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Prenatal Binge-Like Alcohol Exposure Alters Neurochemical Profiles in Fetal Rat Brain

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MAIER, S. E., W-J. A CHEN AND J. R. WEST. *Prenatal binge-like alcohol alters neurochemical profiles in fetal rat brain.* PHARMACOL BIOCHEM BEHAV **55**(4) 521–529, 1996.— The majority of studies examining the effects of prenatal exposure to alcohol on neurotransmitter levels have furnished results that are divergent (increase, decrease or no change). The present study assessed six neurochemical compounds [norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), γ-aminobutyric acid (GABA)] from the same brain tissue. Pregnant Sprague-Dawley rats were given 5.1 g/kg alcohol (by gavage) either daily from embryonic day 1 (E1) through E20 or E20 only. In addition, pairfed/intubated (PF/INT) and ad lib chow (Chow) groups were included as controls. The dams were sacrificed and the fetuses were removed on E20. Binge-like alcohol exposure throughout gestation (E1-E20) produced significantly higher brain to body weight ratios compared with all other groups. Alcohol exposure did not produce changes in NE levels, although the E1-E20 exposure to alcohol reduced the contents of DA and 5-HT compared with the PF/INT and Chow controls. In addition, the E20 alcohol treatment reduced both DA and 5-HT levels compared with the E1-E20 alcohol treatment. DOPAC and 5-HIAA contents were affected by the prenatal treatments insofar as the 5-HIAA levels were decreased in E/1-20 and E20 animals relative to both controls, while the DOPAC levels were decreased in E/1-20, E20 and PF/INT groups compared to the Chow group; however, both metabolites were unaffected by the difference in alcohol treatment duration. Moreover, GABA levels were increased in fetuses exposed to alcohol from E1-E20 compared with all other groups. Collectively, these findings suggest that binge-like alcohol exposure prior to and during neurotransmitter development affects the baseline content of several neurotransmitters. **Copyright © 1996 Elsevier Science Inc.**

Ethanol Gestation Neurotransmitter Rat Brain Blood alcohol concentration Fetal alcohol syndrome Pregnancy

THE spectrum of effects produced by alcohol exposure during gestation is extensive, including Fetal Alcohol Syndrome (FAS) at the severe end of the spectrum (17, 30). Even individuals without conspicuous facial dysmorphology associated with FAS may still be affected by their in utero alcohol exposure and those effects may be manifested as impairments in cognitive or other behavioral performance, possibly due to neurochemical changes in the brain. Since appropriate behavioral functioning relies on the complement of neurotransmitters and their intact neuroanatomical circuits (29), it is of considerable interest to determine how in utero alcohol exposure affects the levels of various neurotransmitters during the course of fetal development.

The examination of neurochemical profiles in response to prenatal alcohol exposure is important for several reasons. First, earlier evidence indicates that specific neurotransmitters can act as regulatory or trophic factors during neurogenesis and synaptogenesis (for a review see 20). The loss of neurons observed in certain brain regions after fetal alcohol exposure may be due in part to a failure of the axons of those cells to make contact with targets in the absence of guidance from a particular neurotransmitter. Second, without fully functioning neurotransmitter systems, the development of certain behaviors will be impeded and may be manifested as behavioral dysfunctions including attention deficits and motor incoordination (3,6). Third, if neurotransmitter levels are altered as a consequence of in utero alcohol exposure, neonates with a history of neurochemical imbalance during development may respond unexpectedly when exposed to various pharmacological agents (illicit or licit drugs) either immediately postnatal (through lactation) or in adulthood (self administration of drugs) (2,8,14,15). Accordingly, the examination of neurochemistry adds a meaningful dimension to the consideration of other known deficits following exposure to alcohol during gestation.

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The central nervous system in the rat develops over the course of gestation with most of the major neurogenesis accomplished during the second trimester equivalent (embryonic (E) day 10 through E21). Similarly, the development of the different neurotransmitter systems occurs mostly in midgestation and levels of the various neurotransmitters can be detected well before parturition (12). For example, serotonin (5-HT) is detectable as early as E13 and reveals the adult pattern of distribution by E19. Dopamine (DA) is found in ventral mesencephalon at E15 and in substantia nigra at E21. Norepinephrine (NE) synthesis is detectable as early as E13, while NE innervation of cortex is most prominent at birth (E22/E23). Therefore, the timing of alcohol exposure during central nervous system development may influence the type and degree of detrimental effects observed in the development of neurotransmitter systems.

While most previous studies that examined basal levels of neurotransmitters following in utero alcohol exposure used the liquid diet method, that methodology suffers from several drawbacks. Animals fed alcohol in a liquid diet have access to the alcohol-containing liquid diet over a 24 h period, thus the pattern of alcohol consumption is more diffuse, rather than condensed into a more common binge-like pattern typical of human alcohol consumption. The difference between the continuous and binge-like alcohol exposure paradigms on brain development has been demonstrated by Bonthius and West (4). Rat pups exposed to a lower dose of alcohol in a binge-like pattern during the third trimester equivalent exhibited more microencephaly than pups exposed to a higher daily alcohol dose but in a continuous pattern. Furthermore, the greatest brain deficits were produced by the alcohol exposure paradigm that induced a high peak blood alcohol concentration (BAC) (4,5). Thus, drinking pattern and BAC, rather than alcohol dose, appear to be primary determinants for inducing detrimental effects on brain development. Finally, in some experiments, the fetuses were removed while under the influence of alcohol (the liquid diet was still accessible to the dam) and an appropriate control for this possible confound was not included (9,10). One of the appropriate controls would be a group exposed to alcohol only on the day of fetal extraction, so that changes in the levels of neurotransmitters following long-term alcohol exposure may be interpreted as the cummulative effects of alcohol exposure during development, rather than the transient effects of alcohol exposure at the time of sacrifice.

In this study, we chose to examine the effect of binge-like alcohol exposure during gestation on the content of several different neurochemical compounds in the fetal rat brain. In order to mimic a human binge-like alcohol drinking pattern and achieve high peak BACs, pregnant rats were intragastrically intubated with alcohol daily. Further, we tested a second group of rats that were treated with alcohol only on the day of fetal extraction in order to differentiate the effect of long term alcohol exposure from the influence of alcohol exposure on our dependent measures. Thus, we were able to contrast the effect of a long (E1-20) and short (E20 only) duration of alcohol exposure on fetal brain neurotransmitters.

METHOD

Subjects

The fetal offspring from matings of adult Sprague–Dawley rats (Harlan Sprague–Dawley Inc.) served as subjects in this study. They were housed in a vivarium with constant temperature (20±1°C) on a 12:12 photocycle cycle (lights on at 0700). All procedures were approved by the University Animal Care Committee and followed the *Guidelines for the Care and Use of Laboratory Animals* published by the National Institute of Health.

Procedures

Alcohol treatments. Each female rat was handled daily for four consecutive days prior to mating, while baseline food consumption and body weight measurements were taken. In addition, on the final two days of baseline measurement acquisition, each rat received practice oral intubations (no alcohol or vehicle solution was administered). For practice intubations and alcohol dosing, a three inch partially curved stainless steel intubation needle (Popper and Sons, Inc., model #7946, New Hyde Park, NY) was used. The rounded tip of the needle was lubricated with a drop of corn oil to facilitate passage down the esophagus.

The mating procedure consisted of housing one male and one female rat together until the female tested positive for sperm by vaginal swabs taken each morning. The day sperm was detected by vaginal smear was designated as embryonic day 1 (E1) to maintain consistency with Altman and Bayer's (1) cumulative findings on the neurogenesis of various brain regions. On E1, females were housed individually and received their randomly assigned treatment the same day. All treatments were executed between 11:30 and 12:30 h daily, where each animal received its respective treatment at the same time each day.

Animals assigned to the Chow group (n = 6) received ad lib access to laboratory chow and tap water. Two groups of animals were intubated with 5.1 g/kg alcohol. One group (EtOH/1-20) received one intubation of alcohol daily from E1-20 (n = 8) and the other group (EtOH/20) received one intubation of alcohol on E20 only (n = 5). Animals assigned to the EtOH/1-20 group had ad lib access to laboratory chow and tap water, while animals in the EtOH/20 group were matched by weight with individual EtOH/1-20 animals on E1 and given the same of amount of lab chow to consume on a given day of gestation. Thus, all EtOH/20 animals were pairfed to EtOH/1-20 animals for the duration of gestation. Animals assigned to the pairfed/intubated (PF/INT) group (n = 6) were intubated once daily from E1-20 with a maltose-dextrin solution (rather than alcohol) that was made isocaloric to the alcohol, plus individual animals in this group were matched by weight on E1 with individual animals in the EtOH/1-20 group and received the same of amount of food as its weight-matched partner in the EtOH/1-20 group for each particular day of gestation. Alcohol (22.5% w/v) and maltose dextrin solutions were made fresh every second day. All animals (including Chow) were weighed and had their food consumption recorded daily from E1 through E20.

Fetal extractions, fetal blood alcohol concentration (BAC) and fetal amniotic alcohol concentration (AAC). On E20, 3 h after their respective treatment, pregnant dams were euthanized by CO_2 inhalation. The dam's blood sample was drawn from a small nick to the tip of the tail immediately after removal from the CO_2 chamber (both EtOH groups). Fetuses were extracted without delay proceeding from cervix towards the ovary and starting with the left arm of the uterus. Fetuses in the EtOH/1-20 and EtOH/20 groups had samples of amniotic fluid taken for determination of AAC. Twenty microliters of blood (dam or fetal) or amniotic fluid was taken from each subject. The samples were immediately placed into

glass vials containing a 200 μl cocktail composed of 0.6 N perchloric acid and 4 mM *n*-propanol in double distilled water, tightly fastened with a septum sealed lid, and stored at room temperature until analysis by head space gas chromatography (Varian Model 3400, Palo Alto, CA).

After AAC samples were taken from the appropriate groups, fetuses from all treatment groups were removed from the amniotic sac, severed from the umbilical cord, blotted dry and weighed to the nearest 10th of a gram. Following decapitation, trunk blood samples (20 μl) were collected from the alcohol treated fetuses (both groups). For the brain weight study, the skin was peeled back from the skull and a small puncture was made at the base of the skull using ultrafine scissors. Heads prepared in this manner were immersed in a 1.0% paraformaldehyde/1.25% gluteraldehyde fixative in phosphate buffer (pH = 7.4). The brains were removed from the heads 24 h later and weighed seven days later after postfixing in the same fixative. For the neurochemistry study, the entire fetal brain was extracted rapidly on ice, placed into individual 1.5 ml microcentrifuge tubes, weighed and stored at −80°C.

HPLC tissue preparation and analyses. At the time of analysis, brain samples were exposed to 200 μl perchloric acid (PCA) containing 200 ng/ml dihydroxybenzylamine (DHBA; internal standard), homogenized by sonication and centrifuged at 13000 g for 5 min. For catecholamine and indoleamine analyses, a volume of 100 μl of original brain supernatant was then transferred to another 1.5 ml microcentrifuge tube, mixed with 100 μl chilled PCA and centrifuged for 2 min. The standards containing the known amount of NE, DA, DOPAC, 5-HT, 5-HIAA and DHBA also were prepared for calibration. Upon the completion of sample preparation, a 20 μl of the supernatant containing the known amount of DHBA and the unknown amount of catecholamines and indoleamines was analyzed using an HPLC technique, with electrochemical detection (ED) at an electrode potential of +0.67V. For γ-aminobutyric acid (GABA) analysis, a volume of 20 μl of the original brain supernatant was transferred to a 0.5 ml microcentrifuge tube to which 60 μl of 5-aminovaleric acid (AVA; 2.5 μg/ml) was added to serve as the GABA internal standard. After vortexing, 20 μl of the above mixture was placed into another 0.5 ml microcentrifuge tube. A volume of 20 μl derivitizing agent was then added into the tube and the mixture was gently vortexed. One minute after the addition of derivitizing solution, 20 μl of sample was analyzed using HPLC method. The HPLC-ED facility and the components of the HPLC equipment, mobile phase and chemicals used were detailed in a paper by Champney et al. (7). The NE, DA, DOPAC, 5-HT and 5-HIAA values were expressed as ng/g of tissue wet weight, while GABA was expressed as μg/g of tissue wet weight.

Data Analysis

The maternal variables of food consumption and body weight were aggregated into 5 blocks across consecutive days (pretreatment = 4 days of baseline, Block 1 = E1-5, Block 2 = E6-10, Block 3 = E11-15, Block 4 = E16-20) and analyzed by repeated measures analysis of variance (ANOVA) using group (EtOH/1-20, EtOH/20, PF/INT, Chow) as the between factor and blocks as the repeated factor. Pretreatment food consumption data were unavailable for some dams, thus the degrees of freedom for the pretreatment food consumption analysis differ from analyses involving other maternal variables. For all offspring data analyses, the litter mean was the unit of analysis. The fetal brain weight, body weight and neurochemistry data were analyzed using one-way ANOVAs with group (EtOH/1-20, EtOH/20, PF/INT, Chow) as the between factor. Student Newman-Keuls (SNK) post-hoc tests were used following a significant treatment main effect. Finally, within each alcohol treatment group, correlations were computed among dam BAC, fetal BAC and AAC to determine the degree of relationship among these variables on E20 after both long (E1-E20) and short (E20) durations of alcohol exposure. To be considered significant, ANOVA and SNK p values must be less than or equal to 0.05.

RESULTS

Maternal Body Weight and Food Consumption

Table 1 shows the values for the body weights and food consumption of the dams. There was no difference in pretreatment body weight or food consumption among the treatment groups. After treatment began, there were no significant differences in body weight among the groups, but the Chow group gained (E20 minus E1) significantly more weight than the EtOH/1-20, EtOH/20 and PF/INT groups [$F(3, 21) = 5.93$ $p < 0.05$]. In terms of food consumption, the Chow group ate significantly more than all other groups on all four blocks of treatment (all p's < 0.05). Since there were no differences in body weight or food consumption among the EtOH/1-20, EtOH/20 and PF/INT groups on any blocks, the pairfeeding procedures were effectively administered.

Fetal Brain and Body Weight

Table 2 presents the relevant data on brain weights, body weights, brain to body weight ratios (Br/Bwt) of fetuses, and number of viable fetuses from all treatment groups. Although the analysis of the fetal brain and body weights did not reveal significant effects, the analysis of the Br/Bwt ratio was significant [$F(3, 20) = 5.80$, $p < 0.01$]. Post-hoc analyses show that the EtOH/1-20 group had a significantly higher Br/Bwt ratio than the EtOH/20, PF/INT and Chow groups, and this increase in Br/Bwt ratio in EtOH/1-20 group likely is attributed to the somewhat lower body weights in this group.

Fetal Neurochemical Profiles

Norepinephrine (NE). There were no significant differences in NE content among the four groups (see Fig. 1).

Dopamine (DA) and dihydroxyphenylacetic acid (DOPAC). An ANOVA on the fetal brain DA content revealed a significant treatment effect [$F(3, 21) = 19.65$, $p < 0.01$]. Figure 2 shows the results of the post-hoc analyses; brain DA levels were found to be significantly lower in the EtOH/20 group than the EtOH/1-20 group, and both alcohol treated groups had lower DA levels than the PF/INT and Chow groups. However, there was no difference between the PF/INT and Chow groups on this measure. The DA metabolite DOPAC also showed a significant treatment effect [$F(3, 21) = 8.44$, $p < 0.01$], but the pattern was different from that of DA (Fig. 2). Post-hoc tests demonstrated that the EtOH/1-20, EtOH/20 and PF/INT groups had significantly less DOPAC than the Chow group. Finally, the ratio of DOPAC to DA was calculated and the ANOVA on this measure showed a significant treatment effect [$F(3, 21) = 6.76$, $p < 0.01$]. Fig 3a demonstrates the results of the post-hoc analysis; the Chow group had significantly higher ratios than the EtOH/20, EtOH/1-20 and PF/INT groups.

TABLE 1

MEAN BODY WEIGHT, WEIGHT GAIN, FOOD CONSUMPTION AND BLOOD ALCOHOL CONCENTRATION FOR THE TREATED DAMS

Group	Pretreatment Baseline	Block 1 (E1–E5)	Block 2 (E6–E10)	Block 3 (E11–E15)	Block 4 (E16–E20)
EtOH/1-20					
Body weight (g)	221 ± 4.7	240 ± 6.0	250 ± 7	270 ± 7	298 ± 6
Weight gain (g)					72 ± 7
Food consumption (g)	18 ± 0.4	13 ± 0.7	17 ± 0.6	19 ± 0.5	20 ± 0.8
E20 peak blood alcohol concentration (mg/dl)					322 ± 33
EtOH/20					
Body weight (g)	232 ± 3.9	257 ± 4	258 ± 4	268 ± 3	299 ± 7
Weight gain (g)					60 ± 12
Food consumption (g)	17 ± 0.6	12 ± 0.7	16 ± 0.9	17 ± 0.5	19 ± 1.0
E20 peak blood alcohol concentration (mg/dl)					278 ± 21
PF/INT					
Body weight (g)	231 ± 5.1	245 ± 4	247 ± 4	262 ± 5	287 ± 11
Weight gain (g)					58 ± 12
Food consumption (g)	20 ± 1.3	14 ± 0.6	17 ± 0.7	19 ± 0.9	20 ± 0.9
CHOW/SC					
Body weight (g)	223 ± 3.9	247 ± 11	265 ± 10	286 ± 10	327 ± 14†††
Weight gain (g)					112 ± 9†
Food consumption (g)	17 ± 0.5	21 ± 0.9†	23 ± 0.5†	24 ± 0.7†	26 ± 0.6†

Data are presented as mean ± standard error of the mean. E20 blood alochol concentration measures were taken at the time of sacrifice.

†Significantly different from all other groups ($p < 0.05$).; ††Significantly different from PF/INT group ($p < 0.05$).

Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). Figure 4 illustrates the significant effect of the prenatal alcohol treatment on fetal 5-HT levels [$F(3, 21) = 22.35$, $p < 0.01$] and the post-hoc analyses revealed that the EtOH/20 group had significantly lower 5-HT levels than the EtOH/1-20 group, while both alcohol treated groups had significantly lower 5-HT content than both the PF/INT and Chow groups. However, there was no difference between the PF/INT and Chow groups in 5-HT content. In addition, there was a significant effect of the treatment on the 5-HT metabolite, 5-HIAA [$F(3, 21) = 8.93$, $p < 0.01$] (Fig. 4). Post-hoc analyses revealed that both alcohol treatment groups had significantly less 5-HIAA than the PF/INT and Chow groups, but the two alcohol groups did not differ. Likewise, the PF/INT and Chow groups did not differ significantly in 5-HIAA levels. The analysis of the ratio of 5-HIAA to 5-HT demonstrated a significant main effect of group [$F(3, 21) = 42.26$, $p < 0.01$] and the post-hoc analyses showed that the ratio for the EtOH/20 group was significantly higher than the ratios of the Chow, PF/INT and EtOH/1-20 groups (Fig. 3b), yet there was no difference in 5-HIAA/5-HT ratios among these three groups.

γ-aminobutyric acid (GABA). The ANOVA on the GABA content revealed a significant treatment effect [$F(3, 21) = 4.87$, $p < 0.01$) and post-hoc analyses showed that the EtOH/1-20 group had significantly *higher* GABA levels than all other groups (Fig. 5). There were no differences among the EtOH/20, PF/INT and Chow groups on this measure.

Alcohol Concentrations

Pearson product moment correlation coefficients were calculated between the various alcohol concentrations from dam blood, fetal blood and amniotic fluid samples. The *n*'s for some correlations involving the alcohol concentration of the

TABLE 2

MEAN BODY WEIGHTS, BRAIN WEIGHTS, BRAIN TO BODY WEIGHT RATIOS AND NUMBER OF FETUSES FOR THE EXPERIMENTAL GROUPS

	Treatment Group			
Variable	Chow	PF/INT	EtOH/20	EtOH/1-20
Fetal body weight (g)	2.40 (0.12)	2.57 (0.23)	2.36 (0.29)	1.88 (0.10)
Fetal brain weight (g)	0.1290 (0.005)	0.1303 (0.004)	0.1204 (0.007)	0.1141 (0.004)
Brain to body weight ratio (%)	5.4042 (0.129)	5.1867 (0.257)	5.2185 (0.241)	6.1191 (0.123)†
Number of viable fetuses per litter	13 (2)	10 (3)	14 (2)	13 (1)

Table values represent the litter mean. Numbers in parentheses are standard error of the mean.

† EtOH/1-20 is significantly different from all other groups ($p < 0.05$).

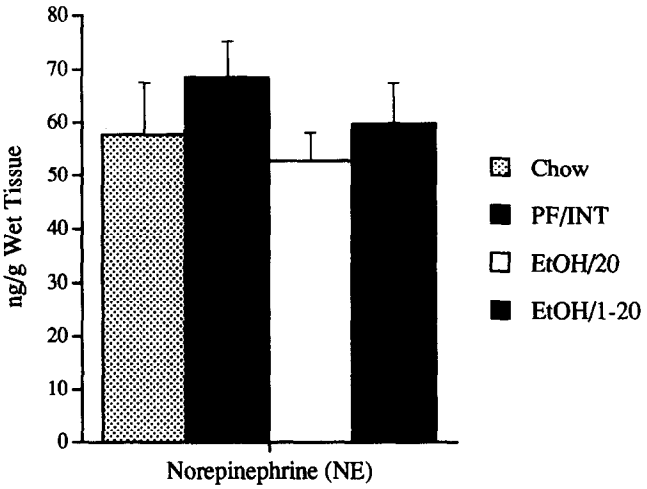

FIG. 1. Mean whole brain norepinephrine (NE) content in fetuses exposed to alcohol in utero and their appropriate controls. Data are expressed as ng/g wet tissue. Vertical bars represent standard error of the mean.

amniotic fluid (AAC) are reduced due to technical difficulties in obtaining samples, which were subsequently resolved. Table 3 shows the correlations between specific variables and within each of the alcohol treatment groups. There was nearly perfect agreement between the dam and fetal BAC, even with relatively small n's within some groups. The dam's BAC and AAC also were highly correlated, yet on average, the correlation between the fetal BAC and AAC was lower than the correlations among the other variables. However, it is important to note that in all cases, the amount of variance accounted for by a linear relationship among the various measures was highly reliable (see Fig. 6).

DISCUSSION

The major findings of the present study can be summarized as follows: (1) the Br/Bwt ratios of fetuses exposed to alcohol throughout gestation (EtOH/1-20) were significantly higher than those of other treatment groups; (2) Among six neurochemical compounds assessed, DA, DOPAC, 5-HT and 5-HIAA contents were significantly reduced in animals of the EtOH/1-20 and EtOH/20 group, although no change in NE content was observed in these alcohol treated groups. On the other hand, the GABA concentration was higher in the EtOH/1-20 group compared with all other groups; (3) The DA, 5-HT and GABA concentrations in the EtOH/1-20 group were different from those in the EtOH/20 group; and (4) The BACs from dams and fetuses were highly correlated, as were dam BACs and AACs, regardless of the duration of alcohol exposure (EtOH/1-20 or EtOH/20).

The results from the brain weight measures indicated no difference in brain growth independent from body growth among any treatment groups. The higher brain to body weight ratio for the EtOH/1-20 group compared with all other groups suggests a 'brain sparing' phenomenon, similar to that reported by Weinberg (31). That is, the somatic growth of the fetus, rather than its brain growth, was affected more by the prenatal binge-like alcohol exposure. Not only was there a definite trend toward lower body weights in the fetuses exposed to alcohol throughout gestation compared with all other groups ($p = 0.06$), but the lower body weights of the fetuses in the EtOH/1-20 group relative to their brain weights can explain why the brain to body weight ratio of this group was significantly higher than all other groups. The lack of alcohol-induced deficits in brain growth independent of body growth

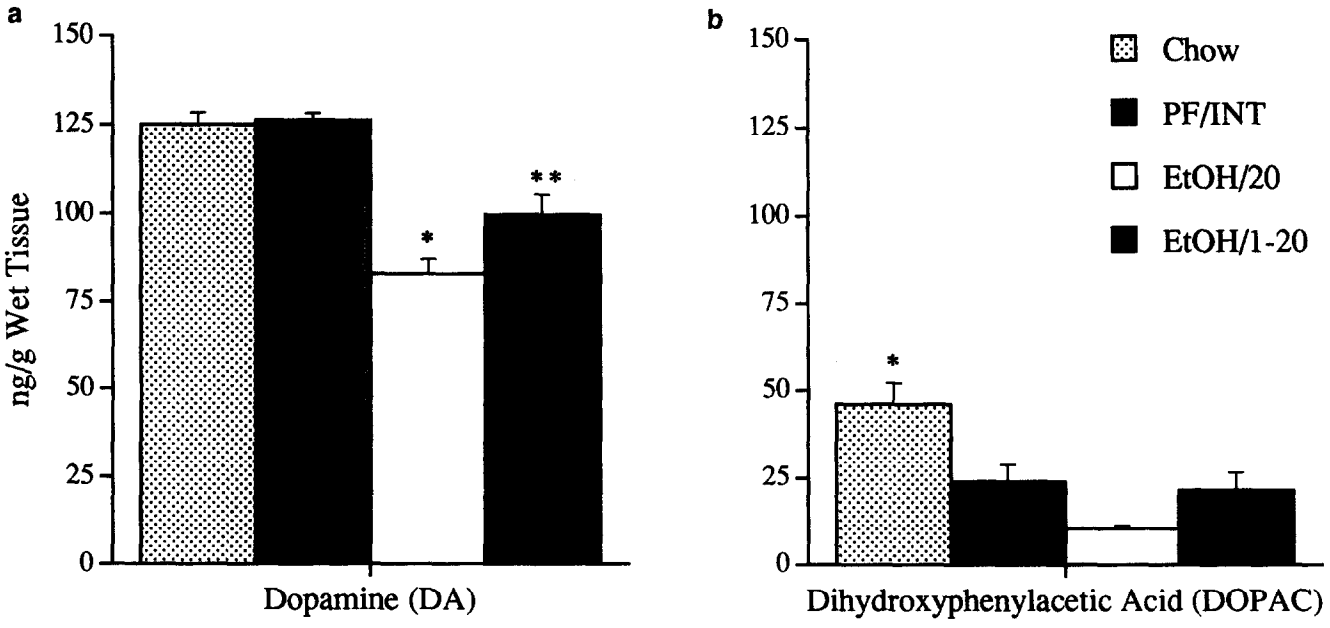

FIG. 2. (a) Mean whole brain dopamine content (DA) or (b) dihydroxyphenylacetic acid levels (DOPAC) in fetuses exposed to alcohol in utero and their appropriate controls. Data are expressed as ng/g wet tissue. Vertical bars represent standard error of the mean. *is significantly different from all other groups ($p < 0.05$). **is significantly different from PF/INT and Chow groups ($p < 0.05$).

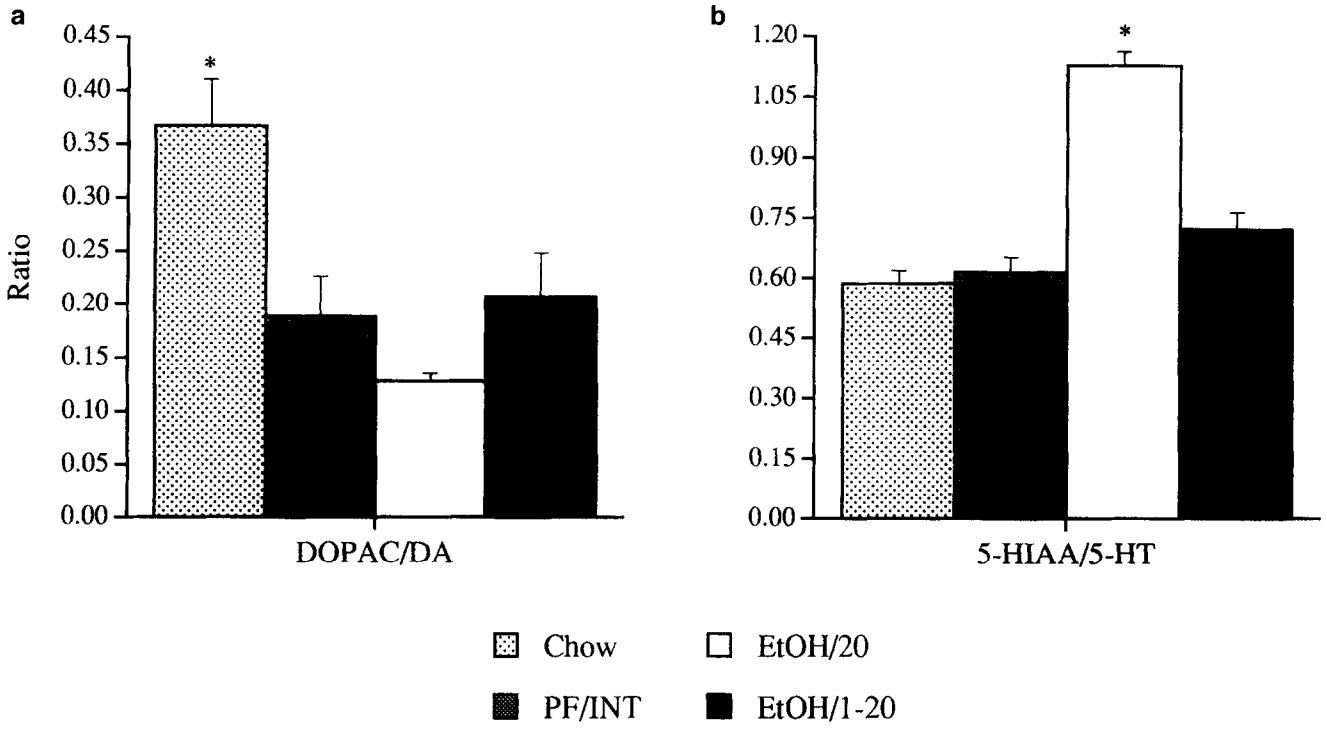

FIG. 3. (a) Mean ratio scores of dihydroxyphenylacetic acid (DOPAC) to dopamine (DA) and (b) 5-hydroxyindoleacetic acid (5-HIAA) to serotonin (5-HT) for the alcohol treatment and control groups. Data are expressed as the ratio of the metabolite (ng/g) to its respective neurotransmitter (ng/g). Vertical bars represent the standard error of the mean. *is significantly different from all other groups ($p < 0.05$).

can be explained in several ways. First, it is possible that there is regional vulnerability to the in utero alcohol exposure and in the present study, weighing the whole brain masked any regional differences. Previous research shows significant regional variation in the volumes of the hippocampus proper, dentate gyrus and vermal cerebellum in response to alcohol exposure during part of the third trimester equivalent in rats (26). Second, because the brain develops over time, it may be

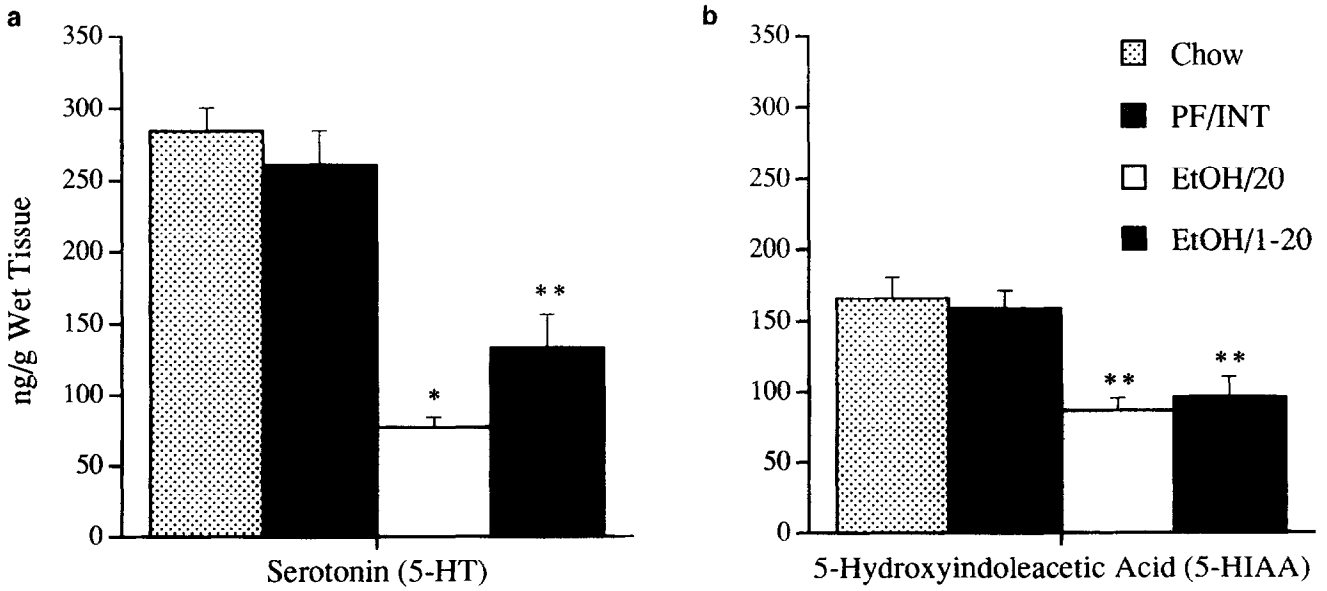

FIG. 4. (a) Mean whole brain serotonin content (5-HT) or (b) 5-hydroxyindoleacetic acid levels (5-HIAA) in fetuses exposed to alcohol in utero and their appropriate controls. Data are expressed as ng/g wet tissue. Vertical bars represent standard error of the mean. *is significantly different from all other groups ($p < 0.05$). **is significantly different from PF/INT and Chow groups ($p < 0.01$).

TABLE 3

CORRELATIONS AMONG THE VARIOUS ALCOHOL CONCENTRATIONS TAKEN FROM BLOOD AND AMNIOTIC FLUID SAMPLES

	Alcohol Treatment Group		
Comparison	EtOH/1-20	EtOH/20	Combined
Dam BAC vs. Fetus BAC	$r = 0.970$ ($n = 8$)	$r = 0.999$ ($n = 5$)	$r = 0.979$ ($n = 13$)
Dam BAC vs. Dam AAC	$r = 0.982$ ($n = 4$)	$r = 0.985$ ($n = 5$)	$r = 0.972$ ($n = 9$)
Fetus BAC vs. Dam AAC	$r = 0.893$ ($n = 4$)	$r = 0.986$($n = 5$)	$r = 0.951$ ($n = 9$)

Numbers in parentheses represent the number of litters.
BAC: blood alcohol concentration; AAC: amniotic alcohol concentration.

more or less vulnerable to teratogenic agents at a given time of development. In essence, a temporal window of vulnerability may exist for alcohol-induced brain weight deficits (or microencephaly: small brain for body size), but the first two trimesters equivalent are not the most vulnerable period for alcohol-induced microencephaly. Several studies have demonstrated frequent and severe microencephaly resulting from alcohol exposure during part of the third trimester equivalent (4,16). In a study comparing the results of alcohol exposure during the first or second or third or all three trimesters equivalent, Maier et al. (23) found that animals exposed to alcohol during part of the third trimester equivalent (postnatal days 4–9) had more severe microencephaly than those exposed to alcohol during all three trimesters equivalent or the combined first and second trimesters equivalent.

Although fetal neurochemical profiles were altered in response to gestational binge-like alcohol exposure, it is unlikely that changes in the levels of DA, 5-HT and GABA following 20 days of binge-like alcohol exposure were due to the presence of alcohol at the time of sacrifice. The comparisons between the EtOH/1-20 and EtOH/20 groups on these three major neurotransmitters demonstrated notably different patterns of change in neurotransmitter contents. Specifically, the average DA, 5-HT and GABA concentrations in EtOH/20 group were found to be much lower than those in EtOH/1-20 group suggesting a differential effect of the two different alcohol exposure regimens (long-term vs. short-term) on these measures. The present results also can be interpreted from a different perspective. The alcohol exposure on E20 represented an acute challenge of the responsiveness of transmitter systems to alcohol suggesting that the dopaminergic, serotonergic and GABAergic systems respond differently to the single alcohol challenge on E20 depending upon prior experience with alcohol. These data may be interpreted as similar to tolerance, whereby the decreased intensity of the response to a given dose of alcohol on E20 is due to the long-term alcohol exposure from E1-19. The results from the analysis of the DA metabolite showing a signficant difference in the DOPAC/DA ratio between the Chow group and all other groups indicates that both alcohol and pairfed treatments have a substantial impact in reducing DA turnover. On the contrary, the 5-HIAA to 5-HT ratio was much higher in the EtOH/20 group compared with all other groups, demonstrating a higher turnover in this group only.

Even though dopamine and serotonin levels were significantly depleted in the offspring of alcohol-treated rats in this

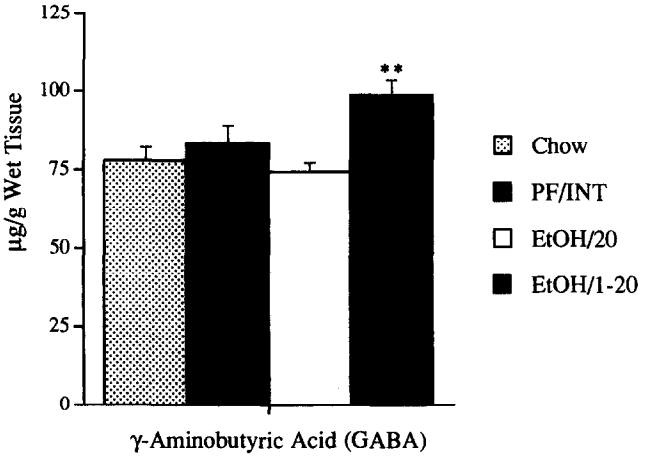

FIG. 5. Mean whole brain γ-aminobutyric acid (GABA) content in fetuses exposed to alcohol in utero and appropriate controls. Data are expressed as µg/g wet tissue. Vertical bars represent standard error of the mean. **is significantly different from all other groups ($p < 0.05$).

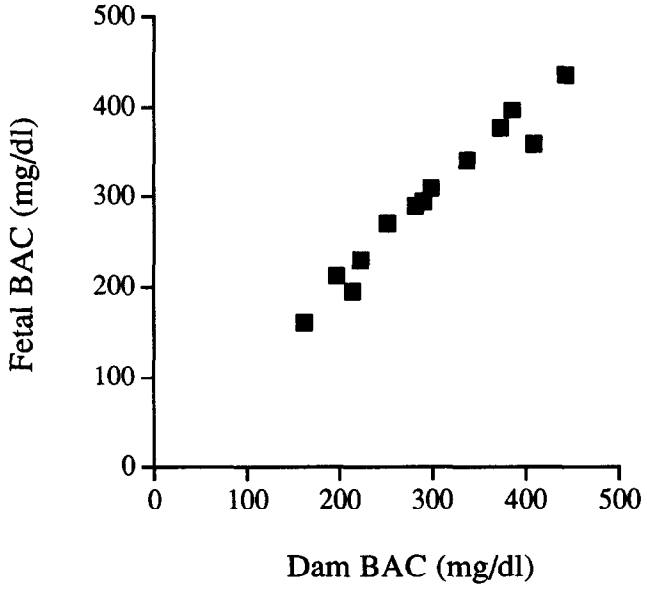

FIG. 6. Correlation between dam BAC and fetal BAC on E20 for all alcohol-treated animals (EtOH/1-20 and EtOH/20) is 0.979 ($n = 13$). The variance accounted for in fetal BAC by dam BAC is 95.8% (i.e., $r^2 = 0.958$).

study, similar to other studies (11,22,27), there were no differences in norepinephrine levels among the four groups, and there was an increase in GABA levels specifically in the EtOH/1-20 group. Detering et al. (10) found no changes in NE levels in whole brain tissue following combined pre- and postnatal alcohol exposure, even though they reported deficits in hypothalamic NE. Similarly, Cooper et al. (9) reported that pre- and postnatal alcohol exposure resulted in NE deficits only in the septal region, but not in cortex, cerebellum, or other regions. Therefore, it seems that alcohol-induced alterations in NE may be limited to a few specific brain regions and the analysis of whole brain or gross brain regions may not be sensitive enough to detect any alcohol-induced effects. With regard to GABA levels, Rawat (28) reported that prolonged maternal ethanol consumption significantly increased fetal and neonatal GABA levels, as we found in this study. However, no obvious change in GABA concentration was reported in other studies (13,25). These studies, taken together with our current findings, confirm that alcohol exposure from E1 through E20 produced significant changes in some major neurotransmitter systems, and these changes were not merely a function of the response to alcohol exposure on E20.

Neurotransmitters can act as trophic or regulatory factors. Early experimental evidence has shown that 5-HT depletion during pregnancy resulted in the retardation of neuronal differentiation in specific neuronal populations within specific brain regions, such as superior colliculus and hippocampus (21). In addition, Lankford et al. (19) showed that DA may act as a growth regulator during neuronal differentiation by affecting the motility of growth cones in embryonic chicken retinas. Furthermore, it has been reported that GABA may affect the expression of some neuron-associated proteins and the ultrastructure of developing cerebellar granule cells in vitro (18,24). In addition, other evidence has shown that neurotransmitters are critical in modulating behaviors ranging from sleep, to basic motor activities to higher cognitive functions. For example, for two specific non-reinforced associative learning paradigms, sensory preconditioning and latent inhibition, it has been shown that NE and DA are the major neurochemical substrates responsible for the performance of these two learning tasks, respectively (8,32). Collectively, these findings suggest that neurotransmitters may act as the developmental signals, regulators and modulators during brain development in addition to their classical roles in neurotransmission. Therefore, any changes in the balance of neurotransmitter concentrations in the developing nervous system in response to prenatal alcohol exposure may subsequently alter the development of neuronal cytoarchitecture and connectivity at either the gross or cellular level.

In summary, these results demonstrate the detrimental impact of prenatal exposure to alcohol on neurotransmitter levels in the fetal rat brain. Since neurotransmitters have wide ranging functions from the perspective of brain development (e.g., as regulatory or trophic factors) to the performance of postnatal behaviors (e.g., as learning and memory modulating factors), it is important to document the impact of prenatal alcohol exposure to account for the consequences of such exposure. While these data do not speak to the mechanisms of the alcohol-induced deficit, they add to the understanding of the spectrum of effects resulting from prenatal exposure to alcohol.

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