keuls post hoc test.

Two of the 10 animals tested for copulatory behavior did not copulate to ejaculation consistently, and their data were eliminated from analysis. Of the remaining 8 animals, one male consistently had much longer postejaculatory intervals (PEI) than the rest. Without that animal's data, the mean PEI values were reduced to 5.1, 6.8, 6.9, 7.7, and 7.8 min for 0, 7.5, 15, 30, and 45 mg/kg, respectively.

There were 5 different copulation trials under the no-drug condition, 3 trials under the 15 mg condition, and 1 trial under each of the 3 remaining conditions. A one-factor repeated measures analysis of variance performed on the 2 multiple-trial conditions showed that there were no significant across-trial changes. Therefore, the multiple data points were converted into means per dose for each animal. A one-factor repeated-measures analysis of variance was then used to compare the animals' performance among the 5 drug conditions. The results are summarized in Table 2.

There were no significant changes in intromission latency, number of intromissions, or ejaculation latency. However, there was a significant increase in the postejacula-

tory interval, $F(4,28)=3.55$, $p<0.025$. A Newman-Keuls test revealed that the postejaculatory intervals under all drug conditions were significantly greater than those under the no-drug condition. The postejaculatory interval under the highest drug dosage (45 mg/kg) was also significantly greater than those under the lower dosages (7.5–30 mg/kg).

DISCUSSION

We found that naloxone did not change the duration of the penile reflex latency. (Naloxone has also been ineffective in altering the latency or duration of tonic immobility in chickens and rabbits, nor has it reversed the potentiation of tonic immobility by morphine or shock in these species [25,29; but see [7].) Our results offer no support for the hypothesis that the duration of the penile reflex latency is regulated by endogenous opiates.

At all doses (7.5–45 mg/kg) naloxone increased the duration of male rats' postejaculatory intervals (PEIs) without affecting other copulatory variables. The absence of a facilitative effect of naloxone on intromission latency or ejaculation latency in this study, contrary to some others (e.g., [10, 18, 20, 21, 24], may have been due to our animals' very fast control-level latencies, which left little room for potentiation. In several studies using naloxone or naltrexone in doses from 2–200 mg/kg, the PEI has also been increased [19, 21, 28] or has remained the same [10, 18, 20, 23, 24]. The decreases in PEI that were expected from antiopiate action have not been observed in any study. The increase in PEI is presently inexplicable. One possibility that cannot be entirely discounted is that the naloxone was exerting an agonistic effect at the relatively high doses used. This hypothesis seems unlikely, however, because behavioral variables other than PEI were unaffected, and because the agitated state described in rats treated with 50 mg/kg naloxone [6] was not manifested in our animals even at 45 mg/kg. Hence, unless (a) the normal antiopiate effect of naloxone and naltrexone is uniquely blocked during the PEI, or (b) β-endorphin is acting via nonopiate receptors, or (c) β-endorphin is acting upon naloxone-insensitive opiate receptors, it appears unlikely that postejaculatory refractoriness is induced by the release of β-endorphin, or terminated by the gradual dissipation of β-endorphin.

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