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Effect of Prenatal Alcohol Exposure on Consumption of Alcohol and Alcohol-Induced Sleep Time in Mice

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RANDALL, C. L., S. S. HUGHES, C. K. WILLIAMS AND R. F. ANTON. *Effect of prenatal alcohol exposure on consumption of alcohol and alcohol-induced sleep time in mice.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 325–329, 1983.—An animal model was used to examine the effect of maternal alcohol administration on behaviors in the offspring which might predispose to alcoholism. Pregnant C3H mice were administered a liquid diet containing 28% ethanol-derived calories (EDC) from Gestation-Day 8 until parturition. Control animals were either pair-fed an isocaloric 0% EDC diet or received standard lab chow and water throughout pregnancy. Offspring were tested for sleep time following a challenge dose of 3.5 or 4.5 g/kg ethanol at 25 or 110 days of age or for consumption of 10% w/v ethanol in a two-bottle choice situation. The results demonstrated that prenatal exposure to alcohol did not affect alcohol-induced sleep time at either testing age or dose and that waking blood alcohol levels were similar across groups. Voluntary alcohol consumption, however, was higher in mice exposed to alcohol *in utero* during the initial week of testing but intake decreased to near control levels by the third week. Whether other alcohol-related behaviors are altered by prenatal alcohol exposure remains to be examined.

Prenatal alcohol C3H mice Sleep time Alcohol preference Alcohol and pregnancy

EVIDENCE accumulated from clinical studies over the last decade has strengthened the argument in favor of a genetic basis of alcoholism. Studies of Danish adoptees raised apart from their biologic parents demonstrated a higher risk of alcoholism if the biologic parent was an alcoholic [8,9]. Recently, these results have been corroborated in a sample of 1775 Swedish adoptees, 913 women and 862 men. There is evidence of a father-to-son [7] and mother-to-daughter [5] inheritance of alcoholism which, in some cases, is independent of the environment. In others, the expression of alcoholism is environmentally determined. Taken together, the adoptee studies argue strongly in favor of the heritability of alcoholism and emphasize that alcoholism in either parent is a risk factor. However, the specific nature of the genetically based predispositional factors is unknown.

Schuckit and his colleagues have begun a search for the biologic basis of alcoholism in male college students with and without a positive family history of alcoholism. Their studies have shown that family-history-positive men have higher blood acetaldehyde levels following alcohol consumption [17], report less intense intoxication with similar blood alcohol levels [14], score higher on the MacAndrew "alcoholism scale" [13], and have a lower muscle-tension response to ethanol [18] than family-history-negative men. No difference has been noted between the groups in alcohol absorption and elimination rates [15] or levels of assertiveness or trait anxiety [16]. Unfortunately, clinical research is limited by the inability to exercise control over possible confounding variables. Animal models afford an excellent alternative approach to study the issue of parental alcoholism and the offspring and they can be used to complement clinical observations.

We used a mouse model to evaluate the effects of maternal alcoholism during pregnancy on two variables possibly related to the development of alcoholism in the offspring: voluntary consumption of alcohol and acute sensitivity to the pharmacologic properties of the drug. Alcohol intake in a two-bottle choice situation and alcohol-induced sleep time were the respective dependent variables. Our mouse model included a pair-fed control group to control for nutritional effects; we fostered the offspring at birth to untreated dams to control for postnatal variables, and we used offspring representing several litters.

METHOD

General Procedure

Parent animals were male and female C3H/He mice obtained from the National Cancer Institute (Frederick, MD) at five weeks of age. Mice were housed five per cage in polypropylene cages (29×19×13 cm) with zinc-plated lids and were maintained on a 12 hr light-dark cycle (0600–1800 light) in temperature and humidity controlled rooms. Wayne Lab Chow and water were available ad lib.

At approximately 10 weeks of age, females were placed

individually in a cage of males and examined daily for the presence of vaginal plugs. The morning of plug identification was referred to as Gestation Day 1 (GD-1). The pregnant females were then weighed and singly housed. On GD-8 and throughout pregnancy, one group of females was given free access to a liquid diet containing 28% ethanol-derived calories (EDC) (5% w/v) as their sole source of food and fluid. Another group of females was allowed limited access to 0% EDC diet. Both groups were fed the 0% EDC diet ad lib on GD-6 and GD-7 to familiarize the animals with ingesting a liquid diet. A pair-feeding procedure was followed such that each animal in the 0% EDC group was matched to a 28% EDC animal and was fed the amount consumed by the 28% EDC animal on a ml/kg/day basis. This procedure required that the 28% EDC animals be at least two days more advanced in pregnancy than matched 0% females. The 0% EDC diet was made isocaloric to the 28% EDC by the addition of sucrose replacing the ethanol. The composition of these liquid diets consisted of chocolate Sustacal (Mean Johnson, Inc.), vitamins and minerals (ICN Nutritional Biochemicals), 95% ethanol or sucrose. Because the diets were isocaloric and were pair-fed on a body weight basis, each female within a pair received the same number of calories for any given day of pregnancy. Diets were administered fresh daily at approximately 0900 hr. All females were weighed again on GD-12 and GD-19 of pregnancy. A third group of animals was included in the experimental design to assess any effect of the liquid diet and/or restricted caloric intake due to pair-feeding. This group received free access to lab chow and water throughout pregnancy. Thus, the three groups of mothers were identified by their treatment during pregnancy: alcohol, pair-fed control, or lab chow. The pups, subsequently, are identified by prenatal history. The three groups are referred to as prenatal alcohol, prenatal pair-fed, and prenatal chow.

Cages were checked twice daily for litters after GD-18. Newborn pups were weighed and litters were culled to six of equal sex, when possible. Offspring were removed from their natural mothers and fostered to untreated surrogate mothers who had recently delivered. Pups were left undisturbed with the exception of being weighed on Day 10 and at weaning on Day 25.

A separate group of ten pregnant females provided additional data concerning circulating maternal blood alcohol levels. These animals received the liquid diet containing 28% EDC beginning on GD-8 as described above. Blood was collected from the retro-orbital sinus on GD-10 and GD-17. Blood samples were taken from five mothers at 0800 and 1300 hours, while samples were taken from the remaining five at 1030 and 1530 hours. Thus, at any one time point, five values were available. Blood alcohol concentrations were determined enzymatically by measuring absorption of NADH at 340 nm on a Gilford 24002 spectrophotometer. The method used was similar to that sold by Cal-Biochem Corp. for alcohol analysis.

Sleep Time Procedure

Two female offspring from eighteen different litters in each prenatal condition were tested for alcohol-induced sleep time. One litter mate was tested at 25 days and the other at 110 days of age, in order to evaluate the influence of testing age. Half of the litters (N=9/prenatal treatment) were given 3.5 g/kg ethanol and the other half were injected with 4.5 g/kg. Thus, littermates received the same dose of alcohol, but at different ages. Alcohol was injected intraperitoneally in a volume of 0.02 ml/gm.

Immediately following injection, each animal was placed in a holding cage until it was unable to right itself twice within 30 sec of being placed on its back. The animal was then placed in a V-shaped plexiglas trough and latency to regain the righting reflex was recorded as sleep time. Righting twice within 30 sec was the criterion used. A 44.7 μl sample of blood was obtained from the retro-orbital sinus of each animal when it regained the righting reflex for spectrophotometric determination of "waking" blood alcohol concentration.

Alcohol Preference Procedure

A maximum of one male and one female from seventeen different litters in each prenatal condition was assessed for alcohol preference beginning on the day of weaning. The mice were housed individually with free access to Wayne Lab Chow and two graduated cylinders. One cyclinder contained 10% w/v alcohol and the other contained tap water. To avoid a position habit, the tubes were alternated daily when fluid consumption was recorded. Fresh alcohol and water were given every fourth day. Alcohol and water consumption were measured daily. The animals were weighed every other day for twenty-one days. Each animal's alcohol preference (ml alcohol/total fluid intake) and relative alcohol intake (g ethanol per kg body weight) were calculated daily and a weekly average was determined for each animal.

Data Analysis

Data from mothers within each treatment group were analyzed separately for the sleep time and alcohol preference experiments because different mothers were used. Dependent variables included alcohol consumption, maternal weight gain, litter size, and litter birth weight. Litter size and litter birth weight were analyzed using the litter mean [1]. For most variables, analysis of variance was used to determine statistical significance at the .05 confidence level. Data were analyzed by comparing the alcohol-fed group with the pair-fed liquid diet group. A separate analysis was performed to compare the two control groups (i.e., pair-fed liquid diet with lab chow group) in order to assess the influence of the liquid diet and/or restricted food intake on the outcome measures. A paired t-test was used to compare maternal alcohol consumption on GD-12 with intake on GD-19. Pup data in the sleep time and preference experiments were analyzed similarly to the maternal data. That is, offspring from the prenatal alcohol and prenatal pair-fed groups were compared and the two control groups were compared in a separate analysis. Individual scores were used as the unit of analysis in both studies.

RESULTS

Sleep Time Study

Female mice administered alcohol during pregnancy consumed 33.3±1.6 g/kg body weight on GD-12. Intake decreased on GD-19 to 26.7±1.5 g/kg, $t(16)=3.11$, $p<0.01$. Peak blood alcohol levels associated with these intakes were between 80 and 120 mg%. Comparison between the alcohol-fed and pair-fed control groups revealed no significant difference in percent weight change during pregnancy, litter size, or litter weight. The average (±SE) weight change for the alcohol-fed mothers was 62±4% and for the pair-fed control

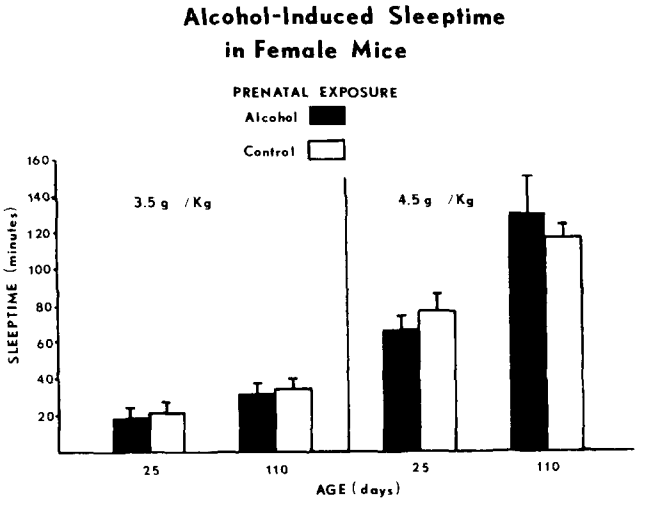

FIG. 1. Effect of prenatal alcohol exposure on sleeptime in female offspring. Mean and (±) standard error of the mean values are presented.

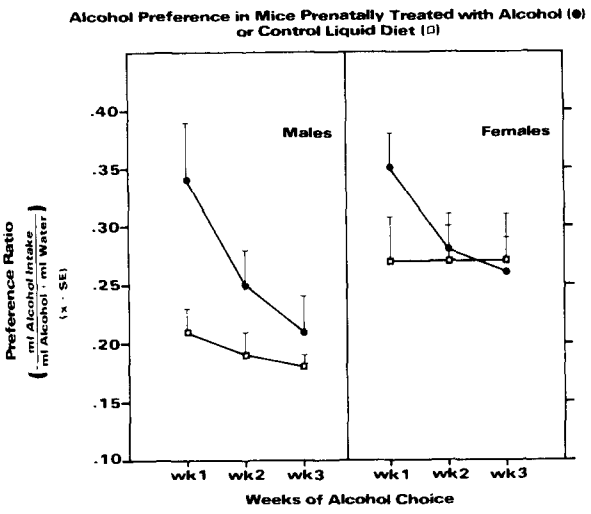

FIG. 2. Effect of prenatal alcohol exposure on alcohol preference in male and female offspring. Data presented for three consecutive weeks as mean and (±) standard error of the mean.

diet group was 56±3%. Litter size was 6.1±0.4 and 6.9±0.5 for the alcohol-fed and pair-fed control, respectively, while pup birth weight was 1.2 g±0.02 for both groups. Comparison between the pair-fed and lab chow control groups failed to reveal any differences between the two control groups on these measures.

Pup Data

A 2 (dose) × 2 (age) × 2 (prenatal condition) analysis of variance revealed significant main effects of Dose, $F(1,26)=74.1$, $p<0.01$ and Age, $F(1,26)=35.5$, $p<0.01$, and an Age × Dose interaction, $F(1,26)=9.73$, $p<0.01$, on alcohol-induced sleep time. There was, however, no effect of prenatal condition on this parameter. No other interactions were significant. These data are illustrated in Fig. 1. The figure clearly shows that mice slept longer at 110 days of age than they did at 25 days of age, regardless of the challenge alcohol dose or prenatal condition. Collapsed across age and prenatal condition, sleep time following 4.5 g/kg alcohol was longer than after 3.5 g/kg. No difference in alcohol-induced sleep time was observed when the two control groups were compared in a similar analysis.

Waking blood alcohol levels were analyzed by a one-way analysis of variance. Samples from groups challenged with 3.5 g/kg at 110 days of age were unavailable for analysis because of a procedural error. The average (±SE) value for 25-day old mice exposed to alcohol in utero and injected with 3.5 g/kg alcohol was 369±10 mg% and 345±30 mg% for pair-fed control offspring. Waking levels for similar aged mice injected with 4.5 g/kg were 339±20 mg% and 364±20 mg%, respectively. Mice injected with 4.5 g/kg at 110 days of age awakened at blood alcohol levels of 379±30 mg% (prenatal alcohol) and 342±30 mg% (pair-fed control). There was no effect of prenatal treatment at either age or alcohol dose. A similar analysis comparing waking blood alcohol levels of the pair-fed and chow control groups also was not significant. These results indicate that prenatal alcohol exposure did not affect subsequent sensitivity to alcohol challenge.

Preference Study

Pregnant mice in the alcohol-fed group consumed (mean±SE) 33.2±1.2 g alcohol/kg body weight on GD-12 and 26.7±1.3 g/kg on GD-19. A paired t-test indicated that the alcohol intake observed near-term was significantly different from that observed earlier in pregnancy, $t(22)=3.02$, $p<0.01$.

Comparison of alcohol-fed and pair-fed control groups by a one-way analysis of variance revealed no difference between the groups on maternal weight gain (60±3% and 56±3%) or pup birth weight (mean=1.3±0.02 and 1.3±0.04). However, litter size was smaller in the alcohol-fed group, $F(1,49)=9.2$, $p<0.01$.

A similar analysis comparing the two control groups indicated that percent maternal weight gain, $F(1,46)=6.45$, $p<0.05$, and pup birth weight was greater for the lab chow mothers, $F(1,99)=17.5$, $p<0.001$. Maternal weight gain for the lab chow mothers was 65±2% as compared to 56±3% for the pair-fed group. Pups born to lab chow mothers weighed 1.39±0.03 g at birth, while pair-fed control offspring weighed 1.24±0.02 g. No difference between groups was noted in litter size.

Pup Data

Average weekly alcohol preference ratio plotted as a function of prenatal condition and sex are shown graphically in Fig. 2. An analysis of variance with repeated measures comparing offspring of alcohol-fed mothers with offspring of pair-fed mothers revealed a significant main effect of Week, $F(2,120)=7.11$, $p<0.01$. While main effects of Prenatal Condition, $F(1,60)=3.70$, $p=0.059$, and Sex, $F(1,60)=3.68$, $p=0.06$ were marginally significant, they failed to reach the 0.05 confidence level. The only significant interaction term was Week × Prenatal Condition, $F(2,120)=3.85$, $p<0.05$. As illustrated in the figure, collapsed across prenatal condition and sex, alcohol preference was higher the first week of testing than in subsequent weeks. The significant Week ×

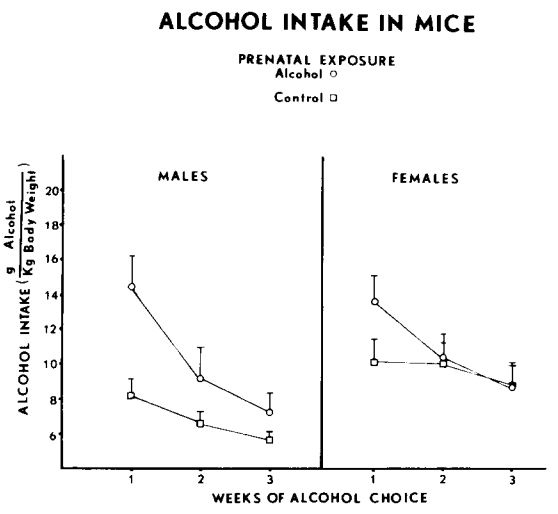

FIG. 3. Effect of prenatal alcohol exposure on alcohol intake of male and female offspring. Intake is presented as g alcohol per kg body weight. Mean and (±) standard error of the mean values are presented.

Prenatal Condition interaction can be explained by the fact that mice prenatally exposed to alcohol tended to have a higher alcohol preference ratio than control offspring, especially in Week 1. A similar analysis comparing offspring from the two control groups did not demonstrate any group difference in alcohol preference.

When alcohol consumption was expressed as a function of body weight, data analysis demonstrated significant main effects of Prenatal Condition, $F(1,60)=4.99$, $p<0.05$, and Weeks, $F(2,120)=24.49$, $p<0.01$, as well as a significant Prenatal Condition × Week interaction, $F(2,120)=7,57$, $p<0.01$. No other terms reached statistical significance. As illustrated in Fig. 3, mice prenatally exposed to alcohol consumed more alcohol than control mice and, collapsed across sex and prenatal condition, alcohol consumption was higher in Week 1 than subsequent weeks. The significant interaction can be accounted for by the fact that mice prenatally exposed to alcohol consumed more alcohol initially than pair-fed control offspring. There were no group differences observed in absolute alcohol intake when the offspring from pair-fed and lab chow control groups were compared in a separate analysis.

DISCUSSION

The present experiment failed to detect an effect of maternal alcohol administration during pregnancy on alcohol-induced sleep time in the offspring of C3H mice at either 25 or 110 days of age. These results agree with those reported by others in C57BL mice [11] and rats [2,4]. Further, the present data imply that testing age is not a salient variable and that CNS sensitivity to the anesthetic doses of alcohol used is not altered by prenatal exposure to the drug. Waking blood alcohol levels were similar across groups. Before an alteration in CNS sensitivity can be ruled out, however, waking brain alcohol levels will have to be measured.

Our results on voluntary alcohol consumption demonstrated an increase in alcohol consumption in mice prenatally exposed to alcohol when the data were expressed as a function of body weight and a marginal, but not significant, increase in consumption when presented as a preference ratio. Regardless of how the data were expressed, increased alcohol intake was most pronounced the initial week of testing, but it tended to decline over time. Females tended to consume more than males. The results cannot be attributed to undernutrition or the liquid diet, per se, since no differences were noted between pair-fed control and lab chow offspring. Our results agree with increased alcohol preference which has been reported in rat pups prenatally exposed to alcohol [6,10] but disagree with others which have failed to find any influence of maternal alcohol administration on this variable [3]. Inconsistent results can be attributed to differences in presentation of the data, age of testing, duration of the testing period, and basic differences in alcohol administration to the pregnant animals. The decrease in consumption over time leads us to conclude that the difference we observed in alcohol intake in mice prenatally exposed to alcohol is reflective of a transitory, non-specific effect. That is, prenatal alcohol exposure may have resulted in offspring with a response inhibition deficit, as has been suggested previously [12] which matured over the three week testing period. It will be recalled that the offspring were tested initially as weanlings. This would explain the sustained increase of a basically unpalatable fluid in week 1 which diminished as the animal matured and became able to refrain from drinking from the alcohol tube. The fact that alcohol preference ratio decreased across testing weeks suggests, in fact, that more water and less alcohol was being consumed. In order to examine this possibility, it will be necessary to test offspring at an older age or to measure voluntary intake of another unpalatable solution, such as quinine, in a choice situation.

With regard to the animal model used to administer alcohol to pregnant mice, it is interesting to note the consistency of alcohol consumption in the mothers across studies. The decrease in intake of the liquid diet near-term which we observed has been noted by others, as well (Riley, personal communication). Although peak blood alcohol levels were intoxicating, the animals never appeared grossly impaired, but they were docile upon handling. The dose used (i.e., 28% EDC) did not result in impaired maternal weight gain during pregnancy or decreased birth weight when the alcohol-fed and pair-fed control dams were compared. The two control groups differed in litter size in one study, but were similar for other maternal variables such as weight gain and birth weight across experiments. Higher doses may yield increased blood alcohol levels and group differences on maternal variables but, in our experience, a significant number of mice will fail to maintain pregnancy or will deliver dead or non-viable pups that are cannibalized rapidly by foster dams.

In summary, the results of the experiment described do not support the notion that alcohol administration during pregnancy affects sensitivity to acute alcohol challenge in the offspring, as measured by sleep time. On the other hand, alcohol intake was elevated in mice exposed to alcohol *in utero*, but the effect was not robust and appears to be transient. This is not to say that prenatal alcohol exposure does not affect the offspring. Studies are underway in our laboratory to examine other alcohol-related behaviors and to evaluate the influence of paternal alcoholism on behaviors in the offspring which are compatible with alcoholism. Our animal model promises to be a valuable adjunct to clinical studies in this area and may be helpful in evaluating treatment strategies.

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