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Effects of Neonatal Exposure to Progesterone on Sexual Behavior of Male and Female Rats

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HULL, E. *Effects of neonatal exposure to progesterone on sexual behavior of male and female rats.* PHYSIOL. BEHAV. 26(3) 401–405, 1981.—Lactating females received daily injections of progesterone or oil, and their offspring were gonadectomized in adulthood and tested for both musculine and feminine sexual behavior elicited by estrogen, progesterone, and testosterone regimens. Male offspring of progesterone treated females exhibited significant impairment of masculine behavior elicited by both estrogen and testosterone. Latency and frequency of mounts and intromissions of those males which did engage in sexual behavior were not significantly different for the two groups. There were nonsignificant trends toward demasculinization of progesterone treated females and feminization of progesterone treated males. Progesterone administered to estrogen primed males failed to facilitate lordosis. There were no progesterone related differences in body weight at any time nor in testis or accessory organ weights of males. The results of this experiment confirm our previous finding of reduced sexual competence of intact male rats exposed neonatally to moderately increased levels of progesterone, and indicate that this effect is not the result of diminished adult hormone levels. Neonatal exposure to progesterone appears to have minimal or no effect on feminine sexual behavior of rats.

Progesterone Sexual behavior Neonatal hormones Rats

THE BEHAVIORAL effects of progesterone on the adult female brain and their loci of action are only beginning to be elucidated (reviewed in [11]). However, almost no evidence is available concerning this hormone's effects during the period of sexual differentiation. Neonatal female rats have been reported to have higher progesterone concentrations than do males [19]. Neonatal progesterone injections have been shown to protect against sterility induced in female rats by neonatal testosterone propionate and in males by neonatal estradiol [7,14]. Diamond, Llacuna and Wong [6] reported that very high neonatal doses of progesterone, as well as of testosterone and estradiol, depressed adult male sexual behavior. It has been proposed that the relatively high levels of progesterone reported in perinatal females helps to protect them against the masculinizing effects of endogenous androgens and estrogens during sensitive periods of differentiation [18,19], although alpha fetoprotein, which binds to estrogen, appears to play the major protective role [16,17]. Recently Weisz and Ward [24] reported no sex related differences in progesterone levels of fetuses or neonatal pups, and we failed to find significant sex differences in progesterone levels of 7 day old oil treated control pups [12]. Whether endogenous levels of progesterone serve a protective function in normal females is thus presently unclear. However, in our studies progesterone levels only slightly higher than the normal physiological range depressed masculine sexual behavior in adulthood [13], shifted performance on learning tasks in a feminine direction [13,20], and increased the activity of types A and B monoamine oxidase (MAO) in whole brain homogenates of day 20 fetuses [21] and of day 7 pups [12]. Progesterone levels of our pups whose mothers received progesterone injections were between 2 and 3 standard deviations above the mean of control animals. Synthetic progestins administered to women during pregnancy have also been reported to increase feminization of girls [10], though boys exhibited little or no demasculinization [15].

The present experiment investigated the effects of neonatal progesterone treatment on both masculine and feminine sexual behavior of both sexes and its effects on relative behavioral sensitivities of gonadectomized animals to estrogen, progesterone and testosterone. Lactating females received daily injections of progesterone or oil, and their offspring were gonadectomized in adulthood and tested for both masculine and feminine sexual behavior elicited by estrogen, progesterone, and testosterone regimens.

METHOD

Animals and Neonatal Treatment

Animals used in this experiment were 20 male and 19 female offspring of five Sprague-Dawley female rats, timemated in this laboratory. (Several additional females failed to produce litters.) Within 24 hours of birth litters were reduced to 4 males and 4 females per litter, except for one litter which contained only 4 males and 3 females at birth. Half the male and half the female siblings of each litter were cross fostered to a dam which received daily progesterone injections (3.3 mg/kg/day in about 0.1 ml oil); the other half were fostered to a dam which received injections of similar volumes of oil. In addition, the number of dams was reduced to 4 in order to provide equal numbers of dams receiving progesterone and oil injections. Pups were tail-marked to indicate both prenatal and postnatal litters. Pups were weighed weekly until weaning at 28 days. After weaning animals were grouped with 2–3 animals of the same sex per cage, and were maintained with ad lib food and water, with lights on from 0700 to 2000 hr.

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TABLE 1
EXPERIMENTAL DESIGN

Estradiol Regimen—Males*				
Days 1–6	7–9	10–12	13–15	16–18
Dose 10 μg/da	10 μg/da	50 μg/da	50 μg/da	50 μg/da
Test ♂ behav. test on day 6	♀ behav. test on day 9	♂ behav. test on day 12	♀ behav. test on day 15	♂ behav. test on day 18
Days 19–22	23–30	31–33	34	35–40
Dose 100 μg/da	100 μg/da	100 μg/da	1 mg progest.	200 μg/da
Test ♀ behav. test on day 22	♂ behav. test on day 30	(no test)	♀ behav. test	♀ behav. test on day 40

Testosterone Regimen—Males		
Days 21 days	1–4	5–7
Dose post-EB	100 μg/da	200 μg/da
Test no treatment	♂ behav. test on day 4	♂ behav. test on day 7

*All males were treated identically in adulthood after having been exposed to maternal injections of progesterone or oil neonatally.

Adult Treatment

Males were castrated beginning at about 150 days of age. Body weights and weights of testes and epididymes were recorded at that time. One week later their light cycle was reversed, so that lights were off from 0800 to 1800 hr. Thirty days after castrations had been completed, males began a daily estradiol benzoate injection regimen. They were divided into 3 squads with equal numbers of progesterone and control animals in each squad. The regimen began on successive days for the 3 groups, such that day 1 of the regimen for squad 2 was day 2 for squad 1, etc. For the first 9 days each male received 10 μg estradiol benzoate in 0.1 ml oil between 0830 and 0900 hr. They were tested as described below for male sexual behavior on day 6 and for feminine behavior on day 9. The dosage of estradiol benzoate was increased to 50 μg/day from day 10 to day 18, and males were tested for masculine behavior on days 12 and 18 and for feminine behavior on day 15. Injections of 100 μg estradiol benzoate were administered on days 19–33, with a feminine behavior test on day 22 and a masculine behavior test on day 30. On day 34 an injection of 1 mg progesterone replaced the estradiol benzoate and was followed by a final test for feminine behavior. Because feminine behavior scores were quite low, estradiol benzoate injections were resumed on day 35 with the dosage increased to 200 μg. A final feminine behavior test was administered on day 40. One week after termination of estradiol benzoate injections males were tested for masculine behavior to ascertain whether they had returned to baseline. Since considerable sexual behavior was exhibited, data from this test were also analyzed in a separate statistical test. Three weeks later a second test verified that levels of masculine behavior had returned to baseline. At that time daily injections of 100 μg testosterone propionate were begun in a similar fashion. A masculine behavior test was given on day 4. Dosage was increased to 200 μg from day 5 to day 7, with a final test for masculine behavior on day 7. Since feminine behavior had been difficult to elicit with estradiol, feminine behavior tests were not given during the testosterone regimen. After completion of behavioral testing males were sacrificed, and body, seminal vesicle and prostate weights were recorded.

Females were ovariectomized beginning at about 210 days of age. Three weeks after the last ovariectomy, 8 daily injections of 10 μg estradiol benzoate in 0.1 ml oil were given, followed by a single injection of 0.5 mg progesterone on day 9. Feminine sexual behavior was tested on days 4, 7 and 9 of the regimen; masculine behavior, on days 5 and 8. One week after termination of the estradiol regimen, females were given a pretest for feminine behavior to verify their return to baseline levels of this behavior. They then received four daily injections of 25 μg testosterone propionate in 0.1 ml oil, with a feminine behavior test on day 2. They received 50 μg testosterone propionate on days 5 through 7, with a masculine behavior test on day 6 and a feminine behavior test on day 7. Dosage was increased to 100 μg on days 8 through 10, with a masculine behavior test on day 9 and on a feminine behavior test on day 10. Body weights were recorded at the termination of behavioral testing. (See Tables 1 and 2 for summary.)

Sexual Behavior Tests

Masculine behavior tests utilized six ovariectomized females which had received a series of 2 or 3 daily injections of 20 μg estradiol benzoate followed by an injection of 0.5 mg progesterone 3 hr before the test. Only 1 female was used for each test day. Tests took place in the experimental animal's home cage with his or her cage mates removed. After five min adaptation time, the receptive female was introduced into the cage. Both copulatory and noncopulatory behaviors of both animals were recorded, as well as time of occurrence of each mount without thrust and each intromission pattern. The test was terminated after 10 min if there were no mounts; otherwise, it continued for 20 min. The first test of males' return to baseline levels of sexual behavior after the estradiol regimen was reduced to 5 min duration because it rapidly became apparent that the animals contin-

TABLE 2
EXPERIMENTAL DESIGN

	Estradiol Regimen—Females*				
Days	1–4	5	6–7	8	9
Dose	10 μg/da	10 μg	10 μg/da	10 μg	0.5 mg progest.
Test	♀ behav. test on day 4	♂ behav. test	♀ behav. test on day 7	♂ behav. test	♀ behav. test
	Testosterone Regimen—Females				
Days	7 days	1–4	5–6	7	8–9	10
Dose	post-EB	25 μg/da	50 μg/da	50 μg	100 μg/da	100 μg
Test	no treatment	♀ behav. test on day 2	♂ behav. test on day 6	♀ behav. test	♂ behav. test on day 9	♀ behav. test

*All females were treated identically in adulthood after having been exposed to maternal injections of progesterone or oil neonatally.

ued to exhibit considerable copulatory behavior. A rating score was calculated for each test, with 1 point given for each mount without thrust and 2 points for each intromission.

Feminine behavior tests utilized three vigorous stud males which were caged singly. The male or female to be tested was introduced into the stud male's home cage, and lordosis ratings were made for each of 10 mounts by the stud male. In some cases vigorous fighting between the stud and test males resulted in termination of the test before 10 mounts had occurred. The following lordosis ratings were used: 0—no lordosis; 1—slight lordosis and head lifting; 2—moderate arching of the entire back and lifting of the head; 3—full arching of back and sharp tilting of the head. An additional point was scored if the position was held after the male dismounted, and still another point, if hopping and darting preceded the mount.

Repeated measure analyses of variance were used to compare frequency of intromission patterns, male behavior rating scores, lordosis rating scores, and latency measures for each hormone regimen within each sex. Body and organ weights, as well as male rating scores on the single post-estradiol "baseline" test, were compared by means of t-tests.

RESULTS

Males exposed neonatally to increased progesterone levels exhibited significant impairment in masculine sexual behavior (see Fig. 1).During the estradiol regimen mean masculine behavior rating scores of progesterone exposed animals were significantly lower than those of controls, $F(1,18)=4.85$, $p<0.05$. The repeated measures factor also produced significant differences, $F(3,54)=10.98$, $p<0.001$, with higher scores on the later tests. The group x test interaction was not significant. The five-minute post-estradiol "baseline" test also revealed lower rating scores of progesterone treated animals (1.8 ± 0.73) compared to controls (5.0 ± 1.33) ($t(19)=2.12, p<0.05$). The testosterone regimen elicited even greater differences. Progesterone treated males exhibited fewer intromissions on the two tests (3.0+2.16) than did controls (13.6 ± 3.76, $F(1,18)=5.98$, $p<0.03$. They also had lower rating scores, $F(1,18)=6.03$, $p<0.03$. Neither the tests factor nor its interaction with the group factor was significant for the intromissions or the rating score analyses. Six of 10 progesterone treated males failed to intromit on either of the tests, while only 1 control male failed to do so ($\chi^2=5.41$, $p<0.05$). Latency and rating scores for those males which did mount and/or intromit were not different for the two groups on any test in the estradiol or testosterone regimen.

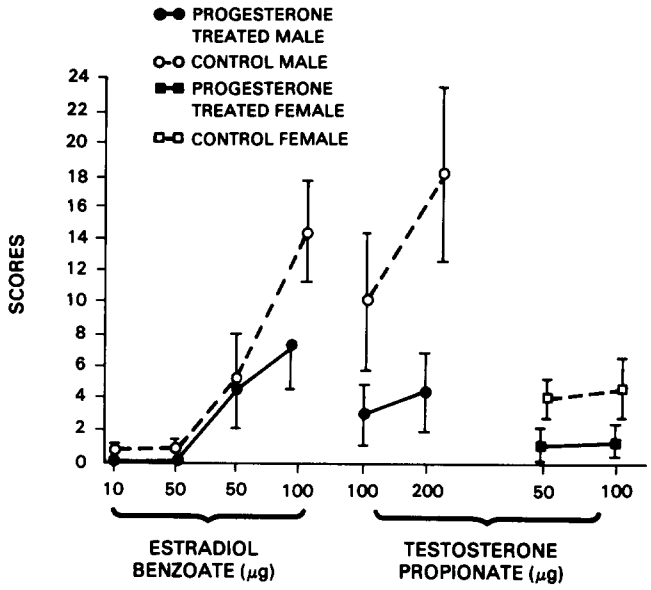

FIG. 1. Mean masculine behavior rating scores of castrated males and ovariectomized females treated neonatally with progesterone or oil. Dosage of estradiol benzoate and testosterone propionate used to elicit the behavior are shown on abscissa. Each mount contributed 1 point to rating score; each intromission pattern, 2 points.

Males exhibited insufficient feminine behavior for meaningful statistical analysis. The only two males which consistently showed lordosis on each of the last three tests were

progesterone treated animals (mean lordosis ratings of 5 and 11). One additional progesterone male and one control exhibited a single lordosis response.

There were no statistically significant differences between progesterone treated and control females in either masculine or feminine behavior tests. There were, however, trends ($0.1>p>0.05$) toward reduction in masculine behavior of progesterone treated females during the testosterone regimen. Mean masculine behavior rating scores for the two tests combined were 2.20 ± 1.38 for progesterone treated females and 8.11 ± 3.26 for controls. Two of 10 progesterone females demonstrated masculine sexual behavior, including mounts without thrusts and intromission patterns, while 4 of 9 controls did so. No female exhibited masculine behavior during the estrogen regimen. Estradiol benzoate elicited similar levels of feminine behavior in the two groups of females. Mean lordosis rating scores for each of the 3 tests were 13.17 ± 2.52 for progesterone treated females and 13.26 ± 3.89 for controls. The single progesterone injection also elicited similar behavior in the two groups: $\bar{X}=33.00 \pm 3.16$ for progesterone females, $\bar{X}=32.56 \pm 5.24$ for controls. The testosterone regimen was less effective in eliciting feminine behavior, but again there were no significant differences between the two groups: 2.23 ± 1.31 and 1.67 ± 1.29 for progesterone and control females, respectively.

There were no significant progesterone related differences in body or organ weights at any time.

DISCUSSION

The results of this experiment confirm our previous finding of reduced sexual competence of male rats exposed neonatally to moderate doses of maternally administered progesterone. The present experiment indicates that this reduced competence is not the result of diminished adult hormone levels. This finding is consistent with the hypothesis that progesterone may modify developing neural circuits, though alterations in sensory mechanisms or in peripheral metabolism of hormones have not been ruled out. The lack of gross morphological differences in adulthood between progesterone exposed and control animals suggests that peripheral structure differences do not mediate the deficit, though fine anatomical examination was not done.

Diamond *et al.* [6] also reported interference with masculinization by neonatally administered progesterone; however, their dosage was very large (5 mg/pup on day 3), and they obtained similar disruption with large doses of estrogen and testosterone. The same dosage regimen used in the present experiment was previously found to raise pup progesterone levels by about 2 standard deviations [12]. The mean plasma progesterone levels of pups whose dams received progesterone injections in that experiment was 3.99 ($\pm$ 0.52) ng/ml; that of control pups was 1.62 ($\pm$ 0.14) ng/ml. Thus, we have demonstrated that a moderate increase in progesterone levels throughout the neonatal period of sexual differentiation can compromise this process.

The behavioral deficits were observed during both the estrogen and testosterone regimens, indicating a behavior-specific rather than a hormone-specific effect. The apparently lower effectiveness of testosterone than estrogen at the 100 μg dose can most likely be explained by the shorter duration of the testosterone regimen at the time of the test. During both regimens there was no significant difference on any measure when only copulating animals were compared. The main difference appears to be the smaller number of progesterone exposed animals responding, rather than diminished performance of those that did respond. This was the case also in our previous study of intact males [13]. The smaller number of progesterone exposed females exhibiting masculine behavior in this experiment also accounts for the slight differences observed in that measure. In all of these groups neonatally administered progesterone appears to raise the threshold for masculine sexual behavior.

Feminine behavior was essentially unaffected by neonatal progesterone treatment. Feminine behavior scores of females were almost identical for the two groups under all adult hormone regimens, and there was only negligible feminization of progesterone treated males. This lack of effectiveness stands in contrast to previous reports of some feminization of girls and essentially no effect on boys whose mothers received progestins during pregnancy [10,15]. That there is a difference in effect between rats and humans is hardly surprising, in view of the gross differences in behaviors observed, time courses of perinatal hormone administration and of developmental sequences, and the particular steroids administered. The relative vulnerability of masculine and feminine behavior patterns to neonatally administered progesterone in this study is, however, somewhat surprising in light of reports that neonatal treatment of intact males with an aromatase inhibitor (which blocks conversion of androgens to estrogens) enhanced the display of feminine behavior but did not affect masculine behavior of rats [5,23]. On the other hand, administration of an aromatase inhibitor together with testosterone to neonatally castrated rats suppressed both masculinization and defeminization [2]. Since one of the possible modes of action of progesterone is to interfere with aromatization (see below), one might expect effects similar to those of aromatase inhibitors, i.e., a greater enhancement of feminine behavior.

Progesterone administered to adult estrogen primed males failed to enhance lordosis responses. It neither increased the number of males responding nor improved the performance of those which did. This result is in agreement with those of Davidson [3,4] and of Aren-Engelbrektsson *et al.* [1], though not with van de Poll and van Dis [22], who reported facilitation by progesterone of lordotic behavior of estrogen primed males. The low levels of feminine behavior displayed by both groups of males in this experiment were probably due to their age at testing. Feminine behavior of neonatally castrated males has been reported to decline more rapidly with age than feminine behavior of females [9].

The precise mode of progesterone's interference with masculinization is not known. It may inhibit aromatization of testosterone to estradiol in the brain, interfere with conversion of testosterone to dihydrotestosterone either centrally or peripherally, or may exert negative feedback on gonadotropin release. On the other hand, it may affect the balance of monoamine transmitters in the brain, thereby influencing the development of patterns of gonadotropin release or of sexual behavior. An indication that monoamines may be involved in the antiandrogenic effects of neonatally administered progesterone is our demonstration that progesterone alters brain MAO activities in fetuses and neonates [12,21]. In addition, adult sexual behavior has been reported to be affected by neonatally administered drugs which alter brain monoamine levels [8].

In summary, neonatally administered progesterone has been shown to interfere with masculine differentiation in rats, and to have little or no effect on feminine behavior. The

impairment is not mediated by alterations of adult hormone levels, and is seen during both estrogen and testosterone replacement of regimens in gonadectomized rats. The precise mode of interference is not known, though progesterone may directly affect metabolism of other steroids, exert negative feedback effects on gonadotropin release, or alter the balance of monoamines.

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