Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 3, pp. 413–419, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00289-4

Maternal Naltrexone Prevents Morphological and Behavioral Alterations Induced in Rats by Prenatal Stress

GILMORE I. KESHET AND MARTA WEINSTOCK[1]

Department of Pharmacology, School of Pharmacy, Hebrew University Medical Center, Ein Kerem, Jerusalem, 91120, Israel

Received 1 February 1994

KESHET, G. I. AND M. WEINSTOCK. *Maternal naltrexone prevents morphological and behavioral alterations induced in rats by prenatal stress.* PHARMACOL BIOCHEM BEHAV **50**(3) 413–419, 1995.—The influence of opioid receptor blockade on the developmental and behavioral effects of prenatal stress was studied. Time-mated dams were implanted with minipumps on day 17 of gestation containing vehicle (V) or naltrexone (NTX, 10 mg/kg/day). Noise and light stress was applied on an unpredictable basis, three times a week throughout gestation to half the dams. Maternal NTX completely prevented the reduction in anogenital distance in prenatally stressed (PS) males and restored the growth rate of both sexes. NTX also decreased the anxiety of PS rats in the plus-maze, increased the opioid component of exploration to control levels, but increased anxiety in control males. NTX did not restore the lower saccharin preference in PS females and decreased it in C females. This suggests that some morphological and behavioral changes induced by prenatal stress could result from excess opioid activity induced by maternal stress.

Anogenital distance Plus-maze Naltrexone by minipump Saccharin preference

SEVERAL studies have shown that prenatal stress (PS) or the administration of alcohol to the pregnant dam can produce alterations in the opioidergic system of the offspring. These alterations include an increase in the levels of methionine-enkephalin and β-endorphin in the hypothalamus (22) or mid- and hindbrain (25) of the newborn rat, and a decrease in μ-opiate receptors in the striatum and nucleus accumbens of the adult rat (11). The maternal stressors also interfere with the normal expression of opioid-dependent behaviors (14,27), such as ultrasonic vocalizations that are induced by maternal separation (13) and saccharin (sweet) preference. Both these behaviors are increased by opiate drugs (3) and decreased by naloxone in control rats (17). Saccharin preference is a sexually dimorphic behavior that is significantly higher in females than in males (29). This sex difference is lost in the offspring of stressed (15) or alcohol-fed dams (18) because the preference is decreased in females and increased in males. The opioid component of exploratory behavior in various forms of novel and stressful environments is also reduced in females by prenatal stress (15).

The reason for the interference with opioid systems by gestational stressors is not clear, but could result from an action of maternal hormones, ACTH, corticosterone, or β-endorphin, which are released in response to the stress (9) and can reach the fetal brain (23). Several independent observations suggest that excess opioid activity occurring in the dam and fetus may explain some of the behavioral abnormalities induced by maternal stress. These include the finding that injection of opiates or β-endorphin to the pregnant dam can induce similar changes in early development (12), behavior (39), the levels of opioid peptides (2) and their receptors (28), to those seen after maternal stress or alcohol. Prenatal stress (32) or opiate administration (34) also produce demasculinization and feminization of sexual behavior in the male offspring. Moreover, three times a day injections of naltrexone to the stressed dam could prevent this alteration of sexual behavior (33).

In previous studies we have shown that maternal stress in the form of noise only produced alterations in offspring behavior and development if it was administered in an unpredictable manner, which prevented the dam from adapting to it (37). Others obtained changes in offspring behavior and devel-

[1] To whom requests for reprints should be addressed.

opment with a much more severe stress, such as restraint with heat and light that was administered on a regular daily basis, because the pregnant dam did not adapt to this stressor as seen by the levels of plasma corticosterone that remained high until parturition (35).

The aim of the present study was to determine whether maternal administration of naltrexone (NTX) could prevent the loss of other opioid-dependent behaviors in the offspring of dams subjected to the unpredictable noise stress. The effect of this antagonist or naloxone on the developing organism was shown to depend on the duration of opiate receptor blockade (30,40). Furthermore, the three times a day maternal injection regime used in the study of Ward et al. (33) is itself stressful and can alter the behavior and reactivity of the HPA system in the offspring (21). NTX was chosen because of its longer duration of action and it was given via osmotic minipumps, which were implanted in the dam on day 17 of gestation. At this time opioid peptides and μ receptors are present in the fetus (5). We took care to establish that the amount of naltrexone released in the dam was sufficient to block opiate receptors throughout the last week of gestation after only one implantation because we wished to avoid the stress of a second operation.

METHOD

Subjects

Virgin female Sprague-Dawley rats obtained from Harlan Sprague-Dawley (Indianapolis, IN) were housed four to a cage on a 12L : 12D cycle and controlled temperature (22 ± 1°C). Food and water were provided ad lib.

Verification of NTX Dose

Experiments were performed in 24 female rats, weighing 234 ± 4 g, implanted subcutaneously (SC) (see below) with osmotic minipumps containing either vehicle, ascorbic acid (V, 16 rats), or NTX plus ascorbic acid (eight rats), each released at a rate of 10 mg/kg/day, to verify that this amount produced continuous blockade of opiate receptors for 7 days. Three days after surgery, four NTX rats and eight V rats were transferred to the test room in which four animals of each group were injected intramuscularly (IM) with morphine 5 mg/kg and the remaining four rats of the V group were given saline (1 ml/kg, IM). Latency of hind paw lick was measured 30 min after injection by a modification of the method described by Weinstock (36), adapted for use with rats. The hot plate was made of copper and was maintained at 55°C, surrounded by a Plexiglas chimney, 40 cm high. The rat was confined to the surface of the hot plate by clear Plexiglas walls 20 × 20 × 36 cm high for a maximum time of 30 s. The apparatus was cleaned with water between each test. The experiment was repeated 7 days after surgery in all groups.

Maternal Treatment

Virgin females were mated with a stud male. When a lordosis response was seen, the pair was left overnight and separated on the following day, which was designated day 1 of pregnancy. Forty pregnant dams were randomly allocated to "stress" and "control" groups and housed singly in small acrylic cages (22 × 17 × 13 cm), maintained at an ambient temperature of 22 ± 1°C, on a 12-h light cycle (lights on at 0700 h), with free access to food and water. Twenty stressed dams were housed in a special acoustic chamber, having the same temperature, light cycle, and humidity, but in which the noise (bell, 90–95 dB) and flashing light stress was applied on a random basis, three times weekly from day 2 of gestation, as previously described (8). None of the dams were handled, except for routine cage cleaning. On day 17 of pregnancy, the dams was anesthesized with halothane and implanted SC with minipumps containing either vehicle or NTX (10/group/treatment). One day before parturition (day 22 of pregnancy) all the dams were transferred to plastic breeding cages (37 × 21.5 × 18 cm) and housed in the same room as control dams.

Litters

Litters were culled to eight pups within 24 h of birth, with equal numbers of males and females whenever possible. A litter that was smaller than eight pups was excluded from the experiment. Anogenital distance and body weight were measured. The anogenital distance of each male was divided by the body weight and expressed as the average value per litter. The mean value for each maternal treatment group was computed from these litter means. Because of the number of experimental groups, the pups were all raised by their biological mothers, a cross-fostering design would have been too complicated both to execute and to evaluate. In a previous study in which prenatally stresssed pups were cross-fostered onto control dams and vice versa, we found that the delays in early motor development were mainly due to the prenatal treatment (7). The pups were weaned at 21 days of age and housed in groups of four by litter and sex. To reduce litter effects, not more than two pups of each sex from each litter were used in any one postnatal test. All experiments were performed on control (C) and prenatally stressed (S) offspring, aged 60–70 days, in a room in the animal house that was maintained under the same conditions of temperature, light cycle, and

TABLE 1

COMPOSITION OF GROUPS OF OFFSPRING FOR THE PLUS-MAZE TEST

Maternal Treatment	Offspring Treatment	Group	Sex (No.)
Control, vehicle	Saline, 1 ml/kg	C-V-sal	Male (9), female (9)
Control, vehicle	Naloxone, 1 mg/kg	C-V-nal	Male (9), female (8)
Control, naltrexone	Saline, 1 ml/kg	C-NTX-sal	Male (9), female (10)
Control, naltrexone	Naloxone, 1 mg/kg	C-NTX-nal	Male (10), female (10)
Stressed, vehicle	Saline, 1 ml/kg	S-V-sal	Male (10), female (8)
Stressed, vehicle	Naloxone, 1 mg/kg	S-V-nal	Male (10), female (9)
Stressed, naltrexone	Saline, 1 ml/kg	S-NTX-sal	Male (11), female (10)
Stressed, naltrexone	Naloxone, 1 mg/kg	S-NTX-nal	Male (10), female (9)

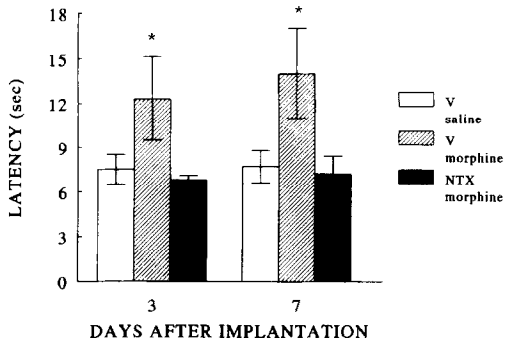

FIG. 1. Effect of chronic naltrexone release from minipump on the analgesic response to morphine. *Significantly different from morphine alone, $p < 0.05$. VEH = vehicle, NTX = naltrexone.

humidity as those in which the rats were housed. The contents of the room remained in identical positions throughout all the experimental procedures to provide the same visual cues for all the animals.

Experimental Procedures

Hot plate test. Each animal received an IM injection of naloxone (1 mg/kg) or saline (1 ml/kg) 15 min before it was placed on the hot plate. Naloxone was used to determine the contribution of endogenous opioid systems to the reaction time to a nociceptive stimulus and other behavioral parameters (see below) because we and others (6) had found this treatment to have a significant effect in control rats in such tests. The latency to lick the hind paw was measured as described above. A cutoff time of 30 s was used to prevent damage to the hind and front paws.

Elevated plus-maze. This test was modified from that described by Pellow and File (20). The experiment was performed in a room illuminated with a red light. The apparatus consisted of two open and two closed arms, each 44 cm long and 10 cm wide, placed 43 cm above the ground. The closed arms had a wooden wall 40 cm on each side, whereas the open arms had rails 1 cm high of clear Plexiglas attached to them to prevent the rats from falling off. Each of 151 animals was placed individually into the maze for 5 min. The total number of entries into any arm and the proportion of time spent in the open arms were recorded. The groups of rats and their treatments are shown in Table 1. All rats were injected with saline or naloxone as above, 15 min before being placed in the plus maze. The tests were carried out between 1300 and 1800 h. The apparatus was carefully cleaned with water and dried after each animal.

Saccharin preference. Eight C or S rats of each sex and maternal drug treatment group were housed individually for 3 days before the experiment in cages equipped with two drinking water bottles. On the fourth day, the tap water in one of the bottles was replaced by a solution containing saccharin (3 mM). The bottles were alternated daily to ensure that there was no preference for a particular position. In preliminary experiments we found that the preference pattern stabilized on the fourth day of saccharin exposure. This day was therefore chosen for the comparison of the effects of the maternal treatments on this behavior. Sweet preference was expressed as the ratio of the intake of saccharin solution on day 4 to the total amount of fluid consumed that day.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) for factors prenatal stress, maternal NTX, pretest naloxone, and sex using the SAS statistical package. Duncan's multiple range test was used for post hoc analysis on group differences. A difference at the level of $p < 0.05$ was considered statistically significant. Data are presented as mean ± SEM.

RESULTS

Verification of NTX Dose

The analgesic effect of morphine (5 mg/kg) was completely blocked 3 and 7 days after implantation of the minipumps containing NTX in the control rats (Fig. 1).

Morphological Data

Exposure to prenatal stress or NTX did not affect the duration of pregnancy or body weight of pups on day 1. Body weight gain by the dam during the first 17 days of pregnancy (by litter mean) did not differ in stress and control groups: C = 74.7 ± 2.6 g, S = 76.1 ± 3.5 g. Maternal NTX tended to decrease the size of litters born to C dams ($p = 0.1$). This

TABLE 2

EFFECT OF PRENATAL STRESS AND NTX ON LITTER COMPOSITION AND PUP WEIGHTS ON DAY 1

	Prenatal Treatment			
	Vehicle		Naltrexone	
	Control	Prenatal Stress	Control	Prenatal Stress
Pregnancy (days)	23.1 ± 0.1	23.2 ± 0.1	23.1 ± 0.1	23.1 ± 0.1
Litter size	12.0 ± 0.8	12.4 ± 0.7	10.6 ± 0.7	11.5 ± 0.5
No. of males in litter	6.8 ± 0.7	5.8 ± 0.6	4.5 ± 0.7*	5.7 ± 0.4
No. of females	5.2 ± 0.5	6.4 ± 0.5	6.1 ± 0.8	5.7 ± 0.3
Weight (g)				
Males	6.7 ± 0.3	6.5 ± 0.2	6.9 ± 0.2	6.6 ± 0.1
Females	6.3 ± 0.2	6.4 ± 0.2	6.7 ± 0.2	6.7 ± 0.1

*Significantly different from V-C, $p < 0.05$.

TABLE 3

EFFECT OF PRENATAL STRESS AND NTX ON RESPONSE LATENCY ON HOT PLATE

	Maternal Treatment			
	Control		Prenatal Stress	
Offspring Treatment	Saline	Naloxone	Saline	Naloxone
Males (time in s)				
Vehicle	8.1 ± 0.8	7.6 ± 1.0	8.1 ± 0.6	9.5 ± 0.8
Naltrexone	10.6 ± 0.5*	9.0 ± 0.7	8.0 ± 0.3	8.7 ± 0.8
Females (time in s)				
Vehicle	8.0 ± 0.5	7.3 ± 0.3	8.4 ± 0.7	9.7 ± 1.0
Naltrexone	9.4 ± 0.7	10.6 ± 0.7*	8.3 ± 0.6	8.5 ± 0.6

*Significantly different from V-C, $p < 0.05$.

appeared to be due to a specific reduction in the number of males in a litter, $F(1, 39) = 4.52$, $p < 0.05$. NTX had no effect on the composition or number of offspring of S dams (Table 2). Prenatal stress significantly reduced the anogenital distance in males (Fig. 2), $F(1, 39) = 11.36$, $p < 0.001$. This was normalized by maternal NTX. Although the body weight (by litter mean) of S-V offspring at the age of 60 days was somewhat lower than that of C-V on day 60 [$F(1, 43) = 3.09$, $p = 0.08$ for males, and $F(1, 43) = 2.05$, $p = 0.15$ for females], this did not reach statistical significance. This delay in growth was also prevented by NTX, which had no effect in C offspring (Fig. 3).

Plus-Maze

The S offspring of V-treated dams made significantly fewer entries into the four arms of the plus maze than C offspring, $F(1, 36) = 6.85$, $p < 0.025$ (Fig. 4A). Pretest naloxone significantly reduced the total number of arm entries in C rats of both sexes, $F(1, 34) = 22.73$, $p < 0.0001$, but not in S offspring. There was a significant main effect of NTX, $F(1, 36) = 55.59$, $p < 0.001$, that was due to a rise in the total number of entries in saline-treated S offspring. Because the total activity was the same in S-V and S-NTX rats after naloxone, but significantly greater in the latter group given saline, it indicated that the opioid component of the behavior was increased by NTX to the level obtained in C offspring. Maternal NTX did not affect exploratory activity in C rats (Fig. 4B). No attempt was made to determine the stage of the estrous cycle in the females before each behavioral experiment, because this is itself a stressful procedure and could well have influenced the behavior. However, as any differences in the behavior of C and S females and their reversal by maternal NTX were also seen in males, it is unlikely that these were due to differences in the stage of the estrous cycle.

S-V rats of both sexes given saline spent significantly less time in the open arms of the plus-maze than C-V, $F(1, 36) = 16.55$, $p < 0.0005$. Naloxone had no significant effect on this parameter in V-treated offspring (Fig. 5A), suggesting that opioid activation did not contribute to an appreciable extent to this behavior. There was an interaction between prenatal stress and maternal NTX, $F(1, 150) = 12.52$, $p < 0.001$, because maternal NTX completely abolished the difference in the response of S-V and C-V and normalized the opioid component of the behavior in the former group. In contrast, NTX reduced the time spent in the open arms by C males given saline or naloxone, $F(1, 150) = 10.26$, $p < 0.005$, but tended to increase that spent by S males (Fig. 5B). This resulted in a

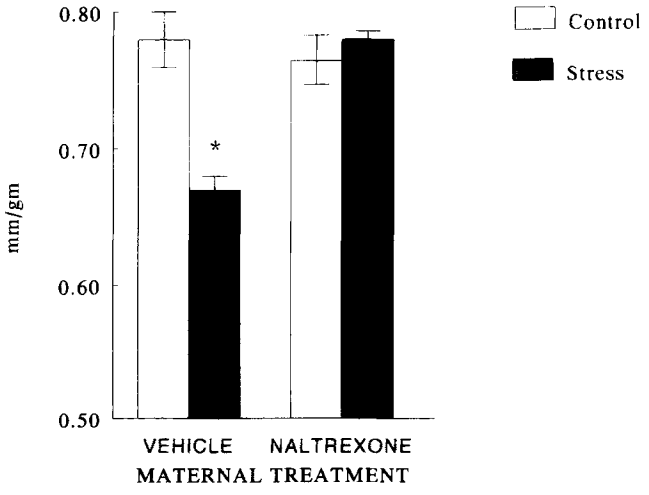

FIG. 2. Effect of maternal naltrexone on anogenital distance in control (C) and stressed (S) male pups. Litter mean, mm/g. *Significantly different from C, $p < 0.001$.

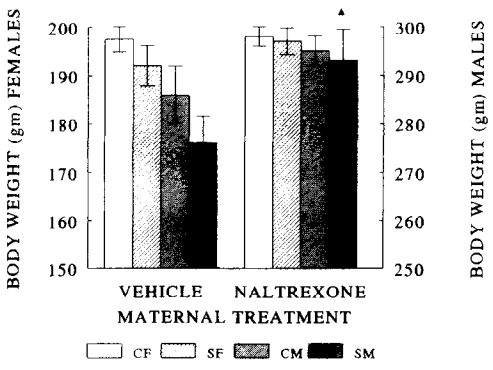

FIG. 3. Effect of maternal naltrexone on adult body weight in C and S offspring. CF = control females, SF = stressed females, CM = control males, SM = stressed males. C-VEH vs. S-VEH, $p < 0.05$; S-VEH vs. S-NTX, $p < 0.05$.

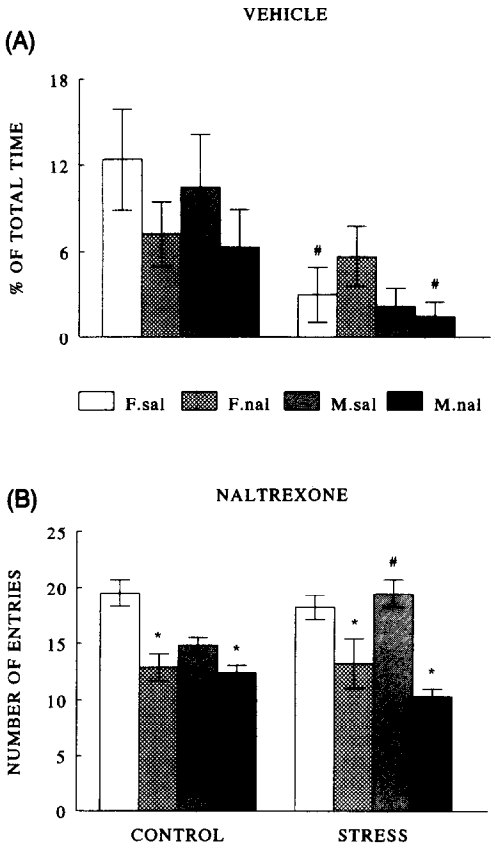

FIG. 4. Effect of prenatal stress and naltrexone on the total number of arm entries in plus maze. (A) Offspring of vehicle-treated dams, (B) offspring of naltrexone-treated dams. *Significantly different from saline, $p < 0.05$; #significantly different from C, $p < 0.05$.

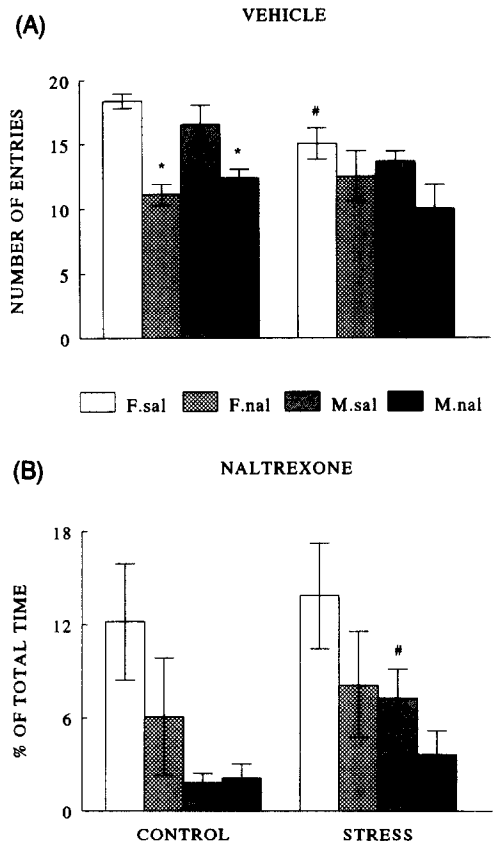

FIG. 5. Effect of prenatal stress and naltrexone on time in open arms of plus maze (percent of total). (A) Offspring of vehicle-treated dams, (B) offspring of naltrexone-treated dams. #significantly different from C, $p < 0.05$.

significant interaction between sex and maternal NTX, $F(1, 150) = 3.62, p < 0.05$.

Saccharin Preference

In line with previous findings by us (15) and others (18), saccharin preference was considerably higher in C-V females than in C-V males [sex main effect, $F(1, 63) = 23.66, p < 0.001$]. Prenatal stress abolished the sex difference by reducing the preference in females and increasing it in males, thereby producing an interaction between prenatal stress and sex, $F(1, 63) = 6.47, p < 0.01$. Prenatal NTX had no significant effect on saccharin preference in C males or S females but reduced it in C females. ANOVA revealed an interaction between sex and maternal NTX, $F(1, 63) = 4.99, p < 0.05$ (Fig. 6).

Hot Plate Test

There was no significant difference in the latency to paw lick after exposure to the hot plate in C-V and S-V offspring of both sexes. Maternal NTX significantly increased the latency in C rats, $F(1, 63) = 4.29, p < 0.05$, particularly in males, but had no effect in S rats (Table 3).

DISCUSSION

The major new findings in this study are that: 1) the reduction in anogenital distance in male offspring and the lower body weight of both sexes at adulthood, induced by unpredictable prenatal stress, were completely normalized by continuous maternal administration of NTX throughout the last week of gestation. 2) Opiate receptor blockade during pregnancy also normalized exploratory behavior of S rats in the elevated plus-maze, as indicated by the increase in the total number of entries into any arm. 3) Maternal NTX restored the time spent by S rats of both sexes in the open arms of this maze to the

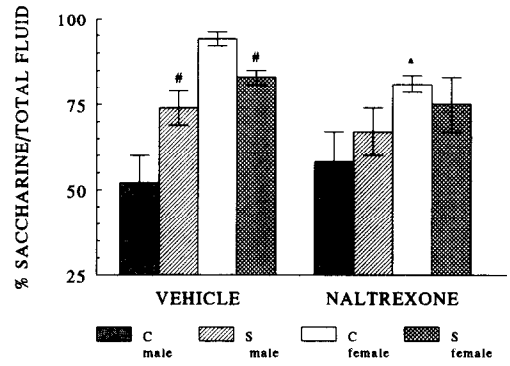

FIG. 6. Effect of prenatal stress and naltrexone on saccharin preference. Measurements made on the fourth day of the test. #Significantly different from C, $p < 0.05$; ▲significantly different from V, $p < 0.05$.

level of that in control offspring. This showed that the opiate receptor blockade during gestation could abolish the heightened anxiety induced by prenatal stress. This may result from a relative lack of μ opioid activity in S offspring at adulthood, because small doses of morphine are known to increase the time spent in the open arms of the plus maze (19). Prenatal NTX may restore μ-opioid activity by preventing the effects of excess opioids during maternal stress.

A reduction of anogenital distance is believed to be due to an inadequate action or release of testosterone and could be an early indication of impaired sexual activity at adulthood. Although sexual activity was not tested in adult rats in our study, others have shown that maternal restraint during pregnancy, which reduces anogenital distance (4), interferes with sexual activity and feminizes the behavior of the male offspring (32). These abnormalities in male sexual behavior appear to be related to a reduction in fetal testosterone release on critical days of male development (34). A similar reduction in fetal testosterone and impairment of sexual activity at adulthood was induced by opiate administration during gestation (34), supporting the suggestion that prenatal stress interferes with male sexual behavior via an opioid-mediated mechanism. This was confirmed by the finding of Ward et al. (33) that injection of naltrexone to the pregnant stressed dam could significantly reduce the feminization of sexual behavior in the male offspring.

Saccharin preference is a sexual dimorphic behavior that is dependent on the influence of androgens during the perinatal period for its expression (31). It is also considered to be an opioid-dependent behavior because it is increased by opiates (3) and blocked by opiate antagonists (17). In the present study, prenatal stress reduced saccharin preference in females and increased it in males, thereby diminishing the sexual dimorphism of this behavior. The increased saccharin preference in S males could have been due to lower androgen activity during the late gestation or neonatal period, whereas the decreased preference in S females could have resulted from a reduction in hypothalamic β-endorphin (38).

In contrast to its effects on morphology, growth rate, anxiety, and exploration, maternal NTX did not influence saccharin preference in S rats, and even reduced it in C females. If maternal NTX had restored opioid activity in S rats, one might have expected it to normalize saccharin preference in S females. A possible explanation for the lack of effect of NTX on this behavior may be found in a recent study of Beczkowska et al. (1), who infused specific agonists and antagonists into the lateral ventricle of rats and showed that δ-, but not μ- or κ-opioid activation, was responsible for inducing saccharin preference. Unlike μ or κ receptors, δ binding sites only begin to appear in the rat about 2 weeks after birth (26). Thus, the latter receptors and opioid peptides activating them may not have been influenced by prenatal NTX.

In previous studies on the effects of maternal opiate antagonists on offspring behavior and development, naloxone was either given by repeated, once (24) or twice daily injections (30) from day 7 or 8 of gestation, or as in our study, by osmotic minipump implanted in the dam on day 17 of gestation (10). Although the first two studies differed in the amounts of naloxone given, no significant effects were induced on litter size, weight of pups, or sex distribution within litters, but growth rate was increased. In the study of Hetta and Terenius (10), a greater neonatal mortality (sex not specified) and reduced growth rate was reported. Although maternal naloxone did not influence the pain threshold of the rats on the hot plate in this study, the response to morphine was nevertheless increased. These apparently contradictory findings can be reconciled if one takes into account the stress induced in the dams by daily saline injections that were given to the controls in the experiments of Vorhees (30) and Shepanek et al. (24), and have been shown to affect offspring behavior (21) in a manner similar to the noise stress used in our study.

We also found that maternal NTX appeared to have some detrimental effects on the male offspring of C rats. It reduced the number per litter and increased their anxiety in the plus maze test and the latency to paw withdrawal on the hot plate. In contrast, no such effects of maternal NTX were seen in S males, and their growth rate was even increased, as in the study of Vorhees (30). This suggests that blockade of normal opioid activity during development may interfere with normal development and differ from that induced by antagonism of the excess opioid activity resulting from maternal stress.

The findings in this study suggest that excess opioid activity in the fetuses of stressed dams, at a critical period of their development, could be responsible for some of the abnormalities induced by prenatal stress. These include a smaller anogenital distance, slower growth rate, diminished sweet preference in females, and greater anxiety in both sexes at adulthood.

ACKNOWLEDGEMENT

This study was supported by a grant from the Anti-Drug Authority of Israel.

REFERENCES

1. Beczkowska, I. W.; Koch, J. E.; Bostock, M. E.; Leibowitz, S. F.; Bodnar, R. J. Central opioid receptor subtype antagonists differentially reduce intake of saccharine and maltose dextrin solutions in rats. Brain Res. 618:261-270; 1993.
2. Bianchi, M.; Marini, A.; Sacerdote. P.; Cocco, E.; Brini, A.; Panerai, A. E. Effect of chronic morphine on plasma and brain beta endorphin and methionine enkephalin in pregnant rats and in their fetuses or newborn. Neuroendocrinology 47:89-94; 1988.
3. Czirr, S. A.; Reid, L. D. Demonstrating morphine's potentiating effects on sucrose-intake. Brain Res. Bull. 17:639-942; 1986.
4. Dahlof, L.-G.; Hard, E.; Larsson, K. Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20:93-195; 1978.
5. DeVries, T. J.; Hogenboom, F.; Mulder, A. H.; Schoffelmeer, A. N. M. Ontogeny of μ-, δ- and κ-opioid receptors mediating inhibition of neurotransmitter release and adenylate cyclase activity in rat brain. Dev. Brain Res. 54:63-69; 1990.
6. File, S. E. Naloxone reduces social and exploratory activity in the rat. Psychopharmacology (Berlin) 71:41-44; 1980.
7. Fride, E.; Soreq, H.; Weinstock, M. Are the effects of gestational stress on motor development and cerebellar cholinesterase activity mediated prenatally? Int. J. Dev. Neurosci. 4:407-413; 1986.
8. Fride, E.; Weinstock, M. The effects of prenatal exposure to predictable or unpredictable stress on early development in the rat. Dev. Psychobiol. 17:651-660; 1984.
9. Guillemin, R.; Vargo, T.; Rossier, J.; Minic, S.; Ling, N.; Rivier, C.; Vale, W.; Bloom, F. β-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science 197: 1367-1369; 1977.
10. Hetta, J.; Terenius, L. Prenatal naloxone affects survival and

morphine sensitivity in rat offspring. Neurosci. Lett. 16:323-327; 1980.
11. Insel, T. R.; Kinsley, C. H.; Mann, P. E.; Bridges, R. S. Prenatal stress has long-term effects on brain opiate receptors. Brain Res. 511:93-97; 1990.
12. Kashon, M. L.; Ward, O. B.; Grisham, W.; Ward, I. L. Prenatal β-endorphin can modulate some aspects of sexual differentiation in rats. Behav. Neurosci. 106:555-562; 1992.
13. Kehoe, P.; Blass, E. M. Opioid-mediation of separation distress in 10 day old rats: Reversal of stress with maternal stimuli. Dev. Psychobiol. 19:385-398; 1986.
14. Kehoe, P.; Shoemaker, W. Opioid-dependent behaviors in infant rats effects of prenatal exposure to ethanol. Pharmacol. Biochem. Behav. 39:389-394; 1991.
15. Keshet, G. I. The involvement of the endogenous opioid system in the effects of prenatal stress. MSc. thesis, Hebrew University; 1993.
16. Kinsley, C. H.; Mann, P. E.; Bridges, R. S. Prenatal stress alters morphine- and stress-induced analgesia in male and female rats. Pharmacol. Biochem. Behav. 30:123-128; 1988.
17. Lynch, W. C. Opiate blockade inhibits saccharin intake and blocks normal preference acquisition. Pharmacol. Biochem. Behav. 24:833-836; 1986.
18. McGivern, R. F.; Clancy, A. N.; Hill, M. A.; Noble, E. P. Prenatal alcohol exposure alters adult expression of sexually dimorphic behavior in the rat. Science 224:896-898; 1984.
19. Motta, V.; Brandao, M. L. Aversive and antiaversive effects of morphine in the dorsal periaqueductal gray of rats submitted to the elevated plus maze test. Pharmacol. Biochem. Behav. 44:119-125; 1993.
20. Pellow, S.; File, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525-529; 1986.
21. Peters, D. A. V. Effect on brain biogenic amines and plasma corticosterone levels. Pharmacol. Biochem. Behav. 17:721-725; 1982.
22. Sanchez, M. D.; Milanes, M. V.; Fuente, T.; Laorden, M. L. Prenatal stress alters the hypothalamic levels of methionine-endephalin in pup rats. Neuropeptides 23:131-135; 1992.
23. Sandman, C. A.; Kastin, A. J. The influence of fragments of the LPH chains on learning, memory and attention in animals and man. Pharmacol. Ther. 13:39-60; 1981.
24. Shepanek, N. A.; Smith, R. F.; Tyer, Z. E.; Dawn Royall, G.; Allen, K. S. Behavioral and neuroanatomical sequelae of prenatal naloxone administration in the rat. Neurotoxicol. Teratol. 11: 441-446; 1989.
25. Shoemaker, W. J.; Baetge, G.; Azad, R.; Sapin, V.; Bloom, F. E. Effect of prenatal alcohol exposure on amine and peptide neurotransmitter systems. Monogr. Neural. Sci. 9:130-139; 1983.
26. Spain, J. W.; Roth, B. L.; Coscia, C. J. Differential ontogeny of multiple opioid receptors (μ, δ and κ) J. Neurosci. 5:584-588; 1985.
27. Takahashi, L. K.; Baker, E. W.; Kalin, N. H. Ontogeny of behavioral and hormonal responses to stress in prenatally stressed male rat pups. Physiol. Behav. 47:357-364; 1990.
28. Tempel, A.; Habas, J. E.; Paredes, W.; Barr, G. A. Morphine-induced downregulation of μ-opioid receptors in neonatal rat brain. Dev. Brain. Res. 41:129-133; 1988.
29. Valenstein, E. S.; Kakolewski, J. W.; Cox, V. C. Sex differences in taste preference for glucose and saccharine solutions. Science 156:942-943; 1967.
30. Vorhees, C. V. Effects of prenatal naloxone exposure on postnatal behavioral development of rats. Neurobehav. Toxicol. Teratol. 3:295-301; 1981.
31. Wade, G. N.; Zucker, I. Hormonal and developmental influences on rat saccharine preferences. J. Comp. Physiol. Psychol. 69: 291-300; 1969.
32. Ward, I. L. Prenatal stress feminizes and demasculinizes the behavior of males. Science 175:82-84; 1972.
33. Ward, O. B.; Monaghan, E. P.; Ward, I. L. Naltrexone blocks the effects of prenatal stress on sexual behavior differentiation in male rats. Pharmacol. Biochem. Behav. 25:573-576; 1986.
34. Ward, O. B.; Orth, J. M.; Weisz, J. A possible role of opiates in modifying sexual differentiation. Monogr. Neural Sci. 9:194-200; 1983.
35. Ward, I. L.; Weisz, J. Differential effects of maternal stress on circulating levels of corticosterone, progesterone and testosterone in male and female rat fetuses and their mothers. Endocrinology 84:1635-1644; 1984.
36. Weinstock, M. Similarity between receptors responsible for the production of analgesia and lenticular opacity. Br. J. Pharmacol. 17:433-441; 1961.
37. Weinstock, M.; Fride, E.; Hertzberg, R. Prenatral stress effects on fuctional development of the offspring. Prog. Brain Res. 73: 319-331; 1988.
38. Weinstock, M.; Matlina, E. Maor, G. I.; Rosen, H.; McEwen, B. S. Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary adrenal system in the female rat. Brain. Res. 595: 195-200; 1992.
39. Zagon, I. S.; McLaughlin, P. J. Behavioral effects of prenatal exposure to opiates. Monogr. Neural Sci. 9:150-159; 1983.
40. Zagon, I. S.; McLaughlin, P. J. Naloxone modultes body and organ growth of rats: Dependency on the duration of opioid receptor blockade and stereospecificity. Pharmacol. Biochem. Behav. 33:325-328; 1989.