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Effect of Natural Progesterone Treatment during Pregnancy on Fetal Testosterone and Sexual Behavior of the Male Offspring in the Mouse[1]

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Abstract. The effect of maternal exposure to progesterone upon the fetal pituitary-gonadal axis and the sexual behavior of the male offspring of mice were studied. Daily injection of progesterone from days 14 to 16 of pregnancy reduced testosterone production in the fetus but caused a significant increase in circulating LH levels. Progesterone-exposed males showed no alteration in anogenital distance or in body weight at any time from birth to adulthood. At 80–90 days of age males from control and progesterone-exposed groups did not differ from each other in testis and seminal vesicle weights. However, in the latter group, there was a marked reduction in the percentage of males that displayed mount, intromission and ejaculation patterns. These findings indicate that in utero exposure to pharmacological doses of progesterone that do not cause abnormalities of male internal and external genitalia may interfere with masculine behavior in adulthood. This alteration could partially be due to diminished peripheral testosterone levels during the prenatal period.

Introduction

A large number of pharmacological agents such as synthetic progestins, β-mimetics and prostaglandin inhibitors has been used in the treatment of premature labor in spite of undesirable maternal and fetal side

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effects. The controversial association of synthetic progestins with possible problems during fetal and postnatal life [Suchowsky et al., 1967] has created new interest in the use of natural progesterone [Ferre et al., 1984]. Although there is no clear evidence concerning the role of progesterone in the fetus [Farquharson et al., 1984], it has been hypothesized that circulating progesterone may serve as precursor for fetal adrenal [Diczfalusy, 1969] and gonadal steroidogenesis [Taylor et al., 1974]. In the newborn, exogenous progesterone only induces slight changes in the development and function of the testis [Tapanainen et al., 1979] but is able to interfere with masculine differentiation [Hull, 1981]. Administration of pharmacological doses of progesterone (5–200 mg) to pregnant rats caused no abnormalities of both internal and external sex organs in the progeny [Revesz et al., 1960]. However, we recently reported that administration to pregnant mice of concentrations of progesterone, in the same range, reduces circulating testosterone levels in the male fetuses [Pointis et al., 1984]. The purpose of the present investigation was to study the effect of this treatment on the fetal pituitary-testicular axis and on the sexual behavior of male offspring in adulthood.

Materials and Methods

Animals

Albino Swiss female and male mice were mated for 1 night. Fertilization was verified by the presence of vaginal plug on the following morning. The average period of gestation in our colony was 19 days. On days 14, 15 and 16 of pregnancy mice received daily subcutaneous injections of 2 mg progesterone diluted in oil. Control females received injections of the vehicle only (200 µl) on equivalent days of pregnancy. On day 17, mice were killed by cervical dislocation and the fetuses were removed. Fetal blood was collected by decapitation and pooled into heparinized tubes, after determining the gonadal sex of each fetus. Plasma from male fetuses, obtained following centrifugation, was stored at −20 °C until processed.

Radioimmunoassays

Testosterone and progesterone levels in fetal plasma were measured using specific radioimmunoassay methods as previously described [Pointis et al., 1979]. The specific antiserum to testosterone was obtained from Institut Pasteur Production and cross-reacts 36% with dihydrotestosterone, but $< 2\%$ with other steroid hormones. Progesterone was analyzed using a specific antibody provided by Roussel Uclaf. The cross-reaction with 5α-dihydroprogesterone, 20β-hydroxyprogesterone and 20α-hydroxyprogesterone was 44, 0.2 and 1.2%, respectively. Plasma luteinizing hormone was measured by a double-antibody ovine system as previously reported [Pointis et al., 1980]. This procedure has been validated for use in the mouse [Beamer et al., 1972].

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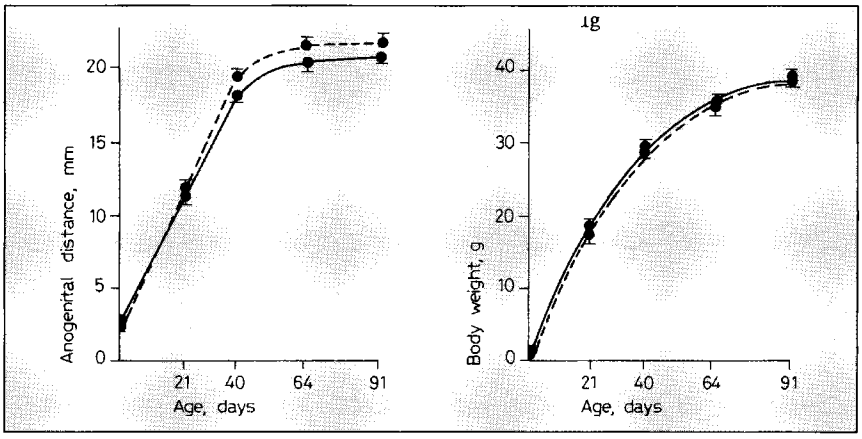

Fig. 1. Effect of progesterone treatment during fetal life (from days 14 to 16 of pregnancy) on the anogenital distances and body weights of the male progeny. Values shown are mean ± SEM. The number of animals studied varied between 20 and 25 in each group. - - - = Control; —— = progesterone-treated.

Sexual Behavior Test

At about 90 days of age the male offsprings of control and progesterone-treated mothers were tested for masculine sexual behavior. Animals were tested for 30 min once a week for 3 weeks. Tests were conducted 3 h after lights off under a red light. Subjects were placed in a plexiglas arena to adapt for 5 min prior to the introduction of a sexually receptive female. Ovariectomized females had been brought into estrus by two subcutaneous administrations of 25 µg estradiol benzoate (dissolved in 100 µl oil) 48 and 24 h before and 0.5 mg progesterone 6 h before testing. The number of males for each group that exhibited the mount, intromission and ejaculation patterns, in at least one of the three mating tests, were recorded.

Results were expressed as means ± SEM and the differences between groups were analyzed using the analysis of variance and Student's t test. Differences in the incidence of masculine behavior were tested for significance by χ^2 tests.

Results

Analysis of variance indicated that body weights and anogenital distances of both controls and males exposed during fetal life to progesterone increased significantly ($p < 0.05$). However, no significant effect of the progesterone treatment could be demonstrated on the two parameters studied (fig. 1). At the time of the autopsy (90–100 days), control

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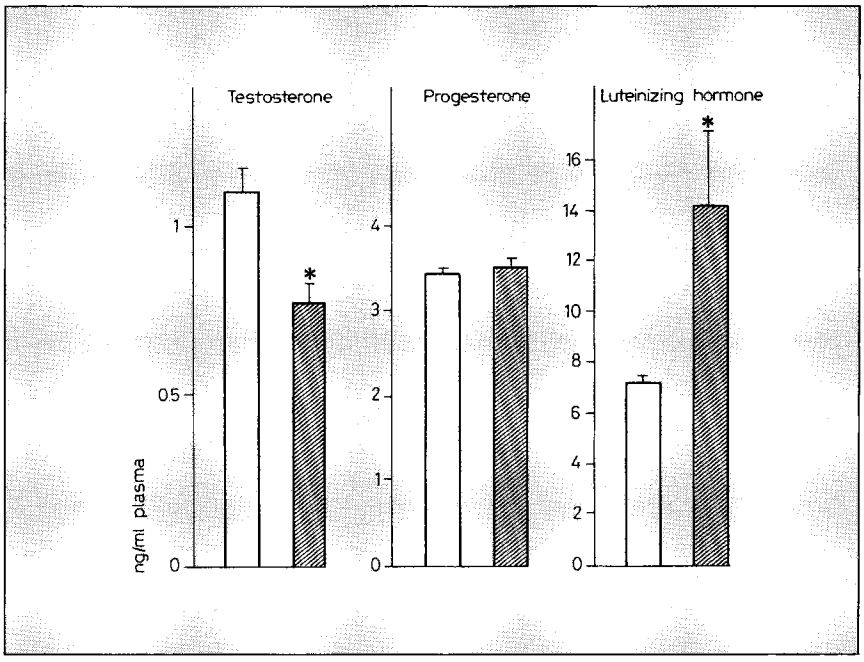

Fig. 2. Effect of maternal exposure to progesterone (from days 14 to 16 of pregnancy) on plasma testosterone, progesterone and luteinizing hormone levels in male fetuses autopsied on day 17. Values shown are means ± SEM of 3 or 4 pools. The number of fetuses per pool varied between 16 and 18. * $p < 0.05$ vs. control group. □ = Control; ▨ = progesterone-treated.

and progesterone-treated group did not differ from each other in paired testis (control: 239.48 ± 38.21 mg; progesterone-treated: 235.68 ± 24.22 mg; means ± SEM; n = 37) and seminal vesicle weights (control: 312.72 ± 94.26 mg, n = 22; progesterone-treated: 316.45 ± 90.60 mg, n = 11). The effect of maternal exposure to progesterone, from days 14–16 of pregnancy, on testosterone, progesterone and luteinizing hormone levels in the circulation of male fetuses is presented in figure 2. Statistical analysis indicated that testosterone levels in fetuses from progesterone-treated mothers were significantly reduced as compared to the control ones ($p < 0.05$). No significant effect of the treatment on circulating progesterone levels in male fetuses was found when animals were autopsied 1 day after the last injection of progesterone. In contrast, at this time, a significant increase ($p < 0.05$) in circulating LH levels was

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Table I. Influence of progesterone treatment during fetal life (from days 14 to 16 of pregnancy) on sexual behavior of the male progeny

	Control		Progesterone treatment	
	No. of animals	%	No. of animals	%
Mount	14/14	100	8/15	53**
Intromission	14/14	100	7/15	47***
Ejaculation	5/14	36	0/15	0*

The proportions of animals in control and progesterone-treated groups displaying mounts, intromissions and ejaculations on at least one of the three mating tests are indicated. * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$ vs. control group.

observed in the progesterone-exposed group. Table I summarizes data on the reproductive performance of the animals in each group. A significantly greater proportion of animals in the control group displayed mount ($p < 0.02$), intromission ($p < 0.01$) and ejaculation patterns ($p < 0.05$) as compared to the progesterone-treated group.

Discussion

The present data indicate that exposure of the male fetus in utero to pharmacological concentrations of natural progesterone is able to reduce masculine copulatory behavior of the male progeny. The fact that, at the time of the autopsy, the testis and seminal vesicle weights of these animals were not significantly reduced as compared to the controls suggests that the reduced competence observed in the present work is not the result of decreased adult hormone levels. It is more likely that this defect is related to events that are programmed during neonatal life. These data are in agreement with those of Hull [1981] who has shown that neonatal exposure to progesterone induces similar impairment of masculine behavior without affecting testis and accessory organ weights of males. Current notions about the sexual differentiation of the central nervous system in the rodent postulate that testosterone, acting during a transient neonatal period, induces masculinization at behavioral [Gorski, 1974], morphological [Raisman and Field, 1973], and biochemical levels [Barraclough,

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1979]. This critical period for sexual differentiation of the brain is close to birth probably starting during the last days of fetal life [Vom-Saal and Bronson, 1980; Gogan et al., 1981] when high levels of testosterone are present in the fetal circulation [Weisz and Ward, 1980; Pointis et al., 1980]. This latter supposition is supported by the facts that (1) male rats exposed to prenatal stress showed low levels of male copulatory behavior, resulting from a decline of circulating testosterone during days 18 and 19 postcoitum [Ward and Weisz, 1980], and (2) demasculinization induced by prenatal stress could be prevented by perinatal androgen treatment [Dörner et al., 1983]. Our results that testosterone levels are reduced in the fetal circulation after exposure of pregnant mice to progesterone strongly support the hypothesis that this treatment modifies the normal process of sexual behavior differentiation by decreasing fetal testosterone production. The possibility that progesterone affects masculinization by altering the balance of biogenic amines, known to be involved in the androgen-dependent sexual differentiation of the brain [Snyder et al., 1979], must also be retained. It seems unlikely that, in the present study, progesterone acts as an antiandrogenic preventing the masculinizing effect of testosterone, since such an effect postulated in nonhuman primate [Resko, 1975] was not verified in rodent [Weisz and Ward, 1980]. The finding that luteinizing hormone levels are increased in the male fetal circulation after maternal progesterone exposure agrees with our previous studies [Pointis et al., 1984] suggesting that the decline of testosterone is not the result of a decreased gonadotropin release. Our current data suggest that the effect of progesterone on fetal testosterone is more likely to be due to an increase in the metabolic clearance rate of testosterone or to a direct effect at the fetal testicular level (either by progesterone or a metabolite of this steroid hormone). This latter possibility has been previously postulated [Tapanainen et al., 1979; Pointis et al., 1984]. *In conclusion,* the present study demonstrates that in utero exposures to doses of progesterone which do not cause abnormalities of male external and internal genitalia lead to irreversible alterations of the process of masculinization of the central nervous system characterized during adulthood by an altered masculine behavior.

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