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Increased surface glycoconjugates of synaptic membranes in mice during chronic ethanol treatment

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Synaptosomal plasma membranes from mice treated chronically with ethanol were incubated with galactose oxidase and [^{3}H]-sodium borohydride, in order to label the exposed galactose and N-acetyl-galactosamine groups of glycoconjugates. The ethanol treatment approximately doubled the amount of exposed sugars. This change may be related to previously observed alterations in the physical properties of neuronal membranes in ethanol-treated mice.

Introduction Neuronal cells contain substantial amounts of gangliosides and glycoproteins with terminal galactose or N-acetyl-galactosamine residues. The precise function of these compounds is unknown, but their high concentration in the brain suggests that they must be important for the function of the membranes. Thus it is reasonable to examine their response to membrane-disordering agents such as ethanol. During chronic administration of ethanol, neuronal membranes become relatively resistant to the disordering effect of alcohols *in vitro* (Chin & Goldstein, 1977a). This physical change must be mediated by some alteration in the chemical composition of the membranes. Here we investigated whether surface glycoconjugates might be among the altered compounds.

In earlier studies (Mahadik, Hungund & Rapport, 1978; Hungund & Mahadik, 1981) we demonstrated that the sensitive galactose oxidase-[^{3}H]-sodium borohydride procedure can monitor surface glycoproteins and glycolipids in rat brain synaptic membranes, as had earlier been shown by Steck & Dawson (1974) for erythrocyte membranes. The principle of the method is the use of galactose oxidase to oxidize the terminal galactose and N-acetyl-galactosamine residues of glycoconjugates; the resulting aldehydes are reduced to their original sugars with tritiated sodium borohydride (NaB^3H_4), thus labelling the terminal galactose and N-acetyl-galactosamine.

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Methods Male Swiss Webster mice were exposed to ethanol vapour for 72 h, using daily injections of pyrazole (68 mg/kg) to stabilize the blood alcohol levels at 1.5 to 3.0 mg/ml (Goldstein, 1972). Control mice received daily pyrazole injections. Concentrations of ethanol in the chamber air and in tail blood were determined enzymatically (Lundquist, 1959). Immediately after ethanol withdrawal, whole brains were removed from the ethanol-treated and control mice, and two brain homogenates were pooled for each preparation of synaptosomal plasma membranes (Jones & Matus, 1974; Chin & Goldstein, 1977b).

Exposed galactose or N-acetyl-galactosamine residues of synaptic membranes were labelled as described earlier (Mahadik *et al.*, 1978; Hungund & Mahadik, 1981) with some modifications. The membranes (800–1000 μg of protein) in a final volume of 2.0 ml potassium phosphate buffer, 100 mM, pH 7.0, were incubated overnight at 37°C with 25 units of galactose oxidase per mg protein. Membranes were washed in 100 mM phosphate-buffered saline, pH 7.0 and incubated with 1.66 mCi of [^{3}H]-sodium borohydride for 15 min at room temperature. Unlabelled sodium borohydride (1 mg) was added to ensure the completion of the reduction of borohydride-reducible groups. The reaction mixture was sedimented at 100,000 *g* for 20 min. The pellet was washed, resuspended, and used for determination of protein and radioactivity (Mahadik *et al.*, 1978).

Galactose oxidase (D-galactose:oxygen 6-oxidoreductase; EC 1.1.3.9, 220 units/mg protein) and sodium borohydride were purchased from Sigma Chemical (St. Louis, MO). Tritiated sodium borohydride (5 Ci/mmol) was from New England Nuclear (Boston MA).

Results The mice that were exposed to ethanol vapour had a mean blood ethanol level of 2.3 mg/ml. This concentration, maintained over 3 days, produces tolerance and physical dependence (Goldstein, 1972). The ethanol-treated mice lost 10% of their body weight, whereas the pyrazole-treated controls lost only 4%.

Table 1 shows that borohydride alone could react

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Table 1 Labelling of membrane glycoconjugates by the galactose oxidase-[^{3}H]-sodium borohydride procedure

	n	*BH*	*GAO + BH*	*Specific labelling*
		(ct min^{-1} mg^{-1} protein $\times 10^{-6}$)		
Ethanol-treated	7	8.8 ± 1.3	14.5 ± 0.9	5.7 ± 0.9*
Control	6	10.2 ± 1.6	13.1 ± 1.6	2.9 ± 0.5

Values are means ± s.e.
n: number of samples, each prepared from two mouse brains.
BH: Nonspecific incorporation of labelled tritium from borohydride in the absence of galactose oxidase. GAO + BH: galactose oxidase incubation followed by treatment with labelled borohydride. Specific labelling: (GAO + BH) − BH. This represents the exposed galactose or N-acetyl-galactosamine.
*$P < 0.05$ by Student's *t* test, vs. controls.

with membranes from ethanol-treated mice and controls. The 9 or 10 million ct/min in each group represent a variety of borohydride-reducible aldehydes and ketones, i.e., the nonspecific binding. The labelling was increased when there had been a prior incubation with galactose oxidase. The specific labelling, calculated by difference, amounted to 2.9×10^6 ct/min in controls and 5.7×10^6 in the membranes from ethanol-treated mice. The amount of exposed galactose or N-acetyl-galactosamine in the membranes from ethanol-exposed animals was double that of the controls. The groups differed significantly ($P < 0.05$).

Discussion These results are similar to those of other workers who measured exposed sialic acid, another component of glycoproteins and glycolipids. (All glycolipids that contain sialic acid have terminal galactose or N-acetylgalactosamine and thus were included in our assays; in addition we can detect glycoproteins that lack sialic acid). Ross, Kibler & Cardenas (1977) reported that two weeks of ethanol treatment produced a 70% increase in exposed sialic acid in rat brain membranes. Noble, Syapin, Vigran & Rosenberg (1976) found that astroblasts grown in 100 mM ethanol for a few months had increased amounts of sialic acid that was releaseable by neuraminidase. The total sialic acid was unaffected. Glycoproteins, rather than glycolipids, were the source of the released sialic acid.

These experiments suggest that the synaptosomal membranes of ethanol-treated mice have undergone some change in chemical and physical properties that allows the glycoproteins or glycolipids to move further into the aqueous phase, thus making them vulnerable to the galactose oxidase. This may be related to our previous demonstration (Chin & Goldstein, 1977a) that chronic ethanol treatment alters synaptic membranes. The modified membrane lipids may force the surface proteins or glycolipids into more exposed positions. The observed changes may be part of the adaptive mechanism that is thought to occur during exposure to alcohols.

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