Pharmacology Biochemistry & Behavior, Vol. 33, pp. 867–873. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Effects of Chronic and Acute Ethanol Treatment During Prenatal and Early Postnatal Ages on Testosterone Levels and Sexual Behaviors in Rats

I. LENA DAHLGREN,*[1] C. J. PETER ERIKSSON,‡ BIRGIT GUSTAFSSON,* CHRISTINA HARTHON,* ERNEST HÅRD*† AND KNUT LARSSON*

**Department of Psychology, Unit of Psychobiology*
and †Department of Psychiatry and Neurochemistry, University of Göteborg, Göteborg, Sweden
and ‡Research Laboratories of the Finnish State Alcohol Company, Helsinki, Finland

Received 3 August 1987

DAHLGREN, I. L., C. J. P. ERIKSSON, B. GUSTAFSSON, C. HARTHON, E. HÅRD AND K. LARSSON. *Effects of chronic and acute ethanol treatment during prenatal and early postnatal ages on testosterone levels and sexual behaviors in rats.* PHARMACOL BIOCHEM BEHAV **33**(4) 867–873, 1989.—This study was prompted by previous findings that prenatal ethanol exposure may interfere with the differentiation of the sexual behavior in rats. Ethanol (6 g/kg) administered daily from day 15 postconception, resulted in elevated testosterone (T) levels on Day 18 in male and female fetuses. No alterations of sexual behavior in the ethanol-treated male offspring were seen under these conditions. However, in ethanol-treated female offspring the onset of regular estrous cycling was significantly delayed. Acute treatment with doses of ethanol, 2, 4 or 6 g/kg, was ineffective in influencing plasma T levels of the fetuses. Acute treatment with 3 g/kg ethanol did not prevent the rise of T levels normally occurring immediately after birth. In adulthood, but not at prepubertal age (Day 30), treatment of male rats with 2 g/kg ethanol caused a depression of plasma T levels. Possible mechanisms affected by ethanol exposure and influencing on the fetal development were discussed.

Ethanol Testosterone Perinatal Sexual behavior

WE previously reported that male rats who were exposed to ethanol prenatally in adulthood showed elevated levels of feminine sexual behavior (20). Feminization of other sexually dimorphic behaviors such as juvenile fighting (26), and preference for sweet solutions (24) following prenatal ethanol exposure has also been reported. These observations suggest that prenatal ethanol exposure may interfere with the normal sexual differentiation of the male.

During a perinatal critical period in the rat extending from approximately 15 days of pregnancy to about 10 days postnatally, testosterone (T) produced by the testes is instrumental in suppressing the potential for feminine sexual behavior, a process called defeminization (17, 23, 34). At the same time, the neural substrate for masculine sexual behavior characteristics is induced, i.e., masculinization. Supposing that ethanol treatment depresses the T secretion in perinatal rats, as it does in adult males (7, 13, 14), we speculated that the impaired defeminization of prenatally ethanol-exposed males may be related to decreased fetal T levels caused by the ethanol treatment.

The first study reported in this communication involved measurement of the T levels of male and female fetuses of mothers exposed to ethanol during the last trimester of gestation. The effects of prenatal exposure to ethanol on sexual behavior of adult males and on the development of estrous cyclicity of females were also assessed in order to obtain a correlate for the possible hormonal effects of ethanol treatment.

In a second study, measurements were undertaken of the T levels following ethanol exposure during the very first hours after delivery since a surge of T has been reported during these hours which normally contributes to produce the normal defeminization of the male (9). A final series of experiments was undertaken where the investigation was extended to include rats of higher ages, who had already completed the critical period of sexual differentiation and passed into late prepubertal age and adulthood.

METHOD

Animals

Animals were offspring of a stock of Wistar rats from Mölle-

[1]Requests for reprints should be addressed to I. Lena Dahlgren, Department of Psychology, Unit of Psychobiology, University of Göteborg, Box 141 58, S-400 20 Göteborg, Sweden.

gaard Breeding Laboratories, Denmark. The colony was maintained under artificial light conditions (light off 1000–2200 hr) at 23°C and a humidity between 50–60%.

Procedure

Chronic treatment with ethanol. For the behavioral experiments, ethanol (6 g/kg/day) was administered to 13 pregnant females by gavage during the period 15–21 days postconception (P.C.), (day of conception = 0). The animals were given ethanol, 2 g/kg, three times per day (900, 1200 and 1500 hr) as a 12.5% w/v solution with 5.5% glucose as vehicle. Fourteen control mothers were treated with the vehicle according to the same schedule as the experimental group. At birth each litter was reduced to 8 pups, 4 females and 4 males.

Immediately after birth, the biological mother was replaced by a foster mother from the same stock who had given birth to pups at the same day or the day before.

The pups were weaned at 25 days of age and housed by sex in groups of four animals in every cage. Between the ages 30 days to 90 days all the animals were weighed every 10 days. Two males were randomly selected from each litter to partake in experiments concerned with the estimation of the age when a complete mating pattern, including ejaculation, could be observed. These same males were also used for observations of the pattern of male sexual behavior in adult age. The two remaining males from each litter were observed for the occurrence of lordotic behavior. Two females were randomly selected from each litter and were observed for the regularity of estrous cycles.

For assessment of the effects of ethanol on T levels in the fetus, 45 pregnant females were treated with ethanol (6 g/kg/day) in accordance with the method described above. Another group of 45 pregnant females were treated with the vehicle and served as controls. On postconceptual days 17, 18 and 19 respectively, 15 mothers were sacrificed by decapitation between 2 and 4 hours after the first treatment on the day of sacrifice. All fetuses were removed from the decapitated mothers and then sex-determined. Afterwards, the fetuses were stored in a freezer, −20°C, for later analysis of their T levels.

Acute treatments with ethanol. The effects that acute ethanol treatment have on T levels in prenatal, neonatal, prepuberal and adult rats were investigated in separate experiments.

To determine the effects on prenatal rats, 45 pregnant females were treated IP on Day 19 P.C. with 2, 4 or 6 g/kg ethanol as 12.5, 25.0 and 37.5% w/v in saline respectively. A control group of 15 females was treated with 0.9% saline. The females were sacrificed by decapitation two hours after treatment. All fetuses were removed from the decapitated mothers and then sex-determined. Blood was collected from each fetus and centrifuged. Blood plasma from all fetuses of the same sex and litter was pooled and mixed with 1 ml distilled water and stored at −20°C.

To determine the effects on neonatal rats, 45 pregnant females were used. They were maintained from the first day after conception in a room with a temperature of +28° C and with an artificial light rhythm (lights on between 0400–1600 hr). From the 22nd day P.C. the females were inspected every 5 minutes between 0800–1400 hr for the occurrence of birth. The mothers were sacrificed by decapitation immediately after the birth of the first pup and the fetuses were removed from the mothers. Within a few minutes six of the males were treated either with ethanol or saline. Two of the pups were SC treated with ethanol, 3 g/kg, as a 18.75% w/v solution in saline. One of these two pups was decapitated 1 hour after the treatment, and a second pup one hour later. Four of the pups were SC treated with 0.9% saline. One of these four pups was decapitated immediately after the treatment, and the other three 1 hr, 2 hr and 3 hr respectively after the treatment. Blood plasma was collected from each pup and stored individually at −20°C.

To determine the effects of ethanol treatment on prepuberal rats, 30–32 days of age, 13 litters were used. Three males were randomly selected from each litter. One of these males was treated IP with 2 g/kg ethanol as a 12.5% w/v solution in saline. A second male was treated with 0.9% saline. Both these two animals were decapitated 2 hr after treatment. An untreated male was decapitated at the same time. Blood plasma, collected from these three animals, was stored separately at −20°C.

Thirty-nine adult males, who were about 90 days of age, were randomly chosen from our colony of rats to partake in the experiment on the effects of ethanol treatment on adult males. They were equally distributed into the experimental and the two control groups and underwent the same treatment as the rats in the preceding experiment on prepuberal rats. The only difference was that a 25.0% w/v solution was used for ethanol treatment.

Behavioral Measures

Masculine sexual behavior and lordotic behavior in males. The males were tested for masculine sexual behavior from 46 days of age, every second day until they showed a complete masculine mating pattern or until they were 70 days old. The tests for sexual behavior followed a procedure previously described (12). The male was presented with a receptive female in the mating arena and observed for the occurrence of mount, intromission and ejaculation. The test was ended after either (a) a maximum of 15 min without mount or intromission; (b) a maximum of 30 min after the first intromission without ejaculation; (c) the first intromission following the initial ejaculation; or (d) a maximum of 15 min without intromission after ejaculation. All males were tested again at the age of 90 days for masculine sexual behavior. This test was performed to provide information on the mating pattern at one specific age level, similar for both ethanol-treated and control rats.

The males were tested for lordotic behavior when they were about 80 days old. Lordosis is an arched curvature of the back which is shown by the female as part of her estrous behavior in response to male mounting. A small proportion of males display the response when mounted by another male. This proportion can be considerably increased by treating the males with estrogen. The tests for lordotic behavior in males were performed in the following way. The male subject to the test was placed in the mating arena, and left alone for 5 minutes for adaptation. Thereafter, a sexually vigorous male was allowed to mount the experimental male ten times. The number of lordotic responses by the experimental male to these 10 mounts were recorded. Two separate tests for lordotic behavior, one to two days apart, were performed.

The female estrous cycle. From the age of 30 days and onwards, the females were examined daily for vaginal opening. The onset of regular estrous cycles was determined by daily inspection of vaginal smears and by testing the reaction of the female to manual palpation of her flanks and lower back. When in behavioral estrus, the female responds to this stimulation with lordosis (depression of the back, raising of the hindlegs and the head, crouching in a motionless position with the legs slightly flexed and the head held parallel to the floor, rapid shaking of the head in the lateral plane and vibrating the ears). Vaginal smears were taken from the day of vaginal opening using a pipette for the suction of desquamated cells from the vaginal mucosa which was moistened by tap water. Tests for estrus were performed daily until the female showed four consecutive four-day cycles, or until the females were 70 days of age. Tests for lordotic behavior and the

collection of vaginal smears were performed at 1400 hr each day.

Testosterone Measurements

Plasma T was measured as described by Ismail *et al.* (21) with a radioimmunoassay kit supplied by Farmos Diagnostica, Oulunsalo, Finland. The mean intra- and interassay precision c.v.% of the T tests were 8.6 and 12.2 respectively. The sensitivity for the method was approximately 0.4 nmol/l. In experiments where fetuses were too small to provide plasma samples, whole fetuses were homogenized with four parts of distilled water prior to execution of the procedures prescribed for the measurement of the plasma T levels. Recoveries (>95%) were not affected by the use of tissue samples.

Statistics

In the statistical analyses of the prenatal effects on T levels and behavioral parameters each litter was represented by a mean value based upon the values of the two males or females observed. Group differences in these mean values were analysed by the Mann-Whitney U-test or the Median test, using two-tailed levels of significance, if not otherwise stated (4). In the experiments on the effects of ethanol on T levels in postnatal animals the control and experimental groups were compared by the *t*-test.

RESULTS

Gestation and Morphological Development

The length of gestation was the same for the experimental group and the control group (Table 1). The ethanol-treated mothers gained body weight at a slower rate than the control mothers during the treatment period.

There was a significant difference in sex ratios between controls and ethanol-exposed pups. The difference can mainly be attributed to the fact that the sex ratio of the control pups was, for some unknown reason, significantly decreased compared to an expected theoretical sex ratio of about 0.5. The sex ratio of the ethanol-exposed pups, on the other hand, did not differ from the expected sex ratio. In comparison to the control groups, the offspring of the ethanol-treated mothers displayed a significantly reduced body weight at birth (Table 1). With advancing age, the difference in body weight between the experimental and control males disappeared, whereas the body weight of the experimental females remained reduced up to adult age (Fig. 1).

Masculine Sexual Behavior and Lordotic Behavior in Males

Ejaculation was first observed in the experimental animals at a median age of 63 days (range: 46–>70) and in the control animals at a median age of 60 days (range: 48–>70) (NS); Mann-Whitney U-test. Seventy percent of the experimentals and 90% of the control males ejaculated before 70 days of age. The animals were tested for masculine sexual behavior at the age of 90 days. No group differences were observed in any of the behavioral components analyzed (Table 2). There were no group differences in the proportion of males displaying lordotic behavior (21% of the experimentals vs. 23% of the controls).

The Female Estrous Cycle

The median age of vaginal opening did not differ between the control and the ethanol-exposed females (Table 3). On the first day of observation, at 30 days of age, vaginal opening was observed in four of the experimental females and in three of the controls. No group differences were seen in the age of the animals at the first observed vaginal or behavioral estrus. The onset of regular vaginal estrous cycling was significantly delayed in the ethanol-exposed female offspring compared to the control females. The ethanol-

TABLE 1

NUMBER OF PUPS PER LITTER AND LENGTH OF GESTATION IN ETHANOL TREATED AND CONTROL MOTHERS (MEAN VALUES AND ±SD)

	Experimental	Control
Number of litters	13	14
Number of pups per litter		
Total	12.1 ± 2.1	10.3 ± 2.97
Sex ratio (proportion of males/litter)	0.52*	0.45
Body weight at birth (g)		
Females	5.2 ± 0.45‡	5.9 ± 0.45
Males	5.5 ± 0.51*	6.0 ± 0.57
Length of gestation (days)	23.1 ± 0.46	22.9 ± 0.48
Weight gain of mothers during treatment period (from Day 15–21 P.C.)	33.7 ± 17.43†	51.4 ± 13.45

Statistical analyses performed by Mann-Whitney U-test.
*$p<0.05$; †$p<0.01$; ‡$p<0.002$.

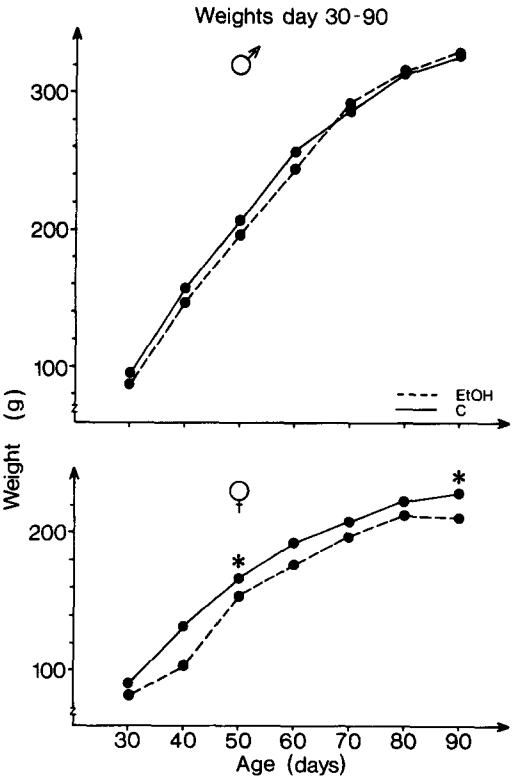

FIG. 1. Development of body weight in offspring of mothers treated with ethanol (6 g/kg/day) or 5.5% glucose solution from Day 15–21 P.C. *$p<0.05$.

TABLE 2

THE SEXUAL BEHAVIOR DISPLAYED BY PRENATALLY ETHANOL-EXPOSED RATS AND THEIR CONTROLS AT THE AGE OF 90 DAYS

	Experimental	Control
Total number of tested males	20	22
Number of animals performing at least one intromission	19	21
Number of animals showing ejaculation	14	18
Intromission latency (min)	0.3 (0.1–5.0)	0.5 (0.1–2.4)
Number of mounts before ejaculation	6 (1–23)	5 (1–19)
Number of intromissions before ejaculation	10 (4–26)	13 (7–17)
Ejaculation latency (min)	10.5 (3.2–15.7)	12.5 (2.8–15.4)
Postejaculatory interval (min)	5.3 (8.8–22.2)	4.1 (7.3–22.3)

The parameters of sexual behavior are based upon sexual performances of animals who displayed at least one intromission (median values and range). Statistical analyses performed by Mann-Whitney U-test.

TABLE 3

DEVELOPMENT OF ESTRUS IN FEMALES PRENATALLY EXPOSED TO ETHANOL AND CONTROL FEMALES

	Median Age in Days	
	Experimental Range	Control Range
Vaginal opening	33 (30–40)	32 (30–37)
First appearance of vaginal estrus	36 (33–45)	34 (30–45)
First appearance of behavioral estrus	44 (37–>70)	41 (36–>70)
Onset of regular vaginal estrous cycles	>70* (38–>70)	52 (36–>70)
Onset of regular behavioral estrous cycles	>70 (45–>70)	>70 (41–>70)
Prolonged duration of vaginal estrus (grand mean of mean number of disturbances/litter)	1.23†	0.67
Total number of tested females	19	23

Statistical analyses performed by Mann-Whitney U-test with the exception for the onset of regular vaginal estrous cycles which was analyzed by the Median test calculating probability in accordance with Fisher's procedure.

Statistically significant differences: *$p<0.05$, two-tailed; †$p<0.05$, one-tailed.

exposed females displayed more disturbances of the vaginal estrous cycles by an increased frequency of prolonged estrous episodes (duration: >1 day) as observed during a 25-day period after the appearance of the first estrus. No group differences were observed in the onset of regular behavioral estrous cycles. It should be noted that only a few animals in each group displayed regular behavioral estrous cycles before the end of the observational period. Obviously, the period of observation which ended when the animals were 70 days old was too short to detect possible group differences in the behavior.

Testosterone

Figure 2 shows the results of T measurements on fetuses of mothers treated with ethanol or glucose from Day 15 P.C. onwards. At all three ages tested, the T levels of both male groups were higher than the levels of the female groups. On Day 18 P.C. the T levels of the ethanol-treated male and female fetuses were elevated when compared to their respective control fetuses. No differences in T levels were observed on Day 17 or on Day 19, P.C.

In the experiment concerned with the acute treatment of pregnant rats with various doses of ethanol on Day 19 P.C., the T levels for each dose of ethanol were higher in the male fetuses than in the female fetuses. A slight, but not statistically significant, increase in T levels with increasing doses of ethanol was observed in the males (Fig. 3).

In the experiment involving the treatment of neonatal male pups with ethanol or saline immediately after birth, the T levels raised to a maximum two hours after birth and thereafter decreased again (Fig. 4). There were no detectable effects of ethanol treatment on T levels.

No statistically significant effects of ethanol treatment on T levels were found in the prepuberal males as can be seen from Fig. 5. In contrast, the T levels were decreased by the ethanol treatment in adult males.

DISCUSSION

Several reports testify to the depressive influence of ethanol on the T production of the adult male rat (7, 13, 14). Although administered in doses high enough to produce reduced plasma T concentrations in 90-day-old males, ethanol still did not diminish T concentrations of fetal animals. Even an acute dose of 6 g/kg ethanol, which is nearly lethal, was without effect on the T levels. Neither was any depressive influence on T secretion seen in

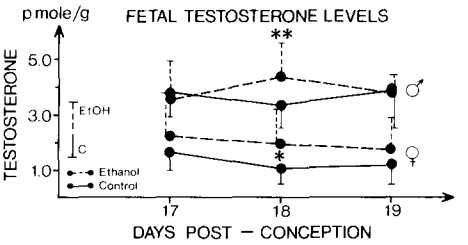

FIG. 2. Testosterone levels in male and female fetuses at indicated ages P.C. The mothers were treated with ethanol (6 g/kg/day) or 5.5% glucose solution from Day 15 P.C. onwards. Mean values and SD are shown. *$p<0.05$; **$p<0.02$.

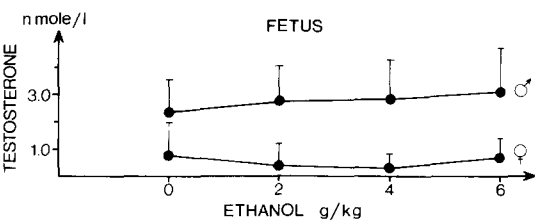

FIG. 3. Effect of treatment of mothers with various doses of ethanol on Day 19 P.C. on plasma testosterone levels of male and female fetuses. Mean values and SD are shown.

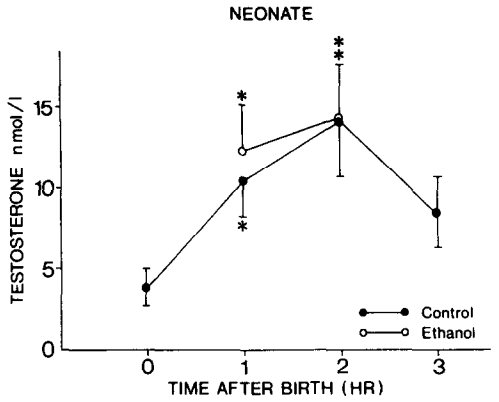

FIG. 4. Plasma testosterone levels in neonatal male rats treated with ethanol, 3 g/kg, or 0.9% saline immediately after birth and decapitated for blood sampling at indicated points of time in hours after birth. Mean values and S.E.M. are shown. $^{**}p<0.01$; $^{*}p<0.05$ compared to controls decapitated immediately after birth.

neonatal or prepubertal rats treated with ethanol.

Both the fetal male and female responded to chronic ethanol administration with significantly elevated T concentrations. In the males, elevated T levels were observed only on Day 18 P.C. whereas T levels were elevated in the females in all age groups, but statistically significant on Day 18 P.C. only. In this context, it is interesting that signs of abnormalities in the female estrous cycle were observed. In line with previous findings (3), the onset of regular vaginal estrous cycling was delayed in the ethanol-treated rats, indicating disturbances in the neurohormonal control of the estrous cycle. Previous studies by Handa *et al.* (19) on the secretory pattern of luteinizing hormone (LH) in prenatally ethanol-exposed females are in line with this contention. The present findings of heightened T levels in the female fetus may be a cause of the delay in the onset of regular vaginal estrous cycling, since exposure to T of the female fetus or neonate may result in aberrations of the estrous cycle (1). Signs of masculinization in female fetuses prenatally exposed to ethanol were previously observed in behaviors characterized by sexual dimorphism like saccharin consumption and maze learning (24).

The T concentrations remained fairly constant throughout days 17 to 19 P.C., a critical period of neural sexual differentiation in this species. In female fetuses, T levels remained very low during this age period. These observations are consistent with results reported by Habert and Picon (18), who, however, found that the mean ratios of male to female T values were about 5. In our study, based on measurements of T in the whole body, the mean ratios were about 2, a value similar to that reported by Slob *et al.* (31). Weisz and Ward (37) reported that the period during which plasma T levels in males were unequivocally higher than in their female littermates was restricted to day 18 P.C. In view of this result, they postulated that, on day 18 P.C. a priming event must sensitize target cells of the brain to subsequent stimulation by testosterone. In the present data, clearcut sex differences in T levels were observed throughout the entire period of gestation studied supporting the view of Habert and Picon (18) that priming on day 18 P.C. is not required for sexual differentiation in male direction. The discrepancies between various reports on the developmental pattern of T secretion during fetal age may reflect strain differences as suggested by Habert and Picon (18). In this context it may be of interest that the Wistar rats bought from Möllegaard Breed Laboratories seem to contain a relatively high proportion of males displaying lordotic reaction when mounted by a stud male (32).

In contrast to present results, McGivern *et al.* (25) recently reported evidence for a depressive influence of ethanol exposure on fetal T levels. In male fetuses of Sprague-Dawley mothers consuming a liquid alcohol diet from Day 14 P.C., McGivern *et al.* (ibid.) noted an absence of the surge of T characterizing normal controls on Day 18 P.C. These workers found that the depressive influence of the chronic ethanol on T levels was restricted to Days 18 and 19 P.C., whereas the treatment was without effect on Days 17 and 20 P.C. The absence of a T peak in normal fetuses in our study renders a detailed comparison between the two studies difficult. Besides the use of different strains, the most apparent difference is the amount of ethanol administered to the mothers (6 g/kg/day) in the present study versus about 14 g/kg/day in the study of McGivern *et al.* (ibid.). In the present study, the choice of dose regimen, 2 g/kg administered three times per day, was dictated by the fact that previous studies on adult rats indicate that 2 g/kg ethanol reliably lowers T levels (7, 13, 14). The amount of alcohol consumed by the pregnant females in the study of McGivern *et al.*; 14 g/kg/day, exerts toxic effects on the morpho-

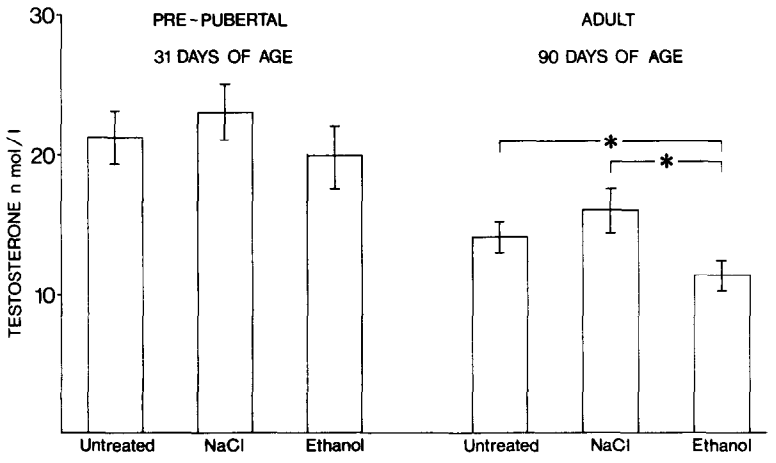

FIG. 5. Plasma testosterone levels in groups of prepubertal male rats, 30 days old, and adult male rats, 90 days old, treated with ethanol, 2 g/kg, or 0.9% saline compared to untreated controls. Two hours after treatment the animals were decapitated for blood sampling. Mean values and S.E.M. are shown. $^{*}p<0.05$.

logical development of the fetal testes and also on the development of the sexually dimorphic nucleus of the preoptic area of the hypothalamus (2). McGivern *et al.* suggest that the high ethanol exposure from Day 14 P.C. also adversely affects the development of testicular receptors for luteinizing hormone. Support for this notion was afforded by in vitro experiments on testes from fetuses on Day 18 P.C., exposed to ethanol from Day 14 P.C., which showed decreased responsiveness to treatment with LH compared to controls previously not exposed to ethanol. For the present study, it is of interest that acute ethanol treatment of normal testes caused an initial increase of T levels which subsided within half an hour. This observation supports the main finding of the present study that acute ethanol treatment of young rats does not induce the decrease of T levels normally observed in adult rats.

The present results demonstrate a peak concentration of testosterone immediately after birth, followed by a fall within a couple of hours. This observation is consistent with the report of Corbier *et al.* (9,10) of high plasma T levels up to 2 hours after birth followed by a fall to minimal levels 4 hours after birth. Castration immediately after birth, before the emergence of the postnatal T peak, resulted in a marked increase in the feminine sexual behavior of the male. Corbier *et al.* (11), therefore, proposed that the transient peak of T secretion seen immediately after birth in the normal male rat might suppress the development of neural tissues mediating feminine sexual behavior.

The origin of T in the fetal and neonatal rat is not clearly elucidated. Steroidogenic cells are detected in the rat testes and measurable amounts of T are produced from day 15 of gestation (15,18). Histochemical and biochemical evidence of steroidogenic activities in ovaries in contrast to the testes do no become detectable until around the end of the first postnatal week (27, 28, 30, 35, 36). Consequently, the ovaries are unlikely to contribute significantly to the high levels of T in female fetuses. This suggests that the male and female fetuses may derive T from different sources. Diffusion of T from male fetuses into the circulation of female fetuses is a possibility (8). A third source of T is the mother. The pregnant rat produces high amounts of T, particularly between days 18 and 20 of pregnancy (5, 16, 37, 38). Sources of androgen production in the pregnant mother are the placenta (6,33), the ovaries and the adrenals (22).

Originally, this study was aimed at examining the hypothesis that sexual deviations after prenatal ethanol exposure, reported in studies from this and other laboratories, were related to depression of the T levels. Under the conditions of ethanol exposure used in this study, no sexual abnormalities were seen in the behavior of the males, which were prenatally exposed to ethanol. In several respects, however, the procedures of ethanol administration differed in present and previous studies. Previously, we administered ethanol to the mother through the drinking water, while in this study, it was given by gavage. The amount of ethanol in this study, 6 g/kg/day, was estimated to be about 20 percent lower than the amount of ethanol used in our previous study. Furthermore, in our previous study, the mother received ethanol not only during the entire period of pregnancy but from the age of her own weaning. In the present study, focusing on the prenatal period of sexual differentiation the mother received ethanol only during the last trimester of pregnancy. Meyer and Riley (26) and McGivern *et al.* (24) administered ethanol by liquid feeding during the entire period of pregnancy. They estimated the amount of ethanol administered to the mother to be about 12–14 g/kg/day, and found signs of feminization in juvenile fighting and in the consumption of sweet solutions exhibited by male offspring prenatally exposed to ethanol.

Both control and ethanol-treated rats showed a relatively high lordosis quotient compared to the untreated controls used in previous studies in this laboratory. In the present study, the lordosis quotient may be due to stress associated with the administration of ethanol by gavage, since stress of the pregnant mother facilitates the elicitation of lordotic behavior in male offspring (12).

Under present regimen of ethanol administration no aberrations were seen with respect to lordotic reactions in male offspring. On the other hand, the estrous cycle showed disturbances suggesting that this feature of sexual function is more vulnerable to the toxic effects of ethanol. Generalizing to the human, this suggests that the menstrual cycle may be particularly sensitive to the influence of prenatal ethanol exposition. In fact, a delay in onset of menstrual cycling of girls of alcoholic mothers was reported by Robe *et al.* (29).

SUMMARY

Acute treatment with ethanol in amounts that decrease plasma testosterone levels of adult male rats did not significantly alter testosterone concentrations in prenatal, neonatal or prepubertal males, suggesting a factor associated with maturation that changes the responsiveness of the endocrine system to ethanol.

Chronic treatment with ethanol, 6 g/kg/day, during the last trimester of gestation caused an elevation of plasma testosterone levels in both male and female fetuses, the cause of which cannot be explained at present.

In adulthood, females prenatally exposed to chronic ethanol treatment showed aberrant estrous cycles presumably associated with heightened testosterone levels during fetal life.

No signs of feminization of the male offspring were seen under present conditions of ethanol treatment.

The present observations indicate a much more complicated picture of the effects of prenatal ethanol exposure on the sexual behavioral and endocrine development than was speculated upon on the basis of previous findings.

ACKNOWLEDGEMENTS

This study was supported by the Bank of Sweden Tercentenary Foundation, Swedish Council for Planning and Coordination of Research, Swedish Medical Research Council (Project No. B85-21X-07202), Swedish Council for Research in the Humanities and Social Sciences, Magnus Bergvall's Foundation, Clas Groschinsky's Memorial Foundation and Wilhelm and Martina Lundgren's Foundation and Anna Ahrenberg's Fund. Johan van der Linde is acknowledged for corrections of style and Madeleine Kröning for the drawings.

REFERENCES

1. Barraclough, C. A. Modifications in reproductive function after exposure to hormones during the prenatal and early postnatal period. In: Martini, L.; Ganong, W. F., eds. Neuroendocrinology. vol. II. New York: Academic Press; 1967:61–99.
2. Barron, S.; Bliss Tieman, S.; Riley, E. P. Effects of prenatal alcohol exposure on the sexually dimorphic nucleus of the preoptic area of the hypothalamus in male and female rats. Alcohol.: Clin. Exp. Res. 12:59–64; 1988.
3. Boggan, S.; Randall, C. L.; Dodds, H. M. Delayed sexual maturation in female C57BL/6J mice prenatally exposed to alcohol. Res. Commun. Chem. Pathol. Pharmacol. 23:117–125; 1979.
4. Bradley, J. W. Distribution-free statistical tests. London: Prentice Hall; 1968.
5. Bridges, R. S.; Todd, R. B.; Logue, C. M. Serum concentrations of testosterone throughout pregnancy in rats. J. Endocrinol. 94:21–27; 1982.
6. Chan, S. W. C.; Leathem, J. H. Placental steroidogenesis in the rat: comparison of normal and giant placentae. Endocrinology 100:

1418–1422; 1977.
7. Cicero, T. J.; Badger, T. M. Effects of alcohol on the hypothalamic-pituitary-gonadal axis in the male rat. J. Pharmacol. Exp. Ther. 201:427–433; 1977.
8. Clemens, L. G.; Gladué, B. A.; Coniglio, L. P. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Horm. Behav. 10:40–53; 1978.
9. Corbier, P.; Kerdelhue, B.; Picon, R.; Roffi, J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology 103:1985–1991; 1978.
10. Corbier, P.; Rhoda, J.; Kerdelhue, B.; Roffi, J. Augmentation de la testosterone endogene dans l'hypotalamus du rat male a la naissance. C. R. Acad. Sci. (Paris) 292:413–416; 1981.
11. Corbier, P.; Roffi, J.; Rhoda, J. Female sexual behavior in male rats: Effect of hour of castration at birth. Physiol. Behav. 30:613–616; 1983.
12. Dahlöf, L. G.; Hård, E.; Larsson, K. Influence of maternal stress on offspring sexual behaviour. Anim. Behav. 25:958–963; 1977.
13. Ellingboe, J.; Varanelli, C. C. Ethanol inhibits testosterone biosynthesis by direct action on Leydig cells. Res. Commun. Chem. Pathol. Pharmacol. 24:87–102; 1979.
14. Eriksson, C. J. P.; Widenius, T. V.; Ylikahri, R. H.; Härkönen, M.; Leinonen, P. Inhibition of testosterone biosynthesis by ethanol. Relation to hepatic and testicular acetaldehyde, ketone bodies and cytosolic redox-state in rats. Biochem. J. 210:29–36; 1983.
15. Feldman, S. C.; Bloch, E. Developmental pattern of testosterone synthesis by fetal rat testes in response to luteinizing hormone. Endocrinology 102:999–1007; 1978.
16. Gibori, G.; Chatterton, R. T., Jr.; Chien, J. L. Ovarian and serum concentrations of androgen throughout pregnancy in the rat. Biol. Reprod. 21:53–56; 1979.
17. Götz, F.; Dörner, G. Sex hormone-dependent brain maturation and sexual behaviour in rats. Endokrinologie 68:275–282; 1976.
18. Habert, R.; Picon, R. Testosterone, dihydrotestosterone and estradiol-17-beta-levels in maternal and fetal plasma and in fetal testes in the rat. J. Steroid. Biochem. 21:193–198; 1984.
19. Handa, R. J.; McGivern, R. F.; Noble, E. S. P.; Gorski, R. A. Exposure to alcohol in utero alters the adult patterns of luteinizing hormone secretion in male and female rats. Life Sci. 37:1683–1690; 1985.
20. Hård, E.; Dahlgren, I. L.; Engel, J.; Larsson, K.; Liljequist, S.; Lindh, A-S.; Musi, B. Development of sexual behavior in prenatally ethanol-exposed rats. Drug Alcohol Depend. 14:51–61; 1984.
21. Ismail, A. A.; Niswender, G. D.; Midgley, A. R., Jr. Radioimmunoassay of testosterone without chromatography. J. Clin. Endocrinol. Metab. 34:177–184; 1972.
22. Jost, A. The fetal adrenal cortex. In: Handbook of physiology. sect. 7. vol. VI. Washington, DC; 1975:107–115. (Am. Physiol. Soc.)
23. Jost, A. Sexual organogenesis. In: Adler, N.; Pfaff, D.; Goy, R. W., eds. Handbook of behavioral neurobiology. vol. 7. Reproduction. New York: Plenum Press; 1985:5–19.
24. McGivern, R. F.; Clancy, A. N.; Hill, M. A.; Noble, E. P. Prenatal alcohol exposure alters adult expression of sexually dimorphic behavior in the rat. Science 224:896–898; 1984.
25. McGivern, R. F.; Raum, W. J.; Salido, E.; Redei, E. Lack of prenatal testosterone surge in fetal rats exposed to alcohol: alterations in testicular morphology and physiology. Alcoholism 12:243–247; 1988.
26. Meyer, L. S.; Riley, E. P. Social play in juvenile rats prenatally exposed to alcohol. Teratology 34:1–7; 1986.
27. Noumura, T.; Weisz, J.; Lloyd, C. W. In vitro conversion of 7-^{3}H-progesterone to androgens by the rat testis during the second half of fetal life. Endocrinology 78:245–253; 1966.
28. Quattropani, S. L.; Weisz, J. Conversion of progesterone to estrone and estradiol in vitro by the ovary of the infantile rat in relation to the development of interstitial tissue. Endocrinology 93:1269–1276; 1973.
29. Robe, L. B.; Robe, R. S.; Wilson, A. Maternal heavy drinking related to delayed onset of daughters' menstruation. Alcohol.: Clin. Exp. Res. 3:192; 1979.
30. Schlegel, R. J.; Farias, E.; Russo, N. C.; Moore, J. R.; Gardner, L. I. Structural changes in the fetal gonads and gonaducts during maturation of an enzyme, steroid 3-beta-ol-dehydrogenase, in the gonads, adrenal cortex and placenta of fetal rats. Endocrinology 81:565–572; 1967.
31. Slob, A. K.; Ooms, M. P.; Vreeburg, J. T. M. Prenatal and early postnatal sex differences in plasma and gonadal testosterone and plasma luteinizing hormone in female and male rats. J. Endocrinol. 87:81–87; 1980.
32. Södersten, P.; Larsson, K. Lordosis behavior and mounting behavior in male rats: effects of castration and treatment with estradiol benzoate or testosterone propionate. Physiol. Behav. 14:159–164; 1975.
33. Sridaran, R.; Basuray, R.; Gibori, G. Source and regulation of testosterone secretion in pregnant and pseudopregnant rats. Endocrinology 108:855–861; 1981.
34. Ward, I. L.; Ward, O. B. Sexual behavior differentiation: effects of prenatal manipulation in rats. In: Adler, N.; Pfaff, D.; Goy, R. W., eds. Handbook of behavioral neurobiology. vol. 7. Reproduction. New York: Plenum Press; 1985:77–98.
35. Warren, D. W.; Haltmeyer, G. C.; Eik-Nes, K. B. Testosterone in the fetal rat testis. Biol. Reprod. 8:560–565; 1973.
36. Warren, D. W; Haltmeyer, G. C.; Eik-nes, K. B. The effect of gonadotrophins on the fetal and neonatal rat testis. Endocrinology 96:1226–1229; 1975.
37. Weisz, J.; Ward, I. L. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306–316; 1980.
38. Weizenbaum, F. A.; Adler, N. T.; Ganjam, V. K. Serum testosterone concentrations in the pregnant rat. J. Steroid Biochem. 10:71–74; 1979.