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Effect of β-Endorphin on Sociosexual Proclivity in a Choice Paradigm

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WIESNER, J B , C A DUDLEY AND R L MOSS *Effect of β-endorphin on sociosexual proclivity in a choice paradigm* PHARMACOL BIOCHEM BEHAV **24**(3) 507–511, 1986 —Intraventricularly-administered β-endorphin (β-END) suppresses receptive and proceptive behaviors of the ovariectomized, estrogen-progesterone-primed rat This rat model was used in a four-way choice paradigm to investigate the behavioral specificity and motivational aspects of sexual suppression by β-END The experimental females showed a preference to associate with a sexually active 'stud' male over the other incentive animals as shown by significantly more approaches to and nose pokes into the stud male compartment β-END did not alter that choice preference Rather, there was a decrease in overall social interaction after β-END treatment, as shown by decreased total nose pokes This overall decrease in social motivation, which was blocked by subcutaneous naloxone injection, was not specific to the stud male It was not a result of akinesia, since total movement among the inner alleys was significantly higher after β-END treatment, an effect also reversed by naloxone These data suggest that intraventricular β-END may act to suppress sociosexual motivation as a whole rather than sexual activity specifically

β-Endorphin Opioids Social behavior Sexual behavior Lordosis Proceptivity Receptivity

STUDIES in this laboratory have shown that the opioid peptide β-endorphin (β-END), administered intraventricularly (ICV), consistently suppresses both receptive and proceptive behaviors of steroid-primed female rats [17, 19, 20] Since proceptivity is suppressed by the peptide, it appears that opioids act to suppress a motivational component of female behavior in addition to suppressing the spinal reflex of lordosis In considering the possible role of endogenous opioid peptides in the control of sexual behavior, it is vital to determine whether the exogenously-administered opioid peptides are acting directly and specifically on the neural mechanisms which regulate sexual behavior Experiments designed to discern the behavioral specificity of the opioid effects have shown that the sexual effects are not secondary to akinesia, and are relatively independent, but not exclusive, of non-sexual behavioral effects of opioids [19,20]

In order to further address the question of specificity and to investigate the motivational aspects of opioid suppression of sexual behavior, the present study utilized a four-way choice paradigm which has been used in this laboratory to determine the influence of endocrine factors on sexual motivation [4,5] Using ovariectomized (OVX) estrogen-progesterone (EP)-primed rats, the 'choice box' technique was employed to measure sexual preference, tendency to seek contact with conspecifics, and general exploratory activity The results indicate that β-END may alter the state of general social motivation rather than sexual motivation specifically

This work has been presented previously in abstract form [18]

METHOD

Subjects

Sprague-Dawley female rats (200–300 g, Simonsen) were maintained in a temperature-controlled colony room with a modified 14 10-hr light-dark cycle (fluorescent lights on 2200 hr, off 1200 hr) in which the dark phase was dimly illuminated by red light Food and water were available ad lib Under equithesin anesthesia, all animals were OVX and stereotaxically implanted with permanent 23G stainless steel cannulae directed into the third cerebral ventricle (cannula placement was verified by gross dissection at termination of the experiment) At least one week after surgery, all animals were subjected to one or two preliminary mating tests in which experimental subjects were selected for their ability to respond to EP-priming by demonstrating high receptivity (lordosis behavior) levels

Steroid Priming

The priming protocol utilized pre-incubated, 15 mm Silastic capsules containing 17 β-estradiol [16], implanted subcutaneously (SC) 50–54 hr prior to testing The animals received SC injection of progesterone (2 5 mg in oil) 4–6 5 hr prior to testing Estrogen capsules were removed following each test session

Apparatus

The Plexiglas choice box (Fig 1) consisted of four inner boxes which formed alleys leading from a center box At the end of each alley was an outer box which held one of the four

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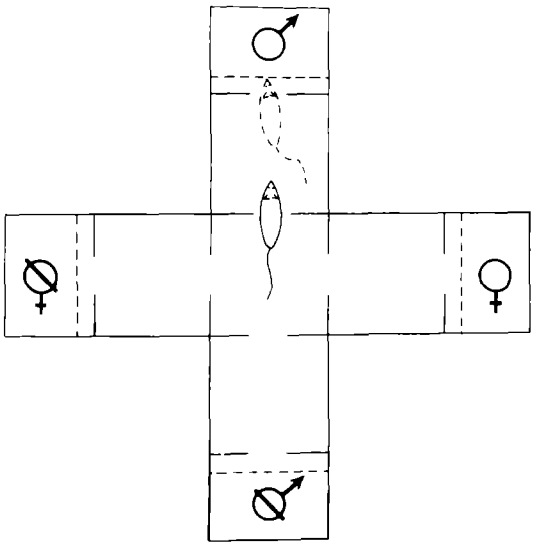

FIG 1 The 'choice box ' Four inner boxes extend at right angles from a center box, from which the experimental animal starts The inner boxes form alleys which lead to four outer boxes holding the incentive animals or choices, indicated by symbols The experimental animal has partial access to each outer box Partial entry into the outer box interrupts a photocell beam, causing 'nose poke' time and frequency (primary measures of preference) to be recorded automatically Photocells lining the alleys record frequency of entrances into each inner box (primary measure of general activity as well as preference)

TABLE 1

Treatment	Choice: Stud Male	Castrated Male	Proestrus Female	OVX Female
	Frequencies of Inner Box Entrances			
SAL	17 8 ± 4 1	15 6 ± 2 7	10 6 ± 1 2	16 0 ± 1 8
β-END	29 1 ± 4 4	20 4 ± 4 1	18 6 ± 2 2	18 5 ± 2 3
Naloxone +β-END	22 2 ± 2 7	15 1 ± 2 6	13 1 ± 1 6	17 7 ± 1 7
	Frequencies of Nose-Pokes			
SAL	41 3 ± 5 7	32 7 ± 8 3	20 6 ± 3 9	25 3 ± 4 3
β-END	35 2 ± 6 5	17 9 ± 3 7	13 9 ± 2 0	16 6 ± 3 0
Naloxone +β-END	49 6 ± 8 1	22 8 ± 4 4	20 9 ± 4 8	21 9 ± 4 3

Newman Keuls comparisons showed that scores were higher for the stud male choice than for each of the other choices ($p<0.001$) Frequencies of inner box entrances were significantly greater following β-END treatment than after treatment with saline or with naloxone+β-END ($p<0.05$) Conversely, nose poke frequencies were significantly lower following β-END treatment ($p<0.05$)

incentive animals (choices) A wire mesh was positioned within each outer box in such a way as to retain the incentive animal and to allow only partial entry of an experimental animal from the other side The alleys were lined with photocells which allowed automated scoring of the following measures for the experimental animal (1) frequency of entrances into each inner box, (2) frequency of partial entrances into each outer box (nose poke frequency), (3) total time in close proximity to the wire mesh of each outer box (nose poke time) The following Long-Evans rats were used as incentive animals (1) an intact 'stud' male which was known to be sexually active, (2) a castrated male, (3) a proestrus female, selected by daily vaginal smearing, (4) an OVX female These were rotated among the outer boxes at each test session to control for possible box-specific preferences The apparatus was cleaned thoroughly prior to each session

In this choice box paradigm, all three variables measured—inner box entrance frequency, nose poke frequency, and nose poke time—are interpreted as measures of preference of the experimental female toward the incentive animal held in the respective outer box This is particularly true of nose poke time and frequency, which reflect a tendency to spend time in close proximity to the incentive animal In addition, the frequency variables may be considered measures of activity, particularly the inner box entrance frequency which reflect movement that is less associated with direct proximity to an incentive animal

Drugs and Administration Procedure

Human β-END (Peninsula Laboratories) was dissolved in physiological saline For ICV infusions, animals were placed under brief (approximately 2 5 min) ether anesthesia A 30G inner cannula, connected with a Hamilton syringe by polyethylene tubing, was inserted into the permanent outer cannula, infusions (1 μl) were performed over one minute, and the inner cannula was left in place for an additional minute Naloxone hydrochloride, a gift of Endo Laboratories, was dissolved in saline and injected SC at the time of ICV infusion

Experimental Procedure

The experimental animals were tested at weekly intervals under EP-primed conditions The animals were habituated to the apparatus for two weekly periods, testing was performed over the following three weeks Each animal received one of the following treatments at each test session in counterbalanced form saline SC+saline ICV, saline SC+β-END (1 μg) ICV, naloxone (2 mg/kg) SC+β-END (1 μg) ICV Thirty minutes after treatment, the animal was placed in the center box for two minutes after which gates were raised allowing access to each area of the apparatus for a period of 15 minutes Testing was done in the dark phase of the light-dark cycle, with the apparatus lit by dim red light

Statistical Analysis

Data were analysed using a two-way analysis of variance for repeated measures (drug treatment × choice) Factors found to be significant were tested with Newman-Keuls comparisons

RESULTS

Analysis of variance yielded significant choice effects for all three variables scored inner box entrances,

TABLE 2

EFFECT OF β-END PERCENT CHANGE FROM SALINE SCORES

Measure	Direction of Change After β-END Treatment	Percent Change			
		Stud Male	Castrated Male	Proestrus Female	OVX Female
Inner Box Entrances	increase	63%	31%	75%	16%
Nose Poke Frequency	decrease	15%	45%	33%	34%
Nose Poke Time	decrease	9%	47%	42%	25%

Effect of β-END on inner box entrances, nose poke frequencies, and nose poke times for each choice, expressed as percent change from saline control scores

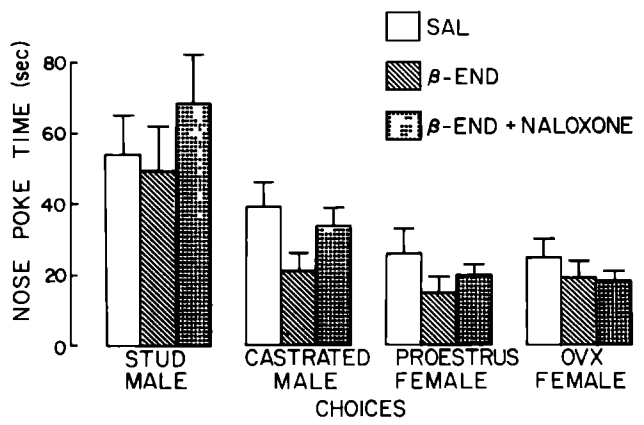

FIG 2 Nose poke times of OVX, EP-primed experimental rats after treatment with saline (SAL), β-END, or β-END in combination with naloxone Nose poke times for the stud male were significantly greater than for each of the other choices, regardless of treatment ($p<0.001$ by Newman Keuls comparisons)

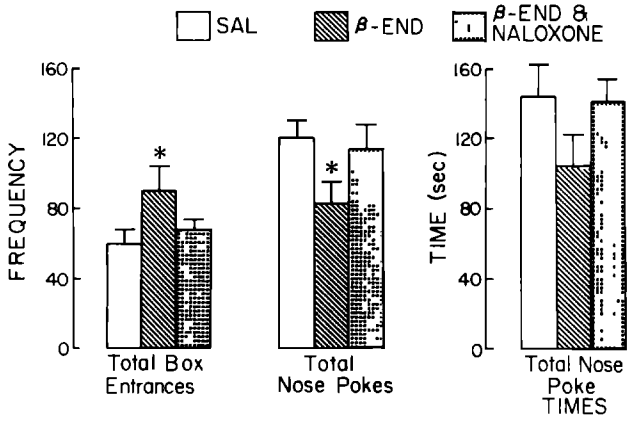

FIG 3 Effect of saline (SAL), β-END, and naloxone+β-END on total inner box entrances, total nose poke frequency, and total nose poke time *$p<0.05$ as compared with both saline and naloxone/β-END treatment

$F(3,39)=11.81$, $p<0.0001$, nose poke frequencies, $F(3,39)=18.66$, $p<0.0001$, and nose poke times, $F(3,39)=13.49$, $p<0.0001$ In addition, treatment effects were significant for frequencies of inner box entrances, $F(2,26)=7.79$, $p<0.005$, and nose poke frequencies, $F(2,26)=4.39$, $p<0.025$ There was no signficiant treatment × choice interaction The distribution of nose poke times is depicted in Fig 2, and frequencies of inner box entrances and nose pokes are summarized in Table 1 As demonstrated by Newman Keuls comparisons, the experimental females exhibited a clear preference for the stud male over the other choices overall inner box frequencies, nose poke frequencies, and nose poke times were significantly greater for the stud male than for each of the other incentive animals ($p<0.001$)

As seen in Fig 3, the total number of inner box entrances was significantly higher after β-END treatment than after saline treatment ($p<0.005$), and this effect of β-END was reversed by concurrent administration of naloxone ($p<0.025$) Conversely, nose poke frequencies were significantly lower after β-END treatment than after saline treatment ($p<0.05$), and this effect was also reversed by naloxone ($p<0.05$) Nose poke times appeared to be altered in parallel with nose poke frequencies, although this difference was not statistically significant

The effects of β-END treatment, expressed as percent change from saline scores, are shown in Table 2 β-END increased inner box entrances and decreased nose poke time and frequency for each of the choices There was no significant alteration of choice preference by β-END treatment according to any of the measurements

DISCUSSION

A major finding of the present study was that ICV treatment with β-END, in a dose which consistently suppresses both receptive and proceptive sexual behaviors in female rats [19,20], did not alter the proclivity of female rats to associate with a sexually active stud male Rather, β-END treatment decreased the tendency of the animals to seek contact with conspecifics in general This decrease in proclivity toward social interaction was not secondary to opioid-induced akinesia, since total movement among the inner boxes was increased, rather than decreased, after β-END

treatment The effects of β-END were apparently mediated via opioid receptors, since the effects were reversed by concurrent treatment with naloxone

The OVX, EP-primed females of this experiment showed a clear tendency to seek contact with the sexually active male over the other choices, as shown by the significantly greater amount of time spent in proximity to the stud male (nose poke time) and the greater frequencies of entry into boxes leading to the stud male The castrated male was the second choice by all measures, but it was not significantly preferred over either female This preference of the stud male is consistent with results of previous studies using this experimental paradigm [4,5] In the latter study, priming with estrogen or estrogen plus progesterone increased the preference of an ovariectomized female for a sexually active male, as shown by an increased nose poke time spent specifically with the stud male Other investigators using different experimental paradigms have reported similar results with steroid-primed rats and with rats in a state of proestrus [6, 8, 9, 12] Such a physiological state which brings the animals to seek sexual contact with another animal has been defined by Meyerson and Lindstrom [12] as sexual motivation In the present study the state of sexual motivation of the experimental females was not specifically diminished by the opioid peptide, as evidenced by the fact that β-END did not disrupt the preference for the stud male This finding was surprising in light of the profound effects of β-END on both receptive and proceptive behavior Interestingly, a study using male rats [11] showed that β-END, while decreasing male copulatory behavior, increased amicable contacts of a male with a female (nuzzling, crawling under, and grooming the female) but decreased contacts with another male Thus, preference for the opposite sex was apparently not diminished by the opioid

Edwards and Pfeifle [6] differentiated between sexual motivation and social motivation, showing that steroid priming did not alter the total amount of time spent with conspecifics (propensity for social interaction) but increased the ratio of that time spent with a sexually active male over a castrated male In the present study, β-END appeared to alter the state of social motivation As indicated by the decreased total nose pokes for all choices, the experimental animals showed less interest in seeking contact with conspecifics This decreased social contact-seeking was not specific to a decreased interest in the stud male, since the level of nose pokes directed toward the stud male was less effected than that directed toward the other choices (see Table 2) Rather, the females showed a decreased interest in all of the choices This opioid effect on social behavior is consistent with previous reports of decreased social behavior in rats and monkeys by methadone [3,14] and increased affiliative behavior in monkeys by naloxone [7] The opioid-induced suppression of social behavior is extremely interesting in light of findings of Herman and Panksepp [10] and Panksepp *et al* [13] These studies found that opiate administration reduced distress and social attachment in infant guinea pigs and puppies which were isolated from their mothers The authors proposed that opiate drugs may replace an endogenous 'endorphin reward normally obtained from socialization

The decreased frequency and time of nose pokes cannot be accounted for by an opioid-induced akinesia, since total movement between the inner boxes was increased rather than decreased The increase may reflect a general hyperactivity which is known to follow a phase of hypoactivity induced by opioid administration [1,15] This hyperactivity was not seen in five minute open-field tests performed 30 minutes after β-END infusion [19,20] The fifteen minute choice box test may have included the phase transition to mild hyperactivity, alternatively, the increase in activity may be environment-dependent

It is not clear why sexual motivation as measured here was not suppressed by β-END, since the opioid exerts profound effects on proceptive as well as receptive behavior As a similar phenomenon was seen in males [11], it appears possible that the sexual effects of β-END may be very specific to certain components of copulatory behavior However, the present results suggest that β-END may suppress sociosexual interaction in general rather than sexual activity specifically Although it is unlikely that decreased social interaction can completely account for the sexual suppression, it is clear that the sexual effects of the intraventricularly-administered opioid cannot be considered behaviorally specific It seems reasonable to hypothesize that the sexual and social effects of β-END, as well as opioid suppression of certain behaviors such as rearing [2, 11, 19, 20], may all result from a more generalized opioid alteration of for example, affect or perception

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