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Prenatal Exposure to Morphine Feminizes Male Sexual Behavior in the Adult Rat

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GAGIN, R., E. COHEN AND Y. SHAVIT. *Prenatal exposure to morphine feminizes male sexual behavior in the adult rat.* PHARMACOL BIOCHEM BEHAV **58**(2) 345–348, 1997.—The endogenous opiate system plays a role in fetal sexual differentiation during development. We examined long-term effects of prenatal morphine on adult sexual behavior in male rats. Pregnant Fischer 344 rats were given increasing doses of morphine (0.75–12.0 mg/day) in slow-release emulsion during gestational days 12–18. Control rats were injected with vehicle and were either pair-fed with morphine rats or ad lib fed. At birth, all litters were culled to eight pups and fostered to naive dams. Testing began when rats were 10–12 weeks old. Masculine behavior was assessed using receptive stimulus females and recording instances of mount, intromission, and ejaculation. Feminine receptivity of the male rats was assessed following castration and priming with ovarian hormones; lordosis quotient of the experimental males was recorded using stimulus male studs. Males prenatally exposed to morphine exhibited normal rates of male copulatory behavior but a significantly higher lordosis quotient, suggesting that prenatal morphine induced long-lasting feminizing effects. © 1997 Elsevier Science Inc.

Prenatal morphine Sexual differentiation Male sexual behavior Opiates Lordosis quotient

SEXUAL development of male rats is normally determined by the presence of androgens released by the testes of the fetus. Several extraneous factors have been reported to affect sexual differentiation in the fetus, including alcohol (15), stress (7,21), opiate agonists (10,18–20,24), and opiate antagonists (5,11). The effects of opiate agonists and antagonists implicate the endogenous opiate system in normal processes of brain sexual differentiation. Opiate influence on neural growth and brain development has been demonstrated in several studies [e.g., (2,26–28)]. Endogenous opiates have been implicated in the feminizing and demasculinizing effects of prenatal stress (11,12,23) and in the modulation of testicular development and fetal androgen synthesis (1,17). The hypothesis that endogenous opiates play a modulatory role in fetal masculinization is congruent with the finding that opiates suppress the hypothalamic–pituitary–gonadal (HPG) axis in adult rats (3,9). Thus, it has been suggested that maternal exposure to abnormally high levels of opiates during critical phases of fetal development enhances the activity of the fetal endogenous opiate system (16) and hence interferes with fetal androgen synthesis and sexual differentiation.

Previous studies on prenatal opiates and sexual behavior (10,20,24) employed discrete injection procedures that typically involved two or three injections per day. The interpretation of such procedures is complicated by possible compensatory reactions [such as receptor downregulation or suppression of endogenous opiate release (14)] that might emerge during drug withdrawal phases. The present study was aimed at investigating the same issue using continuous drug administration. Pregnant rats were injected with morphine dissolved in a slow-release preparation during days 12–18 of pregnancy. Adult offspring were examined on various measures of sexual behavior.

METHODS

Prenatal Treatment

Nulliparous Fischer 344 female rats (Harlan Laboratories, Jerusalem, Israel) 10–12 weeks old and weighing 230–250 g were maintained under standard laboratory conditions (23±1°C; 12 L:12 D cycle, lights on 1900–0700 h). Food and water were provided ad lib (unless otherwise specified). To determine the day of estrus, animals were daily placed with sexually vigorous studs for brief observation periods. Estrus was determined by the occurrence of lordosis behavior. Estrous females were housed with Fischer 344 males for approx-

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imately 16 h. The day of mating was counted as gestational day 0. Females mated on the same day were housed in group cages until day 12 of gestation and then were separated into single cages until parturition. Thirty pregnant rats were randomly assigned to three equal groups and given subcutaneous (SC) injections, once a day for 7 consecutive days, of either morphine or vehicle. Injections consisted of 1 ml of a slow-release emulsion composed of saline (with or without morphine), light white mineral oil, and an emulsifying agent (Arlacel-A; Sigma, Israel) in ratios of 8:6:1, respectively [after (6,8)]. For the experimental (morphine) group, morphine HCl (Teva, Israel) was dissolved in the saline in increasing doses as follows: 0.75, 1.5, 1.5, 3.0, 6.0, 12.0, 12.0 mg/injection; ad lib control animals received vehicle injections and ad lib feeding, and pair-fed control animals received vehicle injections and restricted feeding, corresponding to the average food intake measured on the previous day in morphine-injected dams.

Morphine injections began on day 12 of gestation, because this day just precedes the emergence of opiate receptors in the rat brain (4). The protocol of prenatal morphine administration was based on a pilot study in which we examined various increasing doses of morphine and several schedules of drug administration, including injections up to delivery day. The selected protocol was shown to induce analgesia that lasted at least 24 h after the injection, and yielded a survival rate of $\geqslant$80% of the newborns. Thus, morphine presence, at least up to day 19 [presumably the day of testosterone surge, which is critical for sexual differentiation; see (25)], was guaranteed.

Litters were typically born on day 22–23 of pregnancy; they were culled to eight pups (with both sexes evenly represented when possible) 24 h after birth and fostered to drug-naive dams. At 3 weeks of age, offspring were weaned, housed in cages of three or four rats according to sex and treatment, and maintained under standard conditions.

Postnatal Testing

Measurements of sexual behavior began when rats were 3 months old. Sixty-two male rats of the three prenatal treatment groups were tested. Rats were given three tests of masculine behavior, followed by two tests of feminine receptivity. All tests were conducted during the dark phase of the day (1300–1700 h) in a dimly lit room with temperature controlled to 23 $\pm$ 1°C. Four semicircular observation cages 30 cm high and 60 cm in diameter were used. The front walls of these cages were of Plexiglas, and their bottom was covered with wood shavings.

Masculine behavior was assessed by using stimulus females that had been ovariectomized under ether anesthesia at least 3 weeks prior to testing. Sexual receptivity was induced in these females by two SC injections of 10 μg estradiol benzoate (Sigma Ltd.) 72 and 48 h before testing and an injection of 1 mg progesterone (Sigma Ltd.) 4 h before testing. Both injected hormones were dissolved in olive oil at a volume of 0.1 ml/animal. Male rats were each placed into an observation cage for a 5-min habituation period, following which a stimulus female was introduced. The first test session lasted 30 min; the second and third sessions were 20 min each. Instances of mount, intromission, and ejaculation were recorded by means of a computer-controlled event recorder. The following parameters were derived: a) latency to first behavior (LFB), the time between the introduction of the stimulus female and the first occurrence of either a mount or intromission; b) ejaculation latency (EL), the time from introduction of the stimulus female to the first occurrence of ejaculation; c) mount rate (MR), the average count of mounts and intromissions per minute during the period from introduction of the stimulus female until the occurrence of the first ejaculation; and d) number of ejaculations observed during the test session.

Feminine receptivity was assessed in a subsample of 33 male rats from the three prenatal treatment groups (n = 10, 11, 12 males in the ad lib, pair-fed, and morphine groups, respectively). Following completion of masculine behavior tests, these animals were castrated under ether anesthesia and allowed a 2-week recuperation period. They were primed with 40 μg estradiol benzoate injected SC 72 and 48 h prior to testing and 2 mg progesterone injected 4 h before testing. Sexually vigorous studs and estrous stimulus females (treated as above) were employed in the tests. A stud was placed in an observation cage and allowed a brief contact with a stimulus female. Upon initial arousal of the stud, the female was quickly removed and the experimental male introduced. The stud was allowed 15 mounts over the experimental male, and lordosis reactions were recorded. Feminine receptivity was expressed as lordosis quotient (LQ); for each animal, LQ was computed as the number of lordosis responses per attempted mounts $\times$ 100.

Statistical Analysis

Data were analyzed using one-way analyses of variance (ANOVA) according to prenatal treatment.

RESULTS

Maternal/Litter Data

Food intake of morphine-injected dams was reduced to approximately 35% of food consumption by ad lib dams (3.5–4.0 g per day compared with approx. 10–11 g per day), in accordance with previous reports [e.g., (13)]. There was no difference in the number of newborns among the prenatal treatment groups; average litter size was 8.3 pups. Average body weight at birth ($\pm$SEM) was 8.12 $\pm$ 2.67, 8.94 $\pm$ 2.16, and 8.51 $\pm$ 1.76 g, for morphine, ad lib, and pair-fed animals, respectively. Newborns of morphine-treated dams did not exhibit apparent malformations.

TABLE 1

INDICES OF MALE SEXUAL BEHAVIOR IN MALE RATS PRENATALLY EXPOSED TO MORPHINE OR CONTROL TREATMENTS

	Latency to First Behavior	Mount Rate	Ejaculation Latency	No. of Ejaculations
Ad lib (n = 24)	234.5 (95.16)	1.78 (0.28)	930.5 (88.29)	1.3 (0.30)
Pair-fed (n = 16)	324.1 (120.05)	1.69 (0.37)	926.3 (93.10)	0.9 (0.33)
Morphine (n = 22)	348.4 (95.80)	1.54 (0.24)	920.3 (68.51)	1.1 (0.26)

Values are means (SEM) measured in the third testing session.

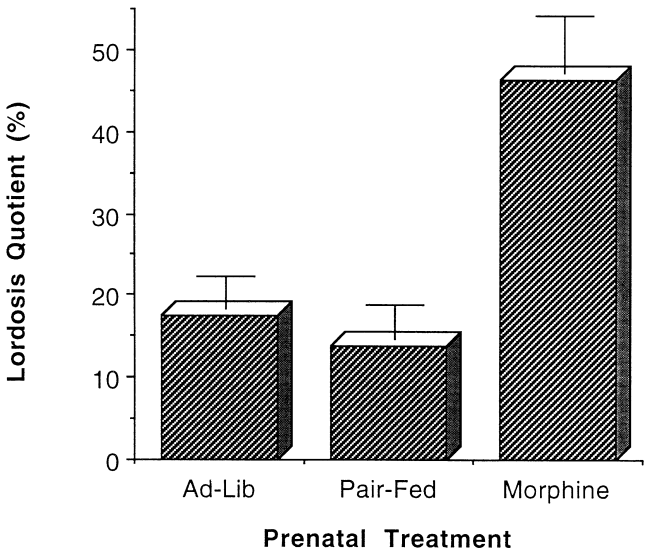

FIG. 1. Lordosis quotient (ratio of number of lordosis responses to number of mounts × 100) in male rats of the three prenatal treatment groups. ANOVA results indicate a significant treatment effect between the morphine and the two control groups. Vertical lines indicate SEM.

Masculine Behavior

No significant differences among the three prenatal treatment groups were observed in any of the parameters of male sexual behavior (Table 1). The percentage of rats not exhibiting ejaculation in the third testing session was 50, 63, and 41% in the ad lib, pair-fed, and morphine groups, respectively.

Feminine Behavior

Mean lordosis quotient scores assessed in male rats of the three prenatal treatment groups are shown in Fig. 1. Feminine receptivity, induced by castration and hormonal priming, was significantly higher in rats exposed prenatally to morphine than in those of both control groups [$F(2, 32) = 11.49$, $p < 0.0002$]. Six males (four pair-fed and two ad lib) exhibited no lordosis response; all of the morphine rats had LQ scores larger that 0. Further analysis did not reveal any significant differences between the two control groups. No instances of soliciting behavior were observed.

DISCUSSION

Male rats prenatally exposed to continuous morphine influence exhibited partial feminization. Copulatory potency was not significantly affected, but female patterns of sexual receptivity were more pronounced in morphine-exposed males than in controls.

Prenatal opiate effects on sexual differentiation have been reported in several studies [for review, see (22)]. However, the results of these studies are not always in agreement. According to Ward and colleagues (24), prenatal morphine induced demasculinization and feminization in male rats; a similar outcome was demonstrated following prenatal β-endorphin exposure (10). In contrast, Vathy and Katay (20) observed suppression of female sexual behavior and a mixed, but mostly enhancing, effect on male performance following prenatal morphine exposure. Opiate-induced feminization was recently supported by the finding that prenatal naltrexone enhanced male sexual behavior and suppressed lordosis quotient in male rats (5). However, the discrepancies between these reports of prenatal opiate effects remain unresolved.

A possible explanation for the discrepancies may reside in the mode of drug administration employed in each case. Dams injected with morphine are likely to alternate daily between periods of drug influence and withdrawal. The possibility of withdrawal effects developing in rat fetuses following daily methadone maternal injections has been discussed by Lichtblau and Sparber (14). Kirby and Holtzman (13) compared the effects on fetuses of equal amounts of morphine administered daily to pregnant rats in either four or two (evenly spaced) portions; morphine tolerance and dependence were demonstrated in fetuses exposed to the four-injection schedule but not in those exposed to two injections per day. Clearly, animals of the two-injection group spent a considerable part of their 12-h daily cycle free of morphine influence. A similar argument was presented by Zagon and McLaughlin (28) in relation to prenatal naloxone treatment. Thus, in assessing the outcome of any study employing discrete administration of opiates, it is difficult to distinguish between the effects of overstimulation and understimulation of opiate receptors. Two of the reports (10,24) employed a three-injection schedule and observed feminization and demasculinization, whereas the third (20) used a two-injection schedule and reported defeminization following prenatal opiate exposure. Given the possible compensatory responses induced by morphine (e.g., reduction in endogenous opiate activity, or receptor downregulation), it could be speculated that animals given morphine injections in 12-h intervals spent a larger portion of the day in a state of opiate understimulation rather than opiate overstimulation. Evidently, the daily ratio of opiate excess to opiate deficiency was lower in these animals than in those receiving similar drugs at 8-h intervals. Therefore, defeminization and masculinization (observed following a two-injection daily schedule) probably resulted from opiate withdrawal rather than opiate stimulation. A similar outcome was obtained following maternal blockade of opiate receptors with naltrexone presented continuously in the drinking water of pregnant dams (5).

In the present study, morphine was administered via a slow-release preparation, which minimized the chance of daily fluctuations in morphine levels and recurrent withdrawal phases. Fetuses may have experienced withdrawal following the termination of drug administration. Because brain sexual differentiation is completed only some time after birth, the possibility of withdrawal effects on this process could not be ruled out.

A plausible mechanism underlying the effect of prenatal opiates is suppression of the HPG axis, as demonstrated both in adult (9) and in fetal (1,17) rats. However, alternative mechanisms not necessarily based on opiate inhibition of gonadotrpin releasing hormone cannot be ruled out at present. One way of testing the involvement of the HPG axis would be to study the effect of continuous prenatal opiates on female development, because sexual differentiation of the female is not dependent on hormonal induction. The effects of prenatal exposure to opiate agonists and antagonists on sexual behavior in females are presently being studied in our laboratory.

Although it attenuated the normal process of defeminization, which typically occurs in male fetuses, prenatal morphine exposure did not have any appreciable effect on the concomitant process of masculinization. A similar outcome was reported following prenatal β-endorphin exposure (10)

and in several prenatal stress studies [e.g., (23)]. Because morphine injection was terminated on gestational day 18, this might indicate that masculinization takes place at a later stage of male fetal development compared with defeminization.

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