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Prenatal Exposure to Prednisone Permanently Alters Fighting Behavior of Female Mice[1]

JUNE MACHOVER REINISCH, NEAL G. SIMON[2] AND RONALD GANDELMAN

Department of Psychology, Busch Campus, Rutgers University, New Brunswick, NJ 08903

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REINISCH, J. M., N. G. SIMON AND R. GANDELMAN. *Prenatal exposure to prednisone permanently alters fighting behavior of female mice*. PHARMAC. BIOCHEM. BEHAV. 12(2) 213–216, 1980.—Female mice born of mothers administered 100 μg prednisone on Days 13–18 of gestation attacked a stimulus male significantly sooner following the commencement of testosterone treatment in adult life than did mice born of control mothers. In a second experiment, significantly fewer prenatally prednisone-exposed females displayed postpartum aggression as compared to controls. In both experiments females of the 100 μg prednisone group showed a reduction in birth weight relative to controls. The effect on body weight did not persist since no differences were observed on Day 21 of life. The data show that prenatal exposure to prednisone permanently modifies the later intraspecific fighting behavior of female mice.

Prednisone Fetal development Mice Fighting Testosterone Lactation Maternal aggression

PARALLEL investigations of humans and animals revealed that full-term offspring of mothers treated with 17α,21-Dihydroxypregna-1,4-diene-3,11,20-trione (prednisone) during pregnancy are significantly reduced in birth weight [12]. Prednisone, having five times the potency of cortisone is reportedly the most widely prescribed corticosteroid in the U.S. [7,10]. Natural and synthetic corticosteroids, administered for their anti-inflammatory, anti-allergic, and immunosuppressant properties, are given routinely in the treatment of such conditions as arthritis, asthma and lupus erythmatosus, among others. In addition, and of relevance to the present study, prednisone has been administered specifically to disinhibit ovulation and support the subsequent pregnancy of women suffering from putative adult adrenogenital syndrome [12]. Since corticosteroids readily cross the placental barrier [1,20], both the incidental and deliberate treatment of pregnancy with these steroids have resulted in the exposure of large numbers of fetuses to augmented adrenal hormone levels.

Based upon the identification of an effect of prednisone on physical development of the fetus [12] and the body of work indicating diverse long-term behavioral and physiological sequellae of early exposure to certain steroid hormones in humans and animals (cf. [8, 11, 13]), developmental studies of mice exposed to prednisone prenatally were initiated. It is reported here that prenatal exposure to prednisone masculinized the subsequent response of females to the aggression-promoting property of androgen (Experiment 1) and reduced the incidence of postpartum aggression, an important component of maternal behavior (Experiment 2).

EXPERIMENT 1

METHOD

Animals

The animals were Rockland-Swiss (R-S) albino mice that had been maintained as an outbred strain in a closed colony. They were kept in 28×18×13 cm translucent polypropylene cages. The cage floors were covered with wood shavings. The mice were provided with food and water in excess and kept under a 12 hr light/dark cycle with lights on at 0600 hr.

Procedure

Sixty-day old nulliparous mice were group-housed with males and examined daily for copulatory plugs. When a plug was discovered the female was housed singly and that day was designed as Day 0 of pregnancy. (Using this procedure, the gestation period is 19 days.) The timed-mated animals were divided randomly among the following groups on Day 12 of pregnancy: (1) no treatment, (2) vehicle, (3) 100 μg prednisone. Prednisone was suspended in 0.05 ml Steroid Suspending Vehicle and each animal was given an SC injection between 0900–1000 hr on each of Days 13–18 of pregnancy. The 100 μg dosage of prednisone was chosen because it was used in the initial study which demonstrated an effect of the compound upon birth weight [12].

On the day of parturition the young of each litter were counted, weighed, culled to 4–6 females, and given to a lactating animal that had delivered within the previous 24 hr and

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[2]Present address: Department of Psychiatry and Behavioral Science, State University of New York at Stony Brook, Stony Brook, NY 11794.

TABLE 1

AGGRESSIVE BEHAVIOR OF FEMALE MICE IMPLANTED AS ADULTS WITH SILASTIC CAPSULES CONTAINING TESTOSTERONE. ONE GROUP OF MICE WAS BORN OF MOTHERS ADMINISTERED 100 μg PREDNISONE DURING PREGNANCY

	Phase I		Phase II	Phase III	
Group	Proportion fighting (%)	Mean* latency (days)	Proportion fighting (%)	Proportion fighting (%)	Mean* latency (days)
Control	15/16 (94)	19.0 ± 0.74	0/15 (0)	14/15 (93)	20.5
Prednisone (100 μg)	14/16 (88)	10.7 ± 1.6	2/14 (14)	11/12 (92)	10.09

*Significant difference between groups ($p<0.001$).

whose own young had been removed. This fostering procedure was used to insure that any effects exerted by prednisone were not mediated by the postnatal behavior and/or physiology of the prednisone-treated mother.

The pups were weighed and weaned on Day 21. They remained in littermate groups until Day 60 when a random sample of females from each treatment condition was chosen. The animals then were housed singly. At that time the mice were ovariectomized and implanted SC with a 10 mm section of Silastic tubing (ID=0.062 mm) containing 5 mg testosterone (T) suspended in .02 ml sesame oil. All surgical procedures were performed while the animals were anesthetized with ether. Tests for fighting behavior were given between 0800–1000 hr and commenced on Day 62. A test consisted of placing an olfactory bulbectomized adult male R-S mouse into the home cage of each animal and observing the behavior of the test animal for 10 min or until a fight occurred (Phase I). Olfactory bulbectomized males served as stimulus animals because, while neither initiating a fight nor retaliating against an attack by fighting, they elicit levels of aggression comparable to those of intact animals [5]. Therefore, any fighting observed is both initiated and terminated by the test animal, eliminating the confounding influence of the stimulus animal's behavior. A fight was recorded if the test animal bit and chased the stimulus animal for at least 5 sec. Animals were tested every other day until they displayed fighting behavior or for a maximum of 15 tests.

The T-containing capsules were removed from the animals the day after fighting was displayed. Three weeks later these animals were tested for fighting behavior every other day for 10 days without exogenous hormone treatment (Phase II). Those animals that did not fight following removal of the implant were given a new T-containing capsule and tested for fighting behavior beginning two days later (Phase III).

RESULTS

Because the two control groups (no treatment; vehicle injected) did not differ on any measure in this or Experiment 2, their data were pooled. A significant difference was observed between the prenatally prednisone-exposed and control animals in body weights at birth, $t(99)=6.46$, $p<0.001$. Mean birth weights were 1.35 g and 1.53 g, respectively. This difference as not observed when the animals were 21 days old. The mean weight for the prednisone-exposed animals was 10.84 g and that of the controls 10.64 g.

The results of the aggression tests are summarized in Table 1. It is apparent that the groups did not differ in the proportion of animals that fought in response to T administration. However, animals exposed to prednisone during fetal development fought significantly sooner following the initiation of T treatment than did controls, $t(27)=4.73$, $p<0.001$. Furthermore, as revealed in Phase II tests, all but 2 mice did not exhibit fighting behavior when tested 3 weeks after the T capsule had been removed. Finally, fighting was reestablished after the mice again were implanted with capsules of T (Phase III), thus demonstrating that the aggressive behavior indeed was mediated by the androgen. It should be noted that the difference between the prednisone-exposed and control animals in the latency to fight following the initial exposure to T (Phase I) was maintained following reexposure to the steroid in Phase III, $t(23)=7.01$, $p<0.001$.

The results of Experiment 1 have shown that female mice exposed to prednisone during fetal development manifest an enhanced responsiveness to the aggression-activating property of T. Since comparably enhanced sensitivity also has been found in mice exposed to T prenatally [16], it may be that prednisone possesses androgenic properties when present during prenatal development. Because prenatal exposure to androgens also disturbs the reproductive activities of female rodents [19], a second experiment was undertaken to ascertain whether prenatal exposure to prednisone would affect fertility, reproductive fitness, and/or maternal behavior of the female.

EXPERIMENT 2

METHOD

Timed-mated pregnant nulliparous R-S mice were given either 50 μg or 100 μg prednisone, only the vehicle, or left undisturbed on Days 13–18 of pregnancy. On the day of delivery, the resulting litters were weighed and culled to 4–6 females and fostered to untreated dams that had delivered within the previous 24 hr and whose own young had been removed. The animals were weaned and weighed on Day 21 and kept in littermate groups until Day 60. At that time a random sample of females from each treatment condition was chosen. Each of these females then was housed singly with an adult R-S male for 10 days.

The resulting litters from the prenatally prednisone-exposed and control animals were weighed, culled to 3 female and 3 male pups, and returned to the dam. Each adult

TABLE 2

THE FERTILITY, LACTATION PERFORMANCE AND POSTPARTUM AGGRESSIVE BEHAVIOR OF MICE FOLLOWING PRENATAL EXPOSURE TO 50 μg OR 100 μg PREDNISONE

Group	Pregnancies (n)*	Mean weight gain per litter between day 0–9 g	Proportion fighting (%)†
Control	14/15 (2)	25.99	11/12 (92)
Prednisone (50 μg)	13/16 (0)	24.18	9/13 (69)‡
Prednisone (100 μg)	16/18 (3)	22.16	4/13 (31)§

*Number of mothers that killed their young or failed to lactate.
†Significant differences among groups (χ^2, $p<0.01$).
‡Not significantly different from controls.
§Significantly different from controls ($p<0.05$).

female was tested for maternal aggression on Days 4–9 of the lactation period. An aggression test consisted of placing an R-S male into the cage of each lactating mouse and its offspring. The stimulus male was left there for 5 min or until the test female exhibited a fight, the criterion for which was 5 sec of biting and chasing of the stimulus male. (It should be noted that the stimulus males for this experiment were not olfactory bulbectomized since intact males never have been observed to attack a lactating female.)

RESULTS

The mean birth weights were 1.46 g, 1.44 g and 1.24 g for the control, 50 μg and 100 μg prednisose-exposed mice, respectively. An overall analysis of variance was statistically significant, $F(2,196)=50.4$, $p<0.001$. Thus, prenatal exposure to 100 μg but not 50 μg prednisone retarded birth weight. There was no significant difference among the 3 groups on Day 21 postpartum. The mean weaning weights were 11.4 g, 10.9 g, and 11.1 g for the control, 50 μg- and 100 μg-exposed animals. The effects of prenatal exposure to 100 μg prednisone, therefore, parallel the findings of Experiment 1.

Data on reproductive performance and maternal aggression are summarized in Table 2. There was no difference in the proportion of animals from each group that mated successfully, in the number of animals that failed to lactate, or that killed young. Furthermore, there was no significant difference in litter weights on the day of birth or on Day 9. However, there was a marked difference in the proportion of animals from each group that displayed maternal aggression, $\chi^2(2)=10.26$, $p<0.01$. The percentage fighting was highest in the control condition (92%), followed by the 50 μg (69%) and 100 μg (31%) prednisone animals. Further comparisons (Fisher's Exact Probability Test) revealed that while the control and 50 μg prednisone females did not differ significantly, the proportion of 100 μg prednisone females which displayed postpartum aggression was significantly lower than that seen in the control group ($p<0.05$).

DISCUSSION

The results have shown that prenatal exposure to prednisone significantly modified two characteristics of the female. First, female mice normally require an extended period of T treatment during adult life to induce male-like fighting behavior [16]. Those exposed to prednisone prenatally responded much more rapidly to the aggression-activating property of T than did controls. Second, lactating mice generally attack conspecifics introduced into their home territory, presumably to protect their young from predation [15]. Female mice exposed to prednisone prior to birth were less likely to display maternal aggression even though fertility, support of pregnancy, and lactation performance were indistinguishable from those seen in untreated dams.

The effects described above may be either a specific consequence of prenatal exposure to prednisone or a generalized effect of reduced weight at birth. The latter must be considered since effects of the corticoid were manifest only in females born of mothers that received 100 μg, a dosage which did affect birth weight. This possibility seems unlikely since other data have shown that females born of mothers that had been treated with ACTH during pregnancy, while lower in birth weight than control offspring, were unchanged in their sensitivity to T as adults [14]. This suggests that the effects of prednisone observed in the present study in all probability are attributable to the influence of prednisone on an aspect(s) of fetal development other than the attainment of body weight in a range normal for the species.

With regard to the enhanced sensitivity to the aggression-promoting activity of T in mice exposed prenatally to prednisone, other manipulations which elevate maternal and fetal corticosteroid levels such as prepartum stress or hydrocortisone or ACTH administration have resulted in the feminization of sexual behavior of male progeny [17], demasculinization of morphology [4] and the reduction of sensitivity to the activational property of androgen on fighting behavior [14]. Female offspring were relatively unaffected by these manipulations. Therefore, it seems likely that the alteration in sensitivity to T observed in the present study is not attributable to the corticosteroid-like activity of prednisone.

Of particular interest is the similarity of the effects of prednisone to those of androgen on the response to T in later life. Either steroid, when given prenatally, significantly enhances a female's subsequent sensitivity to the aggression-promoting property of T. This strongly suggests that prednisone, in addition to its established corticosteroid-like activity, is androgenic. Supporting this suggestion are data showing that prednisone stimulates growth of the seminal vesicles, an androgen-sensitive tissue [3]. However, since prenatal exposure to prednisone did not influence reproductive performance as does T and other androgens, it would appear that prednisone in the dosages used

here either is only mildly androgenic or possesses specific rather than generalized androgenic properties.

Alternatively, the masculinized response of prenatally prednisone-exposed females to testosterone may have been the result of an elevation in unbound estrogen levels in the fetus. Belanger *et al.* [2] reported that prednisolone, the active metabolite of prednisone, depresses the level of estrogen binding alpha-fetoprotein (AFP) in newborn mice and rats. It has been suggested that AFP protects the fetus from high circulating levels of maternal and/or placental estrogen which in its unbound form could masculinize the female during fetal development [9,18]. Further, in the neonate, the addition of prednisolone to subthreshold dosages of exogenously introduced estrogen has been reported to defeminize adult female sex behavior [18]. Since perinatal treatment of female mice with estrogen (like testosterone) results in augmented sensitivity to the aggression-promoting property of T in adult life [6], it also seems reasonable to suggest that the enhanced sensitivity of prednisone exposed females to T was produced by the effect of prednisone on AFP levels which in turn made estrogen available to the fetal CNS.

Prenatal exposure to prednisone also reduced the probability that postpartum aggression would be exhibited. It has been shown that this behavior is initiated by stimulation of the teats by the young [15]. Since lactation was unaffected in the experimental females as evidenced by weight of the young, it would appear that a normal milk let-down reflex was present. Therefore, prenatal exposure to prednisone probably does not affect afferent input from the teats to the central nervous system. The decreased incidence of postpartum aggression in females exposed to prednisone during prenatal development, therefore, may reflect developmental alterations in those areas of the brain which mediate the display of aggression by lactating animals.

In conclusion, prednisone, administered for the treatment of a wide variety of human ailments, has been shown to alter the normal course of development in mice when present during the prenatal period. Implied by this finding is a permanent direct or indirect effect of this synthetic corticoid on the central nervous system. These data, in conjunction with our recent finding of diminished birth weight in the offspring of both mice and humans treated with prednisone during pregnancy [12], suggest that effects reported here may have their counterpart in some behavioral alteration in humans exposed to the compound during prenatal development.

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