Prenatal Alcohol and Stress Interact to Attenuate Ejaculatory Behavior, but Not Serum Testosterone or LH in Adult Male Rats

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Restraint stress reduced blood alcohol levels in pregnant rats given a liquid alcohol diet. The male offspring prenatally exposed to both stress and alcohol failed to ejaculate spontaneously, although they copulated normally following exogenous testosterone (T) administration. Males prenatally exposed only to alcohol or only to stress showed no behavioral deficits. Adult serum T and luteinizing hormone levels were normal in both of the fetal alcohol exposed male groups. It appears that the androgen threshold for ejaculatory behavior is elevated in males prenatally exposed to alcohol plus stress and cannot be realized with normal testosterone titers, but it can be attained with exogenous hormone administration. Presumably the alcohol and stress combination interfered with ontogenetic patterns of T needed to fully masculinize the fetal nervous system.

To display normal adult sexual behaviors, males must have had exposure to adequate amounts of androgen at specific stages of perinatal development (see review by I. L. Ward & Ward, 1985). As a result, factors that alter fetal or neonatal testicular steroidogenesis may have consequences for sexually dimorphic behaviors at later stages of life. Our laboratory has investigated the impact of two prenatal factors, maternal stress (see review by I. L. Ward, 1992) and ethanol, a commonly used and widely abused recreational drug.

The male offspring of pregnant rats exposed to ethanol, to stress, or both had a markedly enhanced potential to display lordosis, a component of the female sexual behavior pattern (I. L. Ward, Ward, Winn, & Bielawski, 1994). The effect of these treatments on male copulatory behaviors was less clear. Male rats prenatally exposed to a combination of alcohol and stress were significantly less likely to ejaculate than control rats. However, in that study, stress alone had no significant effect, and alcohol alone led only to a marginally significant deficit in male copulatory behavior.

The present study (a) reassessed the effects of fetal exposure to alcohol or the combination of stress and alcohol on the development of male copulatory patterns and (b) determined whether any deficiencies that occurred were linked to androgen levels in adulthood. We also measured blood alcohol levels (BAL) on various days of gestation in stressed and nonstressed pregnant rats fed the liquid alcohol diet in use throughout this study.

Experiment 1: Adult Copulatory Potential

Method

Subjects. Sprague-Dawley rats (Harlan Sprague Dawley Inc., Madison, WI) obtained at 45–50 days of age were allowed to adapt for at least 2 weeks before being bred. The temperature-controlled (21 °C) vivaria were maintained on a reverse 12-hr light–dark cycle (lights off at 0700). Except when fed a liquid diet, all rats received ad libitum water and Purina Rat Chow (#5012; Ralston-Purina, St. Louis, MO).

Apparatus and general procedures. Females were mated between 1300 and 1600 hr, by placing them with males until two ejaculations were received (Day 0 of gestation). All experimental dams were maintained on one of two nutritionally balanced liquid diets from Days 10–21 of pregnancy. The first diet contained 5% wt/vol of ethanol (Diet #1265; Bio-Serv Inc., Frenchtown, NJ). This diet derives 36% of its calories from alcohol. The control diet was identical except the ethanol was replaced by an isocaloric amount of maltose dextrin (Diet #1264; Bio-Serv). Liquid diets were presented in drinking tubes that were weighed, cleaned, freshly filled, and reweighed at the same time daily. Water and lab chow were not available when liquid diets were fed. Dams receiving ethanol were maintained in a separate vivarium so that the remaining rats were not exposed to alcohol fumes. Foster mothers were housed in Plexiglas cages (45 × 24 × 20 cm), and experimental dams were placed into single wire mesh cages (24.5 × 17.5 × 17.5 cm). Shredded paper was provided as nesting material on Day 21 of pregnancy.

The stress apparatus consisted of 13 × 5 × 8.3-cm Plexiglas animal holders (A. H. Thomas No. 1123-C30). Two 150-W floodlights delivered approximately 2,150 lm/m² of white light over the surface of the restrainers. The stress treatment was given three times daily (at 0900, 1300, and 1700 hr) for 45 min from Days 14–21 of pregnancy.

Mating and behavioral testing occurred in 27 × 33 × 52-cm
semicircular boxes with Plexiglas fronts located in a room dimly illuminated by red light. Sexual responses were scored using a Datamyte 900 recorder (Electro General, Minnetonka, MN).

All steroids were dissolved in cottonseed oil and were injected intramuscularly.

**Design and behavioral testing procedures.** Two sets of 27 females were pair mated within 48 hr of one another. One set served as foster mothers to the litters of the second. The experimental dams were randomly assigned to the following treatment conditions: (a) ad libitum access to control diet (NC group; \( n = 5 \)); (b) ad libitum access to alcohol diet (NA group; \( n = 6 \)); (c) ad libitum access to control diet while receiving stress treatment (SC group; \( n = 5 \)); and (d) ad libitum access to alcohol diet while receiving stress treatment (SA group; \( n = 5 \)). A yoked control group was included to gauge potential effects that alcohol might exert by decreasing the caloric intake of the dams. Each female in this latter group \( (n = 6) \) was given an amount of control diet calorically matched to that spontaneously consumed daily by individual rats in the alcohol or in the alcohol plus stress groups. The intake of pregnant rats placed on the alcohol diet are equivalent, regardless of whether or not the rats are being stressed (see Figure 1; I. L. Ward et al., 1994), precluding the need for separate yoked control groups. Nevertheless, these rats were yoked to an equal number of NA and SA dams. Mothers were weighed on Days 0, 10, 12, 14, 16, 18, and 20 of pregnancy.

All litters were fostered to untreated chow-fed dams within 6 hr of birth, and 2 male pups from each litter were weighed. The litters were not culled, because there are no significant treatment differences in litter size (I. L. Ward et al., 1994). At 21 days of age, the males were weaned, were weighed, and were housed in triads of the same treatment. Each treatment group contained a total of 18 gonadally intact males.

Tests for sexual behavior under endogenous hormone conditions were given twice weekly beginning when the rats were about 70 days old. We began each test by placing an experimental male with a stud male. After 10 min, the experimental male was moved to the arena of a second stud male for 10 min more. The number of mounts received by the experimental male and any lordosis responses exhibited were recorded.

At the end of each test for lordosis behavior, we determined the ability of each rat to emit the male copulatory pattern by placing each experimental rat with an estrous female for 30 min. The females had been ovariectomized and were made receptive by successive injections of 10 μg estradiol benzoate and 500 μg progesterone given 48 and 6 hr, respectively, before each test. During each test, the occurrence of mounts, intromissions, and ejaculations directed toward the female were recorded, as well as the latency to begin to copulate and to ejaculate and the interval between ejaculation and the first mount of the next copulatory sequence (i.e., postejaculatory interval [PEI]).

After the first 2 weeks (four tests), testing was suspended for 2 weeks and daily injections of 1.0 mg of testosterone propionate (TP) were initiated. Thereafter, biweekly testing was resumed for an additional 3 weeks (six tests) during which TP continued to be injected daily. Consecutive tests for male copulatory behavior were given until an ejaculatory response was exhibited. Once a male ejaculated, no further tests with females were given. Each male was weighed after the last behavioral test.

**Statistics.** Parametric data of the NC, NA, SC, and SA groups (e.g., body weight, diet consumed, litter size, day of parturition, and temporal and quantitative parameters characterizing the test on which ejaculation first occurred) were analyzed using analysis of variance (ANOVA). Significant factors were probed with Tukey tests. Whenever an alcohol-treated group differed from the NC group, a further comparison was made with the yoked control group so that the contribution of maternal caloric intake could be assessed. Chi-square tests were used to analyze differences among the various treatment groups in the proportion of males exhibiting male copulatory behavior patterns. Fisher exact probability tests were used to probe significant chi-square tests.

**Results**

**Intake and weight of dams.** The mean amount of control or alcohol diet consumed by stressed and nonstressed mothers is shown in Figure 1. For the purpose of statistical analysis, gestation was separated into a prestress (Days 11–13) and stress (Days 14–21) period. A 2 (prestress or stress time period) × 2 (alcohol or control diet) × 2 (stress or nonstress treatment) ANOVA revealed a significant triple interaction. \( F(1, 17) = 8.74, p < .007 \). During the prestress period, dams receiving the control diet consumed more than the alcohol diet groups, \( F(1, 17) = 59.38, p < .0001 \). During the stress period, the simple interaction between stress and diet was significant, \( F(1, 17) = 4.17, p < .05 \). The control group (NC) consumed more than the SC (\( p < .05 \)), NA (\( p < .05 \)), or SA (\( p < .01 \)) mothers. There were no significant differences among the SC, NA, and SA groups during the stress period.

The body weights of the four groups of dams during pregnancy are shown in Figure 2. There were no significant differences among the groups on Day 0 or on Day 10 of pregnancy. A 2 (Day 12 prestress or Days 14–20 stress period) × 2 (stress or nonstress treatment) × 2 (alcohol or control diet) ANOVA yielded a significant effect for diet, \( F(1, 17) = 17.29, p < .001 \), and for Treatment × Day, \( F(1,
Figure 2. Mean (± SEM) body weight (in grams) of mothers from the day of impregnation (Day 0 of gestation) through Day 20 of pregnancy.

ANOVA revealed no significant differences among the treatment groups in the day of pregnancy when parturition occurred (Day 22) or in the number of pups in the litters at birth (data not shown). The alcohol groups did not differ from the yoked comparison group on these measures.

Neonatal and weaning measures. Birth weight and the mean body weight for the 18 males in each group at the time of weaning and in adulthood are shown in Table 1. There was a significant interaction between diet and stress at birth, $F(1, 38) = 13.75, p < .0007$, weaning, $F(1, 68) = 30.91, p < .0001$, and at the conclusion of behavioral testing, $F(1, 68) = 13.73, p < .0004$. Probing revealed the SC, NA, and yoked control rats weighed less than the NC males at all stages ($p < .01$). SA males did not differ from NC males at any stage and, at weaning, SA males weighed more than SC and NA males ($p < .01$). No other comparisons among the experimental groups were significant. Neither the NA nor the SA groups differed in weight from the caloric control group with the one exception that at weaning SA males weighed more than the yoked comparison males, $t(17) = 2.84, p < .05$.

Sexual behavior. The proportion of males that ejaculated spontaneously on one of the four tests given before TP treatment is shown in Figure 3. A significant chi-square test ($p < .005$) was followed by Fisher exact probability tests. These revealed that a smaller proportion of SA males ejaculated compared to the NC ($p < .01$), the SC ($p < .01$), or the isocaloric control group ($p < .03$). No other group differences were significant. While most (86%) of the males that failed to ejaculate did not initiate a copulatory sequence, 1 NC and 1 NA male that did not ejaculate did display four intromissions on one of the tests. Two males in the isocaloric control group and 1 each in the NA, SC, and SA groups emitted a few (five to eight) mounting responses but no intromissions. To be considered a copulator, a male had to exhibit a total of at least four mounts during the four tests. A chi-square test showed a significant difference ($p < .005$) among the proportion of copulators in the two control groups (61% each) and the SC (78%), NA (50%), and SA (17%) groups. Fisher tests indicated the SA group to be significantly different from the stress group ($p < .0003$) and the two control groups ($p < .008$). No other differences in the proportion copulating were significant.

The results of the tests following adult TP treatment can be seen in the inset in the upper right of Figure 3. An increased percentage of males ($p < .003$) ejaculated during exogenous androgen treatment in all groups except the SC group. As a result, there no longer were any significant group differences in the percentage of males that had ejaculated when the 5 weeks of daily TP injections had been completed.

For males able to ejaculate, various performance parameters associated with the test on which the ejaculatory sequence was observed are shown in Table 2. There were no significant differences among the NC, SC, NA, or SA groups in latency to the first mount, duration of the copulatory sequence during which ejaculation occurred, the length of

### Table 1

<table>
<thead>
<tr>
<th>Maternal treatment</th>
<th>Birth weight (g)</th>
<th>Weaning weight (g)</th>
<th>Adult weight (g)</th>
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<tr>
<td></td>
<td>$n$</td>
<td>$M$</td>
<td>$SE$</td>
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<tr>
<td>Control</td>
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<td>5.78</td>
<td>0.2</td>
</tr>
<tr>
<td>Stress</td>
<td>10</td>
<td>4.88*</td>
<td>0.1</td>
</tr>
<tr>
<td>Alcohol</td>
<td>12</td>
<td>5.20*</td>
<td>0.2</td>
</tr>
<tr>
<td>Alcohol plus stress</td>
<td>12</td>
<td>5.38</td>
<td>0.14</td>
</tr>
<tr>
<td>Yoked control</td>
<td>12</td>
<td>5.21</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*Significantly different from control group, $p < .01$. 

17) = 28.22, $p < .0002$. On Day 12, there was a significant effect of diet, $F(1, 17) = 17.113, p < .001$, with dams consuming alcohol weighing less than dams consuming the control diet. On Days 14–20, there were significant effects of treatment, $F(1, 17) = 18.80, p < .001$, and diet, $F(1, 17) = 14.44, p < .002$. The NA, SC, and SA groups did not differ from one another during the stress period, but all weighed less than the NC group. Both the NA, $t(4) = 3.19, p < .05$, and the SA groups, $t(4) = 13.86, p < .01$, weighed less than the yoked control group (data not shown), which did not differ from the ad libitum control group.
Figure 3. Percentage of males that displayed the ejaculatory pattern at least once when tested under the influence of testicular androgen (endogenous androgen) or following prolonged treatment with testosterone propionate (exogenous androgen, inset panel). Asterisk denotes significantly different from the ad libitum food, the isocaloric control, and the stress groups.

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of tests</th>
<th>Mount latency (s)</th>
<th>Sequence length (s)</th>
<th>PEI (s)</th>
<th>Mean mounts</th>
<th>Mean intromissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>4.2</td>
<td>262</td>
<td>779</td>
<td>355</td>
<td>28.6</td>
</tr>
<tr>
<td>Stress</td>
<td>17</td>
<td>2.7</td>
<td>232</td>
<td>862</td>
<td>374</td>
<td>18.2</td>
</tr>
<tr>
<td>Alcohol</td>
<td>14</td>
<td>4.1</td>
<td>205</td>
<td>623</td>
<td>336</td>
<td>13.6*</td>
</tr>
<tr>
<td>Alcohol plus stress</td>
<td>18</td>
<td>5.5</td>
<td>283</td>
<td>517</td>
<td>345</td>
<td>19.8</td>
</tr>
<tr>
<td>Yoked control</td>
<td>18</td>
<td>3.6</td>
<td>290</td>
<td>549</td>
<td>325</td>
<td>19.1</td>
</tr>
</tbody>
</table>

*Significantly different from both the control and yoked control groups, p < .05.
replicate previous observations that prenatally stressed males exhibit impaired ejaculatory potentials in adulthood (I. L. Ward, 1972, 1977, 1992; I. L. Ward & Reed, 1985). A normal incidence of ejaculation also occurred in prenatally stressed males observed in I. L. Ward et al. (1994). However, in this latter study, high levels of lordotic behavior were obtained when the males were treated with estrogen and progesterone. There are several methodological features that distinguish the two most recent prenatal stress studies from previous work conducted in our laboratory. First, the dams were maintained on the Bio-Serv liquid diet, which differs in taste, consistency, texture, and caloric content from the previously used diet of Purina Rat Chow. The voluntary caloric intake of stressed dams maintained on the liquid diet has never been compared with that of stressed dams given chow. Second, all litters in the recent work were fostered to nongravid controls at birth. In nature, fostering rarely occurs, but it had to be incorporated into our recent research designs to control for potential deleterious effects that alcohol exposure during pregnancy has on the lactation competence of the mothers. Whether any of these factors alone or in combination contributed to the different outcomes is not presently clear. The attenuation in fetal testosterone levels induced by prenatal stress alone (I. L. Ward & Weisz, 1980, 1984), or alcohol exposure alone (McGivern, Raum, Salido, & Redei, 1988), appears to be sufficient to reliably increase the potential for lordosis in male rats (I. L. Ward et al., 1994). However, a reliable suppression of the ejaculatory potential occurred only when males were exposed prenatally to both stress and alcohol.

The food intake of alcohol-treated dams was considerably lower than that of the control group. However, deficiencies in sexual behaviors shown by the male offspring cannot easily be attributed to early undernutrition, because copulatory patterns displayed by the calorically yoked control group were normal. Also, caloric intake of stressed alcohol-consuming mothers was equivalent to that of nonstressed alcohol-consuming mothers, but only the male offspring of the former showed significant behavioral deficits. Furthermore, there was no relationship between the effects of the prenatal treatments on copulatory behavior and the body weights of the male offspring. Although the offspring of the dams exposed only to alcohol or to stress showed normal ejaculatory potentials, they weighed less than controls at all life stages sampled, that is, birth, weaning, and adulthood. Control rats and rats exposed to alcohol combined with stress had similar body weights, but the SA males did not copulate spontaneously.

Experiment 2: Adult Blood Levels of Testosterone and Luteinizing Hormone

The finding that the impairment in sexual behavior exhibited by male rats prenatally exposed to both alcohol and stress can be reversed by injecting TP suggested that the endogenous androgen levels of these rats might be abnormally low. To assess this possibility, we measured blood levels of testosterone and luteinizing hormone (LH) in a set of adult males at the same age that behavioral testing had occurred in Experiment 1.

Method

Except where indicated, all experimental procedures were identical to those used in Experiment 1. Male offspring from four dams in each of the following five groups were studied: (a) ad libitum control diet with no stress (n = 26 NC males), (b) prenatal stress only (n = 21 SC males), (c) prenatal alcohol alone (n = 14 NA males), (d) prenatal alcohol and stress combined (n = 13 SA males), and (e) nutritional control (n = 43 males). The amount of control diet made available was yoked to daily calories consumed by dams placed on the alcohol diet alone or on the alcohol diet in combination with the stress treatment. The male offspring were housed in triads of like sex and treatment from weaning (Day 21) to 90 days of age (blood sampling) and given ad libitum access to water and Purina Rat Chow.

At 90 days of age, the rats were brought individually into an experimental room, were quickly anesthetized with ketamine (80 mg/kg), and were decapitated with a guillotine. The blood was drained into a test tube and allowed to clot for 30 min. The blood sample was centrifuged for 15 min, and the serum was harvested and stored at -20 °C. Testosterone and LH levels were determined in the same samples using radioimmunoassay (RIA). Testosterone assays were conducted on 50-μl aliquots of unextracted serum. The antibody in the coated assay system (Bio-RAD Diagnostics Group, Hercules, CA) cross-reacted with 10-hydroxytestosterone (28.7%), methyltestosterone (15.1%), and 5-alpha-dihydroxytestosterone (6.6%). The interassay coefficient of variation, based on repeated measurement of testosterone in a pool of male rat serum, was 3.5% (n = 3 assays). The intra-assay coefficient of variation was 8.5% and was determined by measuring duplicate sample agreement.

LH concentrations were measured in duplicate 200-μl aliquots of serum. The antiserum (NIDDK-r-LH-S-11) and standard reference preparation (NIDDK-r-LH-RP-3) were elaborated by A. F. Parlow and made available through the National Hormone and Pituitary Program, the National Institute for Diabetes, Digestion, and Kidney Disorders, the National Institute for Child Health and Human Development, and the U.S. Department of Agriculture. 32P-3 3Iabeled rLH was acquired from Conring-Hazleton (Vienna, VA). Precipitating second antibody (goat anti-rabbit) was acquired from Antibodies Inc. (Davis, CA). The intra-assay coefficient of variation was 7.8%. Interassay variation based on repeated assay of a rat serum pool (n = 2) was 12.8%. There was insufficient serum to measure LH in one stress (SC) sample on which a testosterone value had been obtained.

Results

The testosterone levels found in the blood of the various treatment groups are presented in Figure 4. A one-way ANOVA was significant, F(4, 112) = 5.07, p < .0009. Newman-Keuls tests indicated testosterone levels in the stressed males to be higher (p < .05) than in the ad libitum control, the isocaloric control, and the alcohol plus stress groups. No other differences were significant.

ANOVA, F(4, 110) = 1.70, indicated that there were no significant differences in the mean (picogram per milliliter of serum) LH levels among the ad libitum control (604 ± 119), the yoked control (611 ± 162), the alcohol (660 ± 124), the stress (607 ± 133), and the alcohol plus stress (516 ± 184) groups.

Experiment 2: Adult Blood Levels of Testosterone and Luteinizing Hormone
Discussion

Prenatal exposure to alcohol or to alcohol combined with stress had no effect on adult blood levels of either testosterone or LH. Therefore, the deficient copulatory behaviors of males prenatally exposed to both alcohol and stress cannot be attributed to endogenous adult androgen levels that are too low to activate a normal sexual behavior potential. Rather, the threshold of activation of the neural mechanism that mediates the display of male sexual behavior patterns seems to have been modified in SA males. Only unnaturally high amounts of androgen, such as were administered in the first study, can activate the abnormally high threshold leading to the display of copulatory patterns. The nature of the biological mechanism reflected by this alteration in target tissue sensitivity is not known.

The high blood levels of testosterone found in males derived from stressed mothers maintained on the control diet was unexpected, but it is congruent with the readiness with which this group copulated in the first experiment. Thus, the possibility continues to exist that the CNS of stressed males requires stimulation with higher than normal plasma levels to activate the abnormally high threshold leading to the display of copulatory patterns. The nature of the biological mechanism reflected by this alteration in target tissue sensitivity is not known.

Experiment 3: Maternal Blood Alcohol Level

Male copulatory potentials of rats are severely suppressed by prenatal exposure to alcohol combined with stress but not by prenatal alcohol alone. The cumulative effect of stress and alcohol on sexual differentiation could still be attributed solely to alcohol if stress acted in some way to increase maternal BALs. The findings of Experiment 1 and of I. L. Ward et al. (1994) demonstrated that the stress procedure used does not alter the voluntary intake of ethanol delivered in a liquid diet. However, despite equal intake, BALs might still be higher if stress during pregnancy interfered with ethanol metabolism. The objective of Experiment 3 was to measure BALs in nonstressed and stressed dams maintained on a liquid alcohol diet on several days of pregnancy.

Method

NA and SA mothers were produced as detailed in Experiment 1. Seventy-three pregnant rats were placed on the Bio-Serv alcohol diet from Days 10–21 of gestation. From Days 14–21, 38 of these dams were stressed. Body weight was recorded every other day between days 10 and 20 of pregnancy.

Just before the second stress session on the day of sampling, females were lightly anesthetized with 20 mg ketamine hydrochloride and 3 mg xylazine. A 2- to 4-ml blood sample was drawn from the dorsal tail vein using a heparinized syringe. NA as well as SA mothers were sampled on Day 16, 17, 18, 19, or 20 of gestation. Only one sample was drawn from each rat. The number of mothers in each group were as follows: Day 16, n = 6 per group; Day 17, NA = 6 and SA = 7; Day 18, NA = 8 and SA = 9; Day 19, n = 8 per group; Day 20, NA = 7 and SA = 8.

To determine BAL (milligrams per deciliter), we assayed the blood samples using a standard diagnostic kit from Sigma Chemical Co. (St. Louis, MO; No. 332-A).

Results

The BALs obtained in stressed and nonstressed dams are presented in Figure 5. A two-way ANOVA, 2 (treatment) × 5 (days), yielded a significant main effect for treatment, F(1, 63) = 59.08, p < .0001. The rats receiving stress in combination with alcohol had BALs that were considerably lower than the rats in the alcohol alone group. Neither the days factor nor the interaction of days with treatment was significant.

The large group differences in BAL suggested that the stressed and nonstressed groups may have consumed different amounts of the alcohol diet. This possibility was assessed by computing the amount of alcohol consumed by each rat in relation to its weight (gram of diet × 5%/kg of body weight) during the 24-hr period preceding the time that the blood sample was drawn. These data are shown in Figure 6. A 2 (treatment) × 5 (days) ANOVA yielded no significant effects. However, there was a suggestion in Figure 6 that the stressed rats tended to consume slightly less ethanol than the nonstressed rats. Moreover, the correlation coefficients between BAL and the amount of diet consumed was .78 or higher on 3 of the 5 days for both treatment groups. To further probe the possibility that differences in intake may have contributed to treatment differences in BALs, we
Figure 5. Mean (±SEM) blood alcohol level (milligram per deciliter) on Days 16–20 of pregnancy of rats exposed to alcohol or to both alcohol and stress.

divided the BAL of each rat by ethanol intake per body weight. A two-way ANOVA revealed there still was a significant difference between the NA and SA treatments, \( F(1,63) = 27.79, p < .0001 \). Finally, to rule out any possible contribution of body weight to group differences, we compared the BAL of 12 pairs of NA and SA rats that had been matched for body weight (the group means were within 2 g of one another). This limited comparison indicated that while the groups did not differ either in body weight, \( t(22) = 0.471 \), or alcohol intake, \( t(22) = 0.236 \), the BAL of pregnant rats receiving both stress and alcohol (\( M = 52.95 \) mg/dl) was considerably lower, \( t(22) = 3.007, p < .007 \), than that of dams exposed to alcohol alone (\( M = 152.57 \) mg/dl).

Discussion

The BAL of rats was markedly decreased by exposure to a stressor consisting of restraint coupled with bright lights during Days 16–20 of pregnancy. These data on females are in general agreement with studies reporting the effects of stress on alcohol utilization in males. Alcohol clearance rates are more rapid in the blood of male rats housed in a cold room (2 to \(-5 °C\)) compared to normal (20 to 27 °C) temperatures (Forbes & Duncan, 1961; Platonow & Coldwell, 1966, 1968; Platonow, Coldwell, & Dugal, 1963; Videla, Flattery, Sellers, & Israel, 1975). Similarly, BALs were lowered in rats by a forced swimming procedure (Leikola, 1962) and in pigtailed macaques by exposure to a mild social stressor (Kalhorn, Bowden, & Slattery, 1986). These increased rates of ethanol metabolism seem to involve liver processes. The activity of hepatic alcohol dehydrogenase, the principal enzyme responsible for the oxidation of ethanol by the liver, was markedly elevated in male rats that had been repeatedly immobilized (Mezey, Potter, & Kvetcansky, 1979). An increased rate of ethanol metabolism is congruent with our finding that the BAL of stressed dams given alcohol was lower than that of dams not stressed, although both groups consumed similar amounts of alcohol.

Thus, the severe disruption of copulatory behaviors in males prenatally exposed to a combination of stress and alcohol is not due to higher maternal BALs than in those rats exposed to alcohol alone. In fact, BALs of SA dams on Days 18 and 19 of gestation were approximately half as high as those in nonstressed dams.

General Discussion

If developing male fetuses are exposed to environmental or pharmacological factors that inhibit normal gonadal functioning, the expression of homotypical adult sexual behaviors will be compromised. Stress (I. L. Ward, 1992) as well as several common recreational drugs (see review by O. B. Ward, 1992) have this capability, but few data exist in which potential interactions between these factors were sought. Both ethanol (McGivern et al., 1988) and stress (I. L. Ward & Weisz, 1980, 1984) block a species-specific surge in plasma testosterone that occurs during Days 18–19 of gestation in male rats (Weisz & Ward, 1980). The severe deficit in copulatory potentials found in most males prenatally exposed to a combination of alcohol and stress may reflect even more extensive abnormalities in the pattern of fetal and neonatal plasma androgen levels than result from either treatment alone. Currently, there are no data assessing steroidogenic function in fetal males simultaneously exposed to a combination of maternal treatments.

A variety of complex interactions are known to exist between ethanol and stress exposure that could contribute to the teratogenic effects that the combined treatment (SA group) had on sexual behavior differentiation. Both treatments decrease plasma testosterone levels when adminis-
tered to adult (Chung, 1990; Cicero & Badger, 1977; Mendelson, Mello, & Ellingboe, 1977; Rose et al., 1969; Van Thiel, Gavaler, Cobb, Sherin, & Lester, 1979) or fetal (McGivern et al., 1988; I. L. Ward & Weisz, 1980) males. However, as previously summarized, stress decreases BALs by increasing hepatic metabolism. Moreover, ethanol administration blunts the physiological impact of stress. For example, corticosterone levels in male rats exposed to electric shock are lower in rats that are first treated with ethanol than in shocked males that are not given alcohol (Brick & Pohorecky, 1982; Pohorecky, Rassi, Weiss, & Michalak, 1980). To uncover the probable mechanism through which the combination of stress and alcohol exposure leads to incompletely masculinized sexual behavior potentials in male rats, it will be necessary to assess testicular steroidogenesis throughout the perinatal period during which sexual differentiation is ongoing (Orth, Weisz, Ward, & Ward, 1983; Weisz & Ward, 1980; I. L. Ward & Weisz, 1980, 1984).

In rats, ethanol concentrations equilibrate fairly rapidly in fetal and maternal blood following oral consumption by the dam and are eliminated from both compartments primarily by maternal liver activity (Hayashi, Shimazaki, Kamata, Kakiichi, & Ikeda, 1991; Zorzano & Herrera, 1989). Interestingly, ethanol is cleared more rapidly from maternal and fetal blood than from the amniotic fluid (Hayashi et al., 1991). Thus, the fetus is exposed to alcohol retained in the amniotic fluid for much longer than would be predicted by knowing the BAL of the dam. It, therefore, becomes difficult to predict the extent to which gonadal function is differently affected by maternal ethanol ingestion in the male fetuses of stressed and nonstressed dams. Presumably, the combined treatment causes a greater disruption in the normal ontogenetic pattern of plasma testosterone than does exposure to either alcohol or stress alone.

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