Pharmacology Biochemistry & Behavior, Vol. 31, pp. 63–67. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Neonatal Naltrexone Treatment: Effects on Sexual and Exploratory Behavior in Male and Female Rats

BENGT J. MEYERSON,[1] MARITA BERG AND BIRGITTA JOHANSSON

Department of Medical Pharmacology, University of Uppsala, Sweden

Received 2 September 1987

MEYERSON, B. J., M. BERG AND B. JOHANSSON. *Neonatal naltrexone treatment: Effects on sexual and exploratory behavior in male and female rats.* PHARMACOL BIOCHEM BEHAV 31(1) 63–67, 1988.—The effect of neonatal naltrexone treatment (100 μg SC from day 1 to day 10) on copulatory and exploratory behavior in male and female rats was studied. In the female, neonatal naltrexone treatment enhanced copulatory (lordosis response) and exploratory behavior. An altered response to morphine was obtained; the effect of morphine on copulatory behavior was diminished while morphine's effect on exploratory activity was potentiated. The neonatal naltrexone treatment did not cause analogous effects in copulatory or exploratory behavior in the male rat. These data suggest that opioid mechanisms involved in the female copulatory and exploratory activity are established perinatally and can be influenced by early exposure to an opioid antagonist. It is concluded that there exist sex differences in this respect, both as to the sex-typical copulatory behavior and as to exploratory activity.

Neonatal treatment Naltrexone Morphine Copulatory behavior Exploratory behavior Lordosis response

PERINATAL exposure to synthetic opioids or peptide opioid agonists leads to behavioral effects which suggest that opioids may influence the establishment of the opioid central nervous mechanisms involved in behavioral processes (for bibliography, see [2, 10–13]). Exposure of male or female rats neonatally to β-endorphin or the antagonist naltrexone caused changes in sexual behavior and in the response to β-endorphin and morphine in adulthood [5–7].

The present study concerned the effects of neonatal treatment with the opioid antagonist naltrexone on certain behavioral reactions sensitive to morphine in the adult male and female rat. Endogenous opioids are involved in the regulation of male and female copulatory behavior. β-Endorphin and morphine have an inhibitory influence on copulatory activity in male and female rats [1, 3, 7, 9]. Infusion of the antagonist naloxone into the mesencephalic central gray or intrathecal administration reaching spinal areas induces facilitation of female copulatory behavior [8].

We have recently reported that neonatal exposure to naltrexone affects morphine sensitivity and facilitates lordosis behavior in female rats [7]. We have also demonstrated that neonatal β-endorphin treatment changed certain elements of male copulatory behavior in the adult animal [5]. The present study further tests the hypothesis that opioid mechanisms involved in certain behavioral elements are established perinatally and may be influenced by early manipulation of the opioid system. The effect of neonatal treatment with the opioid antagonist naltrexone on the copulatory and exploratory behavior in adult male and female rats was studied. The further aim of the study was to compare the influence of the neonatal treatment on hormone dependent sex-dimorphous and nonhormone dependent forms of behavior, the expression of which is the same in both sexes.

METHOD

Laboratory and Breeding Conditions

The animals used (32 females, 20 males) were born in the laboratory: the mothers (n=7) were Sprague-Dawley females which were 15–17 days pregnant (412±14 g) upon arrival at the laboratory from a commercial breeder (A-LAB, Sollentuna, Sweden, transported to the laboratory by us under special care). According to our routine the range of litter size accepted is 6–8 animals with both sexes represented (in the present study: 2×6 and 5×8 pups). The animals were kept under controlled environmental conditions, which implied housing under forced ventilation, controlled temperature (21±1°C) feeding with commercial rat food pellets, tap water available ad lib, and a reversed light cycle from day 23 after birth (light on between 0900 p.m. and 0900 a.m.). Behavioral tests were performed during the dark period under dimmed red light conditions.

Neonatal Treatment

The pregnant mothers were randomly alotted as to treatment of litter. Naltrexone (NX) was given subcutaneously

[1]Requests for reprints should be addressed to Bengt J. Meyerson, Department of Medical Pharmacology, P.O. Box 593, S-751 24, Uppsala, Sweden.

TABLE 1

BODY WEIGHTS OF MALE AND FEMALE RATS TREATED NEONATALLY WITH NALTREXONE (NX) OR SALINE (Sal)

Treatment		Body Weights Days After Birth 7	22	60
Neonatal				
Females	NX	15 ± 0.4†	58 ± 1.5	215 ± 3.8*
n=16	Sal	13 ± 0.3	56 ± 1.0	201 ± 2.6
Males	NX	14 ± 0.5	57 ± 1.2†	323 ± 5.2*
n=10	Sal	16 ± 0.9	63 ± 0.9	346 ± 8.9

Mean ± s.e.m. Student's *t*-test for differences between NX vs. Sal. *$p<0.05$, †$p<0.01$. Nested ANOVA on effect of treatment and with days as subordinate classification, females, $F=4.29$, $p<0.01$, males, $F=3.32$, $p<0.05$.

TABLE 2

THE EFFECT OF MORPHINE ON LORDOSIS RESPONSE (LR) IN OVARIECTOMISED FEMALE RATS

Adult Treatment		Lordosis Response Neo Sal LM	LR%	Neo NX LM	LR%
Morphine 1.5 mg/kg SC		33	13 ┐ †	56 ┐ *	69*
Saline	0.2 cc/animals SC	60	81 ┘	85 ┘	81

N=16 in each treatment. Statistical difference between Neo NX and Neo Sal or as indicated. *$p<0.05$, †$p<0.01$. The LR was activated by estradiol benzoate, 10 μg/kg and progesterone 0.4 mg/rat. The hormones were given 48 hr apart. The tests were conducted 5 hr after progesterone and 60 min after morphine (saline) treatment.

(SC) 100 μg/injection from day 1 (day 0=day of birth) to day 10 in a volume of 50 μl of saline (Neo NX). The dose was choosen on basis of results achieved in a previous report [7] and given per animal instead of related to body weight in order to keep the daily handling at a minimum. Control groups were treated analogously but given saline (Neo Sal) instead of NX.

Preexperimental Procedure

The animals were weighed on days 7, 14, 22 and 60; weaned at day 22 and sex-separated at day 40.

Females were inspected for vaginal opening from day 30–35 and ovariectomised under Brietal® (methohexital natr.) anesthesia at day 70–75. Tests for exploratory and copulatory behavior were performed starting 2 weeks after ovariectomy.

The males were placed together with sexually receptive females (ovariectomised females treated with estradiol benzoate 25 μg/animal and 48 hr later progesterone 1 mg/animal) at an age of 70–80 days. All males achieved ejaculation within 20 min.

Behavioral Tests and Injected Materials

Lordosis response (LR) was activated by estradiol benzoate (Sigma) followed 48 hr later by progesterone (Sigma). The doses of estrogen were chosen to give a response rate which permitted an increased response as well as a decreased response to be detected, namely 5 μg/kg and 10 μg/kg respectively. The progesterone treatment was kept constant at 0.4 mg/rat. The LR was calculated as the percentage of females which showed a clearcut lordosis response on two consecutive mounts or as the L/M, ratio that is number of LR/number of mounts × 100. Note that the L/M ratio applies only to the animals which show a positive LR response according to the percentage score. The two measures provide us with information as to the occurrence of the lordosis response and the rate of response in animals which fulfill the response criterion.

Male Copulatory Behavior

The male was transferred to an observation cage (40×60×40 cm) with a Plexiglas front and a floor covered by wood shavings. Exploratory behavior was recorded for a period of 10 min (see below). An estrous female was then placed into the observation cage and copulatory behavior recorded:

Mounting: clasping the flanks of the female and performing pelvic thrusts;

Intromission: mount ending with a vigorous backward lunge;

Ejaculation: prolonged mount with intense clasping of female followed by a slow dismount;

Mount and intromission latencies were calculated from the beginning of the test. Ejaculation latency is the time from first intromission till ejaculation occurred. Postejaculatory interval is the interval between the ejaculation and the subsequent mount with intromission.

Exploratory Behavior (for Details see [12])

The occurrence, duration (D), frequency (F) and latency (L) of the following behavior were recorded by means of a microcomputer (ABC-80 Luxor). The values are expressed in sec (D,L) or as scores (F) for each time the behavior was initiated.

Sniffing=rapid movements of whiskers while the animal explores;

Intense sniffing=directed sniffing at a particular object;

Rearing=standing on the hind legs;

Investigation=intense sniffing directed at a particular object which is picked up by the animal.

Statistics

For comparing groups with a different neonatal treatment the Mann-Whitney U-test was used. When comparisons involved animals acting as their own controls the Wilcoxon test was applied. The Chi square test or Fisher exact probability test was used for comparing measurements at a normal level.

RESULTS

Body Weight and Time for Vaginal Opening

The data in Table 1 represent the body weights at the time during neonatal treatment, at weaning and after puberty. NX

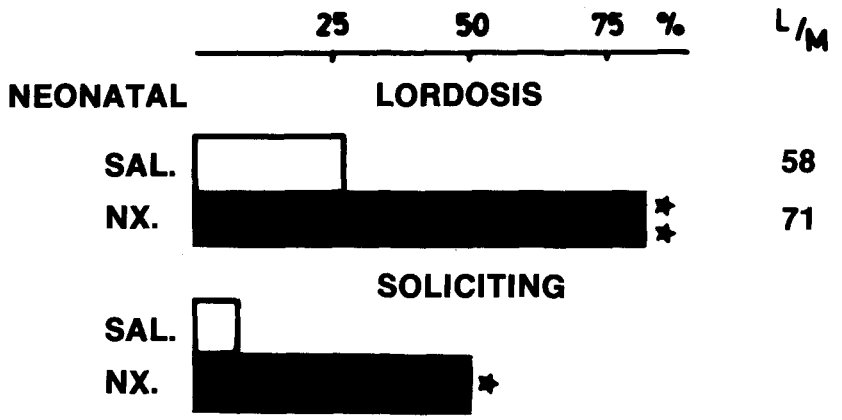

FIG. 1. Lordotic and soliciting behavior in neonatally naltrexone- (NX) or saline- (SAL) treated female rats. Adult treatment: estradiol benzoate 5 μg/kg SC followed 48 hr later by progesterone 0.4 mg/rat. Tests were conducted 4 and 6 hr after the progesterone injection. For naltrexone treatment see the Method section. n=15–16.

TABLE 3

THE EFFECTS OF MORPHINE ON EXPLORATORY BEHAVIOR IN OVARIECTOMISED *FEMALE* RATS TREATED NEONATALLY WITH NALTREXONE (NX) OR SALINE

		Exploratory Behavior											
Treatment		Rearing			Sniffing			Int. Sniffing			Investigating		
Neonatal	Adult	L	F	D	L	F	D	L	F	D	L	F	D
NX	Morphine	2	55	102	<1	49	388	153*	1†	3*	146*	9	36*
	Saline	4*	89*	184	1	50	318	160†	3†	5†	308	3	7
Saline	Morphine	2	63	124	<1	50	368	274	2	6	223	5	13
	Saline	7	75	181	<1	51	348	261	2	3		[a]	

N=7–8 per treatment group.
*$p<0.05$, †$p<0.01$. Statistical difference between Neo NX and Neo Sal or as indicated.
[a]Only 14% of the animals displayed investigating behavior.
Morphine 1.5 mg/kg SC (resp. saline), was given 60 min before test.

treated females weighed slightly more than corresponding controls at Day 7. At weaning, there was no significant difference between test animals and controls. Again at day 60 Neo NX females weighed slightly more. In males, no significant difference was seen at day 7 but at weaning and day 60 Neo NX males had lower body weights than the control males. Neo NX females did not differ from control females in the average time for vaginal opening (day 33, data not shown).

Lordosis Behavior

Significantly more neonatally NX-treated females showed LR and soliciting behavior (Fig. 1). Among the responders the L/M ratio was only slightly increased in the early naltrexone treated group. This difference was not statistically significant.

Morphine 1.5 mg/kg clearly inhibited the LR in the early saline treated group (Table 2). The effect was significantly less pronounced in the Neo NX-treated females.

Exploratory Behavior, Females

All scores of the intense sniffing behavior were significantly different in the Neo NX compared to Neo Sal group (Table 3). The early naltrexone-treated animals had shorter latency, higher frequency and longer duration of this behavior. A similar difference was seen in the rearing performance. The occurrence of investigating was low (Neo Sal 14%, Neo NX 38%, adult saline). After morphine treatment, the occurrence of the investigating behavior increased (Neo Sal 70%, Neo NX 100%) which permitted a difference between the Neo NX females and the Neo Sal groups to be detected. The investigating behavior had a longer duration and shorter latency of onset in the Neo NX compared to Neo Sal females.

TABLE 4

THE EFFECTS OF MORPHINE ON COPULATORY BEHAVIOR IN *MALE* RATS TREATED NEONATALLY WITH NALTREXONE (NX) OR SALINE

Treatment		Copulatory Behavior									
		Mount			Intromission			MF + IF	Ejaculation		
Neonatal	Adult	%	ML	MF	%	IL	IF	min	%	EL	PEI
NX	Morphine	60	75 ‡	18	40 *	143 ‡	10 ‡	3.7	40	385	288
	Saline	90	15	13	90	15	14	3.1†	80	465	315
Saline	Morphine	70	55 †	17	60	135 †	7 ‡	2.9	40	218	275
	Saline	100	13	15	80	25	11	2.1	70	750	310

N=10 per treatment group. The data are given as median values.
*$p<0.05$, †$p<0.02$, ‡$p<0.01$, for statistical difference between neonatal NX/saline or as indicated.

TABLE 5

THE EFFECTS OF MORPHINE ON EXPLORATORY BEHAVIOR IN *MALE* RATS TREATED NEONATALLY WITH NALTREXONE (NX) OR SALINE

Treatment		Exploratory Behavior											
		Rearing			Sniffing			Int. Sniffing			Investigating		
Neonatal	Adult	L	F	D	L	F	D	L	F	D	L	F	D
NX	Morphine	6	13 ‡	44 ‡	2	31 *	349	105	3 *	7 *	90 †	8 †	85 ‡
	Saline	6	42*	130	<1	38	344	37	4	16	156	5	15
Saline	Morphine	4	11 ‡	26 ‡	<1	29 ‡	361 ‡	280	2 *	5 *	116	10 †	50 ‡
	Saline	3	58	147	<1	41	276	111	6	19	95	4	11

N=10 per treatment group; NX=naltrexone.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$, for statistical difference between neonatal NX/saline or as indicated.
Morphine, 3 mg/kg SC, was given 60 min before test.

Male Copulatory Behavior

Only very minor differences appeared in copulatory behavior between the Neo NX and Neo Sal groups. The mounts + intromissions per minute score was higher in the Neo NX group (Neo NX-saline vs. Neo Sal-saline, Table 4). Morphine generally increased the latency times of mounts and intromissions. There were no obvious differences in this respect between the two neonatal treatments. The latency time before ejaculation became shorter after the morphine treatment. However, the results are not statistically significant because so few males achieved ejaculation.

Exploratory Behavior, Males

No very marked difference can be seen between the Neo NX and Neo Sal groups (Neo NX-saline vs. Neo Sal-saline, Table 5). Morphine decreased rearing and intense sniffing and increased investigating activity. These effects were seen in the Neo NX as well as the Neo Sal group.

DISCUSSION

It should at the outset of this discussion be made clear that drug treatments given during the neonatal period may cause nonspecific permanent effects if the dosage or handling, including prenatal care, exceeds a certain level or is stressful to the animal. The prenatal care was not fully controlled by us as the mothers were purchased as pregnant. Considering the minor differences in body weights between Neo NX and the vehicle treated groups, the normal time for puberty as indicated by vaginal opening, and the fact that the influence on adult behavior was selective, we are able to state that the Neo NX treatment did not cause early disturbances of a more general or debilitatory character. We find it unlikely, however not possible to completely exclude that the neonatal naltrexone treatment protects or adds to a stressful effect of laboratory handling, pre- or/and postnatally.

This study confirms our previous report [7] that neonatal naltrexone treatment facilitates the estrogen/progesterone activated copulatory behavior in adult female rats and that the inhibitory effect of morphine on this particular behavior is reduced in early NX treated female rats. Neonatal β-endorphin treatment influences various elements of sociosexual behavior in the adult male rat [5,6]. The present

investigation shows that in *the male*, copulatory performance was not affected by neonatal naltrexone treatment. In the adult male rat, morphine (and β-endorphin) [3] has an inhibitory effect on copulatory activity. The effect of morphine on copulatory activity was, however, not modified by neonatal NX treatment as was the case with the lordosis response in the female. The results suggest a difference in effect of early NX treatment on opioid related mechanisms involved in sex-typical copulatory behavior. However, the absence of any effect on adult male copulatory behavior by the NX treatment could mean that we have not used the right treatment schedule regarding the critical period of time and/or dosage in order to influence the male behavior. Opioid mechanisms might also be involved in the two forms of behavior at different organisational levels, including different types of opioid receptors and regions of the CNS.

Another interesting comparison is the one between nonhormone dependent exploratory behavior and copulatory behavior. The Neo NX treatment facilitated certain forms of exploratory activity; this is analogus to the LR. For several types of exploratory behavior the effect of morphine was more obvious in the neonatal naltrexone-treated groups. Thus, in contrast to lordotic behavior, exploratory behavior became more sensitive to morphine. Such an effect has also been reported by Hetta and Terenius when measuring morphine-induced analgesia [2]. The effect of morphine was inhibitory except for the investigating behavior which was stimulated. For this behavior also, after morphine treatment, there was a difference between the two neonatal treatment categories. This means that the effect of early naltrexone treatment is not restricted to forms of behavior inhibited by morphine treatment. In this context it is worthwhile to note that the sniffing behavior, which is not influenced by morphine, was unaffected by the early NX treatment.

In the male rat the neonatal naltrexone and saline groups exhibited similar degrees of exploratory activity. The investigating behavior was significantly increased by morphine treatment in the male also, whereas the other forms of exploratory activity were decreased. Despite the similar response to morphine by both sexes, the male morphine response was not affected by the neonatal NX treatment unlike the females. The lordotic response and various components of male copulatory behavior are so different that we can expect that the opioid mechansims involved may differ with respect to organization. The motor expression of exploratory activity, however, is the same in males and females, although considering the variety of behavior the profile might differ somewhat between the sexes. The overall similarity of exploratory behavior in both sexes means that there is less likelihood of detecting a sex difference in exploratory behavior, unlike copulatory behavior, after neonatal naltrexone treatment. However, it should be noted that although the motor performance of exploratory behavior is the same in both sexes, the functional basis for this behavior, and thus the central nervous representation, may still contain important differences.

It is concluded that neonatal exposure to naltrexone influences the adult expression of the female copulatory response (LR) as well as various forms of exploratory activity in female rats. The response to morphine is altered by the early naltrexone treatment in females with consequences for morphine sensitive behavior. From this follows that during a developmental period opioid mechanisms can be influenced by compounds with affinity for opioid receptors with consequences for the adult response to those particular peptidergic mechansims. To put the present results into a physiological coherence we adopt as working hypothesis that peptides may, during a critical period of the development, act as stimuli for their own receptor properties.

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