Effect of Prenatal and Postnatal Exposure to Ethanol on Rat Central Nervous System Gangliosides and Glycosidases

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We investigated the effect of maternal alcohol consumption on cell number, gangliosides and ganglioside catabolizing enzymes in the central nervous system (CNS) of the offspring. Virgin female rats of the Charles Foster strain were given 15% (v/v) ethanol in drinking water one month prior to conception and during gestation and lactation. At 21 days postnatal age, the offspring were sacrificed and the brains were separated into cerebrum, cerebellum and brain stem to investigate possible regional variations. Compared to controls, wet weight of cerebrum, cerebellum and brain stem, and of spinal cord was decreased in the pups exposed to alcohol. DNA and protein contents were also found to be lowered in all the CNS regions of the pups exposed to alcohol. Conversely, maternal alcohol consumption was found to increase the concentration and the content of total ganglioside *N*-acetylneuraminic (NANA) in CNS of the pups. In addition, alcohol treatment was found to induce alterations in the proportions of individual ganglioside fractions. Interestingly, these alterations are somewhat different than those observed in the neonatal brain and spinal cord of the pups subjected to prenatal alcohol exposure. The alterations in the proportions of ganglioside fractions were shown to be region-specific. Maternal alcohol consumption resulted in decreased activities of sialidase, β-galactosidase, β-glucosidase and β-hexosaminidase. The results suggest that the alcohol-associated increases in ganglioside concentration may be at least partly due to the decreased activities of ganglioside catabolizing enzymes.
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Alcohol consumption during pregnancy is detrimental to the fetus, and the nervous system has been shown to be affected most (1). Clusters of abnormalities observed in children exposed to alcohol *in utero* are described as fetal alcohol syndrome (FAS). The complete spectrum of abnormalities of FAS includes pre- and postnatal growth retardation, microcephaly, facial dysmorphology and central nervous system (CNS) dysfunction (2,3). To study the effects of alcohol on rat brain development, it is necessary to expose the offspring to alcohol during both pre- and postnatal periods, as rats, unlike humans, are born relatively immature with respect to their brain development (4).

The plasma membrane appears to be the primary target of the effects of alcohol; hence much research in this area has been focused on lipids, which are major constituents of neural membranes. However, most studies have been confined to cholesterol and phospholipids, which are important for maintaining membrane integrity and fluidity. Neural membranes (5), particularly those of brain, also are rich in gangliosides (6) which play an important role in a wide variety of cellular events, ranging from cell differentiation (7) to neuronal transmission (8). Despite their suggested importance, information on the effects of alcohol on CNS gangliosides is scanty and is mostly restricted to adult rats. There are no studies on the effects of pre- and postnatal alcohol exposure on the enzymes involved in the catabolism of gangliosides.

We have reported the effects of prenatal exposure to alcohol on rat brain gangliosides at birth. However, growth spurt in rat brain development (9) and the rapid phase of accumulation of gangliosides (10) occur during the postnatal period. As the brain is a heterogeneous organ, the present study was designed to follow the effects of pre- and postnatal exposure to alcohol on the cell number and protein content, and on gangliosides and their catabolizing enzymes in cerebrum, cerebellum, brain stem and spinal cord of pups at weaning.

MATERIALS AND METHODS

Experimental design and animals. Virgin female rats of the Charles Foster strain weighing 180–200 g were divided into two groups. A control group (CT) was given a 20% casein diet and drinking water *ad libitum,* and a second group of rats (AL) was given 20% casein diet and 15% alcohol (ethanol) in drinking water *ad libitum.* The rats were fed a semi-synthetic diet containing 20% sucrose, 20% casein, 7% ground nut oil (peanut oil), 0.3% DL-methionine, 0.2% choline bitartrate, 48% sago, 3.5% mineral mixture, and vitamin mixture (11). Both groups of rats were maintained on the respective regimen for four weeks prior to breeding and during gestation and lactation. After birth, pups were culled randomly and nursed in litters of eight. Pups were sacrificed by decapitation, at 21 days postnatal age, after the body weights had been recorded. Intact whole brains and spinal cords were quickly dissected and the weights were recorded. Whole brains were separated into cerebrum, cerebellum and brain stem on ice-cold glass plates. Tissue samples for DNA, protein and enzyme assays were processed immediately; the samples for ganglioside analysis were stored until use in a chloroform/methanol (1:1, v/v) mixture at −20°C.

Estimation of maternal plasma alcohol levels. Tail blood samples from the mothers were collected in the morning after birth. Alcohol was assayed using alcohol dehydrogenase (12). Blood ethanol concentration was found to be 87.3 ± 10 mg/dL (mean ± SD).

Estimation of DNA and protein. DNA was extracted by the method of Schneider (13) and was estimated according to the method of Burton (14). Protein was estimated by the method of Lowry *et al.* (15) using bovine serum albumin as standard.

Analysis of gangliosides. Total gangliosides were isolated and purified by the method of Seyfried *et al.* (16), and ganglioside *N*-acetylneuraminic acid (NANA) was assayed by the method of Warren (17) as modified by

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Abbreviations: AL, pups exposed to alcohol; BS, brainstem; C, cerebrum; CB, cerebellum; CNS, central nervous system; CT, control pups; FAS, fetal alcohol syndrome; NANA, *N*-acetylneuraminic acid; SC, spinal cord.

Skoza and Mohos (18). The details of these methods are described elsewhere (19). The ganglioside nomenclature used is that of Svennerholm (20). For the separation of individual gangliosides (GQ_1, GD_{1b}, GD_{1a}, GT_{1b} and GM), 20 × 20-cm glass plates coated with silica gel G were developed by one ascending run with chloroform/methanol/water containing 0.02% $CaCl_2 \times 2H_2O$ (58:38:9, v/v/v). Ganglioside bands marked after exposure to iodine vapors were scraped from the plates and NANA in the individual ganglioside fractions was estimated as described earlier. Since the concentration of monosialogangliosides such as GM_2 and GM_3 was extremely low, the bands corresponding to all monosialogangliosides were pooled and the fraction was designated GM. The bands were confirmed as gangliosides by using resorcinol-HCl reagent on a duplicate set, and the identity of individual ganglioside bands was confirmed by simultaneously running a commercially obtained reference bovine brain ganglioside mixture (Sigma, St. Louis, MO) (19).

Enzyme assays. A 10% (w/v) homogenate for enzyme assays was prepared by homogenizing the tissue with ice-cold, glass-distilled water for three minutes at 2000–3000 rpm using a Potter-Elvehjem homogenizer while cooling with ice. Activities of β-D-galactosidase (EC 3.2.1.23) and β-D-glucosidase (EC 3.2.1.21) were assayed according to the method of Gatt and Rapport (21). Total β-D-hexosaminidase (EC 3.2.1.30) activity was assayed according to the method of Frohwein and Gatt (22). Units for the abovementioned enzymes are defined as nmol of *p*-nitrophenol liberated per minute under the assay conditions used. Sialidase (EC 3.2.1.18) activity, using endogenous substrate alone, was assayed according to the method of Irwin *et al.* (23). The enzyme unit for sialidase is defined as nmol of NANA liberated per minute under assay conditions. Statistical comparison between group means was by Student's *t*-test (24). Differences between values were considered significant for $p < 0.01$.

RESULTS

In preliminary studies we found that providing a 15% (v/v) alcohol solution as the sole water source had no effect on the total calorie or water intake of rats (data not shown). However, higher concentrations of alcohol did cause a significant decrease in food and water intake. The data obtained for tissue weights, and DNA and protein levels in the CNS regions of 21-day-old pups exposed to alcohol *in utero* and during lactation are presented in Table 1. Pre- and postnatal exposure of rat pups to alcohol caused significant deficits in cerebrum, cerebellum, brain stem and spinal cord weights as compared to controls. The tissue weight loss was more severe in brain stem followed by cerebellum and cerebrum, in that order. The deficits in tissue weights were 11, 15, 29 and 16% in cerebrum, cerebellum, brain stem and spinal cord, respectively.

DNA content (mg DNA/total tissue) showed a significant decrease in all CNS tissues of the pups exposed to alcohol when compared to the control pups. DNA content in cerebrum, cerebellum, brain stem and spinal cord of the alcoholic pups was 84, 88, 83 and 66% of control values, respectively. These deficits in DNA contents were reflected in the reduced cell number. The reductions in cell numbers of pups exposed to alcohol were 16, 12, 17 and 34% of controls in cerebrum, cerebellum, brain stem and spinal cord, respectively. Similarly, protein content (mg/total tissue) showed a significant decrease in all CNS regions.

TABLE 1

Effect of Alcohol on DNA and Protein Levels in the Central Nervous System[a]

Tissue	Group	Tissue weight (g)	DNA		Protein		Cell number (in millions)
			mg/g tissue	mg/tissue	mg/g tissue	mg/tissue	
C	CT (n=6)	1.098 ±0.006	1.85 ±0.007	2.03 ±0.02	123 ±0.33	135 ±0.99	327 ±4.0
	AL (n=6)	0.980[b] ±0.002	1.730 ±0.02	1.70[b] ±0.02	121 ±0.35	118[b] ±0.42	274[b] ±3.2
CB	CT (n=6)	0.186 ±0.002	9.2 ±0.04	1.7 ±0.016	104 ±0.4	19.3 ±0.19	244 ±2.4
	AL (n=6)	0.159[b] ±0.001	9.50[b] ±0.06	1.50[b] ±0.014	101 ±0.22	16.2[b] ±0.05	227[b] ±3.0
BS	CT (n=6)	0.153 ±0.012	2.14 ±0.043	0.326 ±0.005	104 ±1.0	15.8 ±0.12	52.6 ±0.84
	AL (n=6)	0.108[b] ±0.002	2.47[b] ±0.037	0.270[b] ±0.005	100 ±0.07	11.0[b] ±0.17	43.5[b] ±0.8
SC	CT (n=6)	0.182 ±0.03	2.52 ±0.04	0.458 ±0.008	100 ±0.67	18.3 ±0.23	73.9 ±1.31
	AL	0.153[b] ±0.001	1.98[b] ±0.02	0.304[b] ±0.003	101 ±0.4	15.4[b] ±0.1	49.0[b] ±0.5

[a]CT, control pups born of mothers fed a 20% casein diet. AL, alcoholic pups born of mothers fed 15% alcohol; C, cerebrum; CB, cerebellum; BS, brainstem; SC, spinal cord; n, number of observations; values are mean ± SE.

[b]Values significantly different from the CT group if value of $p < 0.01$.

TABLE 2

Effect of Alcohol on CNS Gangliosides at Weaning[a]

Tissue	Group	Total gangliosides		Ganglioside species (% of total ganglioside NANA)				
		μg NANA/g tissue	μg NANA/tissue	GQ_1	GT_{1b}	GD_{1b}	GD_{1a}	GM
C	CT (n=8)	706 ±4.6	791 ±11.8	9.9 ±0.4	21.1 ±0.32	14.1 ±0.37	43.8 ±0.49	11.1 ±0.49
	AL (n=6)	954[b] +17	924[b] ±14	4.1[b] ±0.5	29.4[b] ±0.3	14.4 ±0.56	38.2[b] ±0.19	14.0[b] ±0.30
CB	CT (n=8)	398 ±6.5	71.1 ±1.5	6.7 ±0.5	44.3 ±0.2	10.3 ±0.3	26.5 ±0.39	12.5 ±0.38
	AL (n=8)	587[b] ±14	93.8[b] ±1.5	8.9[b] ±0.3	36.0[b] ±0.2	13.8[b] ±0.3	24.6 ±0.37	16.5[b] ±0.3
BS	CT (n=8)	395 ±13	59.6 ±2	3.3 ±0.3	34.7 ±0.3	22.1 ±0.7	22 ±1	17.8 ±0.7
	AL (n=6)	635[b] ±25	70.9[b] ±3	7.4[b] ±0.5	42.3[b] ±0.67	21.4 ±0.26	20.1 ±0.41	8.8[b] ±0.4
SC	CT (n=8)	291 ±4	56 ±1	18.6 ±0.2	31.6 ±0.8	13.3 ±0.6	22 ±0.4	14.6 ±0.5
	AL (n=8)	371[b] ±11	57.5 ±1.2	15.0[b] ±0.4	29.6 ±0.4	17.5[b] ±0.4	27.6[b] ±0.6	10.0[b] ±0.3

[a]CT, control pups born of mothers fed a 20% casein diet; AL, alcoholic pups born of mothers fed 15% alcohol; C, cerebrum; CB, cerebellum; BS, brain stem; SC, spinal cord; n, number of observations; values are mean ± SE.

[b]Values significantly different from the CT group if value of $p < 0.01$.

Administration of alcohol to rats during gestation and lactation caused an increase in total ganglioside NANA concentration (μg NANA/g tissue) in cerebrum, cerebellum, brain stem and spinal cord of the pups when compared to controls (Table 2). The increase in total ganglioside concentration was 35, 47, 61 and 27%, respectively, in cerebrum, cerebellum, brain stem and spinal cord, compared to control values. Despite reduced tissue weights, the content of total ganglioside NANA (μg NANA/total tissue) was significantly higher in cerebrum, cerebellum and brain stem of alcoholic pups when compared to controls.

Alcohol was also found to induce alterations in the proportions of individual ganglioside fractions, and the changes were found to be region-specific. In general, alcohol-induced alterations in the proportions of GQ_1, GT_{1b} and GD_{1a} were more pronounced. In cerebrum, the proportions of GT_{1b} and GM showed an increase, GQ_1 and GD_{1a} showed a decrease, and GD_{1b} was unaffected by exposure of pups to alcohol. On the other hand, in cerebellum, alcohol increased the proportions of GQ_1, GD_{1b} and GM and decreased the proportion of GT_{1b} without affecting the proportions of GD_{1a}. However, in brain stem the proportions of GQ_1 and GT_{1b} showed a significant increase and GM showed a decrease in pups exposed to alcohol. The proportions of GD_{1a} and GD_{1b}, which were unaltered in brain stem, showed an increase in the spinal cord of the alcoholic pups. However, the proportions of GQ_1 and GM showed a decrease in spinal cord due to alcohol.

Sialidase, β-glucosidase, β-galactosidase, pH 3.1 and β-hexosaminidase activities decreased in cerebrum, cerebellum, brain stem and spinal cord of the pups due to maternal alcohol consumption (Table 3). A similar decrease was observed in the β-galactosidase, pH 4.5 activity in cerebrum, cerebellum and brain stem following exposure to alcohol. However, alcohol was found to increase β-galactosidase, pH 4.5 activity in the spinal cord of alcoholic pups as compared to control pups. Sialidase, β-hexosaminidase, β-glucosidase, β-galactosidase, pH 3.1 and β-galactosidase, pH 4.5 activities, in cerebrum of alcohol exposed pups were only 65, 70, 69, 91, and 89% of control values. The corresponding values in cerebellum, brain stem and spinal cord were 78, 85, 75, 84 and 94%; 66, 61, 75, 78 and 77%; and 70, 88, 82, 93 and 77%, respectively.

DISCUSSION

In the present study rat pups were exposed to alcohol by feeding mothers with 15% alcohol one month before gestation, and during gestation and lactation. Alcohol consumed by the mother during gestation reaches the fetus as there is no placental barrier to alcohol (25); after birth, pups were exposed to alcohol through the mother's milk (26).

Alcohol-associated deficits in DNA content observed in the present study correlate well with the reported decrease in the incorporation of labeled thymidine into fetal tissues (27) and with impaired cellular multiplication observed *in vitro* due to alcohol (28). Alcohol also was found to decrease the levels of zinc and folate (29,30), which are required for DNA synthesis. Taken together the studies suggest that alcohol-associated cell loss may be due to

TABLE 3

Effect of Alcohol on Rat CNS Ganglioside Catabolizing Enzymes at Weaning[a]

Tissue	Group	β-D-Galactosidase[c] Activity at pH 3.1	β-D-Galactosidase[c] Activity at pH 4.5	β-D-Glucosidase[c]	β-D-Hexosaminidase[c]	Sialidase[c]
C	CT (n=10)	222 ±0.9	128 ±0.5	210 ±4.5	2930 ±32	13.1 ±0.41
	AL (n=10)	202[b] ±3.0	114[b] ±1.5	145[b] ±4.5	2053[b] ±64	8.5[b] ±0.78
CB	CT (n=10)	422 ±4.4	219 ±1.7	137 ±2	2897 ±39	7.9 ±0.78
	Al (n=10)	356[b] ±6.5	206[b] ±4.0	92[b] ±2.5	2460[b] ±46	6.2 ±0.61
BS	CT (n=10)	388 ±5.7	225 ±2.5	171 ±3.1	2695 ±60	8.9 ±0.14
	AL (n=10)	301[b] ±3.7	173[b] ±2.7	128[b] ±1.8	1652[b] ±43	5.9[b] ±0.11
SC	CT (n=10)	330 ±7.3	155 ±2.9	184 ±3.2	1985 ±39	8.1 ±0.76
	UN (n=10)	306[b] ±5.5	168[b] ±2.0	150[b] ±1.4	1742[b] ±43	5.7[b] ±0.36

[a] CT, control pups born of mothers fed a 20% casein diet; AL, alcoholic pups born of mothers fed 15% alcohol; C, cerebrum; CB, cerebellum; BS, brain stem; SC, spinal cord; n, number of observations; values are mean ± SE.

[b] Values significantly different from the CT group if value of $p < 0.01$.

[c] Enzyme units/g tissue.

impairment in DNA synthesis. Postnatal alcohol exposure has been shown to induce neuronal cell loss in hippocampus and cerebellum (31) and to delay the maturation of oligodendrocytes (32). Deficits observed in CNS cell numbers of the alcoholic pups are in line with a 29% cell loss in the cerebellum reported by Burns *et al.* (33) following the administration of alcohol (4 g/Kg body weight) between 6-16 days postnatal. In the present study, the cell loss was found to be 16, 12, 17 and 34% in cerebrum, cerebellum, brainstem and spinal cord, respectively, when compared to the controls. Alcohol also was found to decrease the protein content. This loss in protein content could be attributed to alcohol related impairment in the uptake of amino acids across the placenta (34,35) and in protein synthesis (36).

In contrast, the concentration of total ganglioside NANA (μg NANA/g tissue) and content (μg NANA/total tissue) was higher in all brain regions following exposure to alcohol. This increase in ganglioside concentration is in agreement with the reported increase in adult mouse (37) and rat (38) brain ganglioside concentration following chronic alcohol administration. In this context it may also be relevant to note that chronic alcohol treatment increases the intracellular fluid volume (39,40) and that maternal alcohol consumption increases water and sodium content in the fetus (41) and increases brain cell size of the offspring (33).

Although alcohol tends to fluidize biological membranes, chronic exposure to alcohol increases order in membranes by increasing the concentration of cholesterol (42). It is believed that the increase in cholesterol content, following chronic exposure to alcohol, is to confer rigidity to the membranes to counteract the fluidizing effects of alcohol. An increase in gangliosides has been shown to reduce the integrity of lipid bilayers (43) and to increase the sensitivity of liposomes to alcohol (44). Alcohol also was found to increase the content of unsaturated fatty acids of phospholipids in the synaptosomal fraction (45). Such an increase in the unsaturated fatty acids would also increase membrane fluidity.

It is of interest to note that the alcohol induced alterations in the proportions of individual gangliosides that were observed in the present study are somewhat different than the alterations observed in the neonatal pups subjected to alcohol exposure *in utero* (19). In addition, the changes in the ganglioside proportions are region-specific. For example, trisialoganglioside GT_{1b} showed an increase in cerebrum and brain stem and a decrease in cerebellum following exposure to alcohol. On the other hand, disialoganglioside GD_{1a} showed a decrease in cerebrum and an increase in spinal cord due to alcohol. Polysialoganglioside GQ_1 showed a decrease in cerebrum and spinal cord and an increase in cerebellum and brain stem of pups exposed to alcohol. Monosialogangliosides (GM) showed an increase in cerebrum and a decrease in the myelin rich regions, such as brain stem and spinal cord, due to alcohol. This decrease in monosialoganglioside (GM) is consistent with the reported decrease in monosialoganglioside GM_1 in myelin of 24-day-old rat pups born of mothers fed 6.6% (v/v) ethanol diet prior to breeding and during gestation (46). In addition, postnatal alcohol exposure was found to delay the acquisition of myelin by rat optic nerve (47).

In conclusion, it would appear that the changes in gangliosides observed can have profound implications in various surface events, such as cell differentiation, cell-to-cell interaction, and synaptogenesis, as gangliosides are located on the outer layer of the plasma membrane with their negatively charged polysaccharide chains protruding towards the extracellular space.

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