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Naltrexone Blocks the Effects of Prenatal Stress on Sexual Behavior Differentiation in Male Rats

O. BYRON WARD, EDWARD P. MONAGHAN AND INGEBORG L. WARD

Department of Psychology, Villanova University, Villanova, PA 19085

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WARD, O. B., E. P. MONAGHAN AND I. L. WARD. *Naltrexone blocks the effects of prenatal stress on sexual behavior differentiation in male rats.* PHARMACOL BIOCHEM BEHAV **25**(3) 573–576, 1986.—The male offspring of rats stressed three times daily during days 14–21 of pregnancy were more likely to show lordotic behavior when tested in adulthood than were control males. This feminization of sexual behavior was not observed if the mothers were injected with the opioid antagonist naltrexone before being stressed. These data suggest that endogenous opioids released under conditions of stress can alter the normal process of sexual behavior differentiation in the fetal male rat.

Endogenous opioids Prenatal stress Sexual behavior differentiation Naltrexone Fetal male rat

EXPOSING a pregnant rat to environmental stress during the last trimester of gestation leads to feminized patterns of sexual behavior in the male offspring (see reviews [30,32]). The behavioral alterations shown by prenatally stressed male rats are believed to be the result of a suppression of the surge in testicular steroidogenic enzyme activity, i.e., 3β-hydroxysteroid dehydrogenase (3β-HSD) [22], and plasma testosterone titres [33,34] which normally occurs in male rats on days 18 and 19 of gestation. Adequate levels of testosterone are required during perinatal development if adult sexual patterns are to be fully masculinized and defeminized (see review [32]). The mechanism by which stress changes the steroidogenic activity of the fetal testes is presently unknown. One potential candidate is the endogenous opioid system.

Opioids are released when rats are subjected to conditions of stress [16,18]. The involvement of opioids in the etiology of what is known as the Prenatal Stress Syndrome is suggested by the fact that the endocrine and behavioral consequences of exogenous opiate administration during gestation mimic those of prenatal stress. Injections of the synthetic opiate methadone to pregnant rats causes a decline in the plasma testosterone titres of their male fetuses, an effect which can be blocked by pretreating the mother with the opiate antagonist naloxone [28]. Maternal treatment with morphine beginning on day 16 results in lower than normal levels of fetal testicular 3β-HSD activity on days 19 and 20 of gestation [3]. Furthermore, male rats exposed prenatally to morphine show feminized sexual patterns in adulthood [35]. More direct evidence of endogenous opioid involvement in the Prenatal Stress Syndrome is the finding that treatment of pregnant rats with naltrexone prior to the administration of stress prevents the reduction in 3β-HSD activity normally found in the Leydig cells of 19 day old fetuses from stressed mothers [35]. If the Prenatal Stress Syndrome is mediated by physiological changes which involve stimulation of fetal opioid receptors, then adult patterns of sexual behavior should be normal in the male offspring of pregnant rats given naltrexone prior to being stressed. The present study was designed to test this prediction.

METHOD

Procedure

Thirty-five female rats (Harlan Sprague-Dawley), 60 to 100 days old, were mated by placing them with a male between 1000 and 1300 hr until 2 ejaculations were observed to have occurred (day 0 of gestation). Each female then was housed in a polycarbonate cage (24×20×45.5 cm) with water and Purina rat chow available ad lib. The vivarium was maintained at a temperature of approximately 23°C, and on a reverse light/dark cycle (lights off between 0800 and 2000).

Pregnant females were assigned randomly to the following groups: No Injection-Stress (N=6), No Injection-Control (N=5), Saline-Stress (N=5), Naltrexone-Stress (N=7), Saline-Control (N=6), and Naltrexone-Control (N=6). The injection and stress treatments began on day 14 of gestation and continued daily through day 21. Animals were injected subcutaneously with 1 ml/kg of saline or with 2 mg/kg of naltrexone hydrochloride at 0830, 1230, and 1630 hours. The stress treatment consisted of placing mothers at 0900, 1300, and 1700 hours into individual 13×6×8 cm Plexiglas animal restrainers. The restrainers were placed under two 150-W flood-lights, delivering approximately 2,150 lm/m^2 [31,33]. Each stress session lasted 45 min. On day 21 of gestation, all animals were provided with nesting material (shredded paper).

TABLE 1

PERCENTAGE OF INDIVIDUAL MALES AND LITTERS THAT RESPONDED ON AT LEAST ONE OF FOUR TESTS FOR LORDOSIS BEHAVIOR AND THE MEAN HIGHEST LQ SCORE OF THE RESPONDING ANIMALS

Maternal Injection	Maternal Treatment	N	Percentage Responding Animals*	Mean Highest LQ	Number of Litters	Percentage Responding Litters*
None	Stress	24	46	57	6	67
None	Control	24	12.5†	64	5	0‡
Saline	Stress	20	75	64	5	80
Naltrexone	Stress	30	40§	64	7	28.5¶
Saline	Control	20	35#	60	6	33**
Naltrexone	Control	27	33	70	6	0

*An animal was considered to be a responder if it displayed an LQ of at least 40 on one or more test. Litters were counted as being responsive if 50% or better of the male members met the criterion set for individual animals.

Significantly different from the Non-Injected Stress group: †$p<0.0005$; ‡$p<0.004$.

Significantly different from the Saline-Injected Stress group: §$p<0.00005$; ¶$p<0.005$; #$p<0.0001$; **$p<0.018$ (Binomial tests).

When the litters were 21–23 days old, pairs of male pups from the same treatment group were weaned into wire suspension cages (35×18×18 cm). Littermates were housed together, whenever possible. No females were maintained in the vivarium in which the experimental animals lived after weaning.

Beginning at 60 days of age, 4 weekly tests for male behavior were given. These tests consisted of placing the experimental male into a 53×33×23 cm semi-circular observation chamber and adding an estrous female for a period of 30 min. Incidents of mount, intromission and ejaculation behavior were scored [31].

All males then were castrated under Chloropent anesthesia (Fort Dodge Labs., Inc.). One week after castration, weekly intramuscular injections of 50 μg estradiol benzoate, followed 44 hours later by 1.0 mg of progesterone were initiated. Both hormones were suspended in cottonseed oil. Weekly tests for female sexual behavior were instituted following the second set of injections. Approximately 4 hours after the progesterone injection, the experimental animal was placed into an observation chamber containing a vigorous stud male. The test continued until the experimental male had been mounted at least 10 times. To accrue 10 mounts some animals were placed with several stud males. A lordosis quotient (LQ) was computed for each of the 4 tests for female behavior. The LQ consists of the number of lordosis responses performed by the experimental animal divided by the number of times he was mounted × 100 [30].

RESULTS

While 81% of the males had at least one test during which they exhibited no lordotic behavior at all, a number of these animals displayed high levels of lordosis on one, or more, of the four tests for female behavior. Therefore, the test on which the highest LQ score occurred was selected as representing each subject's maximum potential for lordosis. Males with an LQ of 40 or higher on at least one test were designated as responders. Seventy percent of the responders had an LQ of at least 30 on two or more of the four tests. A majority of the males in the control groups were almost completely non-responsive, i.e., showed not a single lordotic response, or, at best, lordosed only once out of the 40 or more mounts received during the four tests.

The percentages of responding males in each treatment group are summarized in Table 1. Injecting the mothers three times daily during the last week of pregnancy resulted in a significant elevation in the percentage of male offspring showing lordotic behavior in adulthood. The Saline-Injected Control group had more responders than the Non-Injected Control group (Binomial test, $p<0.014$). Further, the injection effect seemed to summate with the effects of restraint and light stress since more Saline-Injected Stressed animals exhibited lordosis than did Non-Injected Stressed animals ($p<0.002$). For these reasons, further analyses of treatment effects were restricted to differences between the two non-injected groups and to comparisons among the various injected groups.

As in previous studies [30,31], a comparison of the male offspring from the non-injected stressed and control mothers revealed that the prenatal stress treatment produced a significant increase in the number of animals capable of showing lordosis behavior ($p<0.0005$).

Prenatal stress also increased the percentage of responding males from saline-injected mothers compared to those from saline-injected control mothers ($p<0.0001$). Administration of naltrexone to the pregnant dam blocked the increase in lordotic behavior induced by restraint and light stress. The percentage of males displaying the female sexual pattern was significantly smaller in the Naltrexone-Injected Stress group than in the Saline-Injected Stress group ($p<0.00005$), but did not differ from either the Naltrexone-Injected Control group or the Saline-Injected Control group.

While naltrexone blocked the effects of the restraint and light stress procedure which followed drug administration by 30 min, the drug had no apparent effect on the potentiation of lordosis induced by the injection procedure, i.e., there was no significant reduction in the percentage of responding males from Naltrexone-Injected Control mothers compared to those from Saline-Injected Control mothers. The failure of naltrexone to block the effects of injecting the dam does not

rule out the possibility that the injection procedure also may have constituted an opioid-mediated stressor. In the case of the injection, the drug is given simultaneously with the potential source of stress, thereby not allowing sufficient time for onset of effective drug action to block the stress effect.

The mean LQ of responders for the single test in which each animal showed the highest LQ score are also shown in Table 1. While the groups differed in the percentage of animals capable of performing the lordosis pattern, there were no group differences in the highest score obtained from the responding animals.

It has been argued that in species giving birth to multiple offspring, the assessment of prenatal treatments should be based on litter effects rather than on data taken from individual subjects [1,12]. For this reason, the percentage of litters in which at least 50% of the males showed an LQ of 40 or better on one or more test also are presented in Table 1. Analysis of the litters generally revealed the same group differences as had analysis of the data from individual subjects. More stressed litters responded than did control litters in both the uninjected ($p<0.004$) and injected conditions ($p<0.018$). Naltrexone given 30 min prior to each stress session blocked the stress effect when compared to Saline-Injected Stressed litters ($p<0.005$). Control and Naltrexone-Stress litters did not differ from one another.

On the four tests for male sexual behavior, an animal had to have performed 5 or more mount, intromission or ejaculation responses to be considered a copulator. The percentage of animals that met this criterion in the six treatment groups ranged from 85–100. Binomial tests revealed no significant differences among the groups using either litter means or individual subjects as the data unit.

DISCUSSION

The present findings support the hypothesis that the alterations in sexual behavior differentiation characteristic of the Prenatal Stress Syndrome are mediated by physiological changes which involve stimulation of fetal opioid receptors. In males shielded by naltrexone from the effects of increased opioid activity, gestational stress did not potentiate female lordotic patterns. These behavioral data are congruent with a previous report that the stress-induced reduction in the activity of 3β-HSD in fetal Leydig cells is blocked by pretreating the animals with naltrexone [35]. Presumably, naltrexone promotes a normalization of gonadal steroidogenic activity in stressed fetuses, allowing sufficient testosterone to be produced to defeminize adult sexual behavior potentials.

It is well known that insufficient androgen exposure during prenatal development leads to increased levels of lordotic behavior in male rats, (see review [32]). The finding that methadone given to pregnant rats lowers plasma testosterone levels in their fetuses [28] suggests an altered androgenic milieu to be the likely mechanism through which endogenous opioids influence the process of sexual behavior differentiation [35].

The site of action where opiates may be acting to influence fetal testosterone production is unclear. In adult male rats, intense stress [15,29] or exogenous administration of opiates [5, 7, 9, 20] result in decreased plasma levels of LH and testosterone. Since endorphins are present in adult rat testes [19,27] and morphine added to testicular homogenates reduces the activity of 3β-HSD [23], the possibility exists that opiates exert a direct inhibitory action at the level of the testes. More likely, the critical action involves a higher level of the hypothalamic-pituitary-gonadal axis. Reductions in plasma testosterone titres following morphine injections are preceded by a decline in LH levels [7,9]. However, morphine does not block LHRH-stimulated release of LH from the pituitary [6], suggesting that the pituitary probably is not the primary site of opiate action. Rather, there is considerable indirect evidence that opiates reduce plasma testosterone levels in adult rats through a central action at the level of the hypothalamus [6, 8, 13, 20, 25].

While no comparable data exist for fetal rats demonstrating that opiates alter testosterone levels by acting on the CNS, the opioid and neuroendocrine components required for a central action are developed in fetal rats during the last week of gestation. Opioid receptors appear in the rat brain by day 15 postconception [10], and high levels of endorphin have been found in a variety of brain sites by day 16 [4]. Furthermore, LHRH is present in the fetal brain by day 12 [2], and LH is detectable in the pituitary by day 12 [21] and in plasma by day 16 postconception [26]. Testosterone production by fetal testes may depend on hypothalamic-pituitary stimulation since decapitation from day 17 of gestation on or encephalectomy from day 18 on results in a loss of Leydig cells [14]. While maternal stress reduces plasma levels of LH [26] in fetal male rats, naltrexone reversal of this effect or of the stress-induced reduction in testosterone [33,34] has not been attempted.

The origin of the endogenous opioids which influence sexual differentiation of fetal males also is unclear. Synthetic narcotics, such as methadone, cross the placental barrier when administered to pregnant rats, appearing in a variety of fetal organ systems, with preferential uptake in the CNS [24]. It is possible that endogenous opioids released by the dam under conditions of stress enter the circulation of her fetuses. Alternatively, the stress experienced by the mother may activate, through pathways not yet characterized, the endogenous opioid system of the fetuses. This point cannot be resolved at the present time. In any case, these data provide the first evidence that fetal exposure to elevated levels of endogenous opioids can have long term behavioral consequences. That fetal exposure to exogenously administered opiates induces long term effects is already well known.

The expected deficiency in male copulatory behavior often found in prenatally stressed male rats [30–32] was not obtained. This is in agreement with a few reports in which stressed males showed enhanced levels of lordosis behavior but patterns of ejaculation were normal [11,36]. An increase in lordosis patterns seem to be the most reliable consequence of prenatal stress on behavior. The factors responsible for the variable ejaculatory patterns shown by prenatally stressed males are not well understood. Recent research suggests that various social factors experienced during prepuberal development interact with prenatal hormonal events to determine adult potentials for the male copulatory pattern [17,31]. Whether these inadvertently affected the male behavior potentials of the animals in the present study is not known. However, since both control and stressed males showed normal levels of copulatory behavior, it was not possible to assess whether naltrexone could block the deficiency in ejaculatory behavior which typically occurs in prenatally stressed male rats.

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