PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 1–5 (1987)

Chronic Ethanol Administration Alters Hepatic Surface Membranes as Evidenced by Decreased Concanavalin A Binding (42508)

JORDAN P. METCALF, CAROL A. CASEY, MICHAEL F. SORRELL, AND DEAN J. TUMA

Liver Study Unit, Veterans Administration Medical Center, and the Departments of Internal Medicine and Biochemistry, University of Nebraska Medical Center, Omaha, Nebraska 68105

Abstract. The effects of chronic ethanol administration on the hepatic surface membrane were examined. The binding of the lectin, concanavalin A (Con A), to isolated hepatocytes was used to ascertain changes in the hepatic plasma membrane, especially in regard to glycoprotein composition, due to chronic ethanol feeding. Hepatocytes, isolated from rats fed ethanol for 5 to 7 weeks, had a decreased ability to bind Con A when compared to hepatocytes from either the pair-fed controls or *ad libitum* chow-fed rats. Since decreased Con A binding was more apparent at high Con A concentrations, reduced lectin binding likely reflected changes in the composition of surface membrane glycoproteins in the livers of the ethanol-fed rats. When ethanol (50 m*M*) was added to the incubation medium containing hepatocytes from ethanol-fed rats, pair-fed controls, or chow-fed rats, no effects on Con A binding were observed. These results indicate that chronic ethanol administration induces changes in the oligosaccharide chains of plasma membrane glycoproteins in the liver. Such alterations may play a role in the pathogenesis of alcoholic liver disease.

The toxic effects of ethanol on the hepatocyte manifest themselves in many different ways. It is known that ethanol deranges such varied hepatocellular processes as amino acid uptake (1), lipid (2), and drug metabolism (3, 4), and protein and glycoprotein synthesis and secretion (5–8). With regard to the effects on glycoprotein metabolism, alcohol appears not only to alter glycoprotein synthesis, but also to affect the transport of these glycoproteins to their appropriate subcellular or extracellular destinations (9–12). One would expect that this disordering of glycoprotein trafficking would effect an alteration in cellular membrane composition, particularly in the various surface receptors and cell markers which are largely composed of glycoproteins. In this regard, several groups have shown that chronic ethanol administration results in altered receptor number or affinity for the estrogen (13), glucagon (14), and insulin (15) receptors. In this study, we examined the effects of ethanol on cell surface glycoproteins using the carbohydrate probe concanavalin A (Con A). Con A is a lectin with a molecular weight of 110,000 that has specific binding affinity for α-mannosyl and α-glucosyl residues of glycoproteins (16). Since cell surface glycoproteins have substantial amounts of these residues, alterations in the assembly or the trafficking of these glycosylated proteins should result in altered surface binding of Con A. Therefore, we investigated the effects of chronic ethanol administration on the lectin binding properties of the hepatic plasma membrane.

Materials and Methods. Young male Sprague–Dawley rats were obtained from Small Animal Supply Co. (Omaha, NE) and were fed Purina rat chow until they reached 140–150 g. The rats were then divided into three groups. The first group was fed Purina rat chow *ad libitum* until the time of the experiment (5–7 weeks). The remaining rats were housed in individual cages and fed the Lieber–DeCarli liquid control diet (17) (Bio-Serve, Inc., Frenchtown, NJ) for 5 days. These rats were then weight matched and paired so that one rat received the liquid diet containing ethanol as 36% of total calories while the second animal was pair-fed the isocaloric control diet in which ethanol was replaced isocalorically with carbohydrate. The rats were pair-fed for 5–7 weeks. In the 48 hr prior to death, the animals were fed the liquid diets in two divided doses and in the last 24 hr prior to the experiment the diet was divided into three portions in order to minimize differences in

0037-9727/87 $1.50

feeding patterns prior to the preparation of hepatocytes.

Hepatocytes were prepared according to the method of Seglen (18) with the following modifications. The livers were perfused *in situ* with 0.05% collagenase Type IV (Sigma Chemical Co., St. Louis, MO) for 10–15 min. The liver capsule was then incised and suspensions of the cells were agitated for 10 min at 37°C. The resultant cell mixture was then filtered through nylon mesh (Small Parts Inc., Miami, FL) and purified by repeated centrifugation and washing. The cell pellets wre resuspended in Eagle's medium and viability was determined by the trypan blue dye exclusion test. Cell viabilities averaged 80, 77, and 74% for the chow-fed, pair-fed control and ethanol-fed groups, respectively. Cells were then placed on continuous Percoll (Pharmacia AB, Uppsala, Sweden) gradients which were prepared at an initial density of 1.06 g/ml. Use of the Percoll gradient resulted in a yield of cells which were more than 85% viable in all cases. The resultant cell suspension was then diluted to 50 mg wet wt cells/ml and pipetted into reaction flasks containing various concentrations of [^{3}H]concanavalin A (Amersham, Arlington Heights, IL) in a total volume of 1 ml Eagle's/Hepes buffer.

Con A binding studies were conducted according to the method of Brewster *et al.* (19) as adapted to determine binding to whole cells. Total and nonspecific Con A binding were directly measured by preincubating the cells with either the buffer or the inhibitory sugar, α-methyl-mannoside (1.0 *M*), respectively, prior to the addition of the cells to the reaction flasks. Specific binding was calculated as the difference between total and nonspecific binding. Binding was performed at 0–4°C for 120, 180, and 240 min at which time 0.2-ml aliquots were removed and added to 8 ml of Eagle's/Hepes buffer to quench the reaction. The cells were then centrifuged for 10 min and washed twice in 4 ml of buffer and the pellets frozen overnight at −20°C. Pellets were then solubilized in 0.5 ml of 1.0 *N* NaOH by heating in a 60°C water bath for 30 min. Aliquots were withdrawn for protein determination by the method of Lowry (20) and for determination of cell associated radioactivity by liquid scintillation counting. Cell aliquots were also used for measurement of DNA content by the method of Burton (21) and for the determination of cell number by an automated Coulter counter. Results of binding were expressed as femtomoles of Con A bound per microgram of protein, per microgram of DNA, and per 10^6 cells. Statistical analysis was carried out using Student's *t* test.

Results. To characterize the Con A binding properties of hepatocytes, we initially conducted binding studies using cells from Purina chow-fed animals. The effect of Con A concentration on the extent of binding is shown in Fig. 1A. Total and specific binding increased rapidly with increasing Con A concentrations up to 100 μg/ml, at which point binding increased much more gradually. Nonspecific binding was less than 15% of total binding at all concentrations tested. The effect of time of incubation on Con A binding to hepatocytes indicated that binding was essentially completed by 180 min of incubation (Fig. 1B).

The binding of Con A to hepatocytes prepared from ethanol-fed rats, pair-fed controls, and chow-fed rats was then determined. For these experiments, we chose two concentrations of Con A (1 and 100 μg/ml), and binding was measured after 120, 180, and 240 min of incubation. The binding data in these experiments were expressed as femtomoles of Con A bound per million cells or per microgram of DNA since hepatic DNA content is probably unaffected by ethanol feeding whereas both liver weight and liver protein content are known to be increased (11). During the 240-min incubation there was a slight decrease in cell viability. Initially, cell viability was over 85% for cells from all three experimental groups, but viability decreased to 69, 71, and 65% for the chow-fed rats, pair-fed controls, and ethanol-fed groups, respectively, after 240 min of incubation. When hepatocytes were incubated with the high concentration of Con A (100 μg/ml), the total binding of Con A to cells obtained from ethanol-fed rats was significantly lower than the binding to cells from either the pair-fed controls or the chow-fed animals (Fig. 2A). On the other hand, no differences in total binding among the three groups were observed when cells were incubated with the low Con A concentration (1 μg/ml; data not shown). Nonspecific binding did not differ significantly among the three groups tested at either concentration or at any

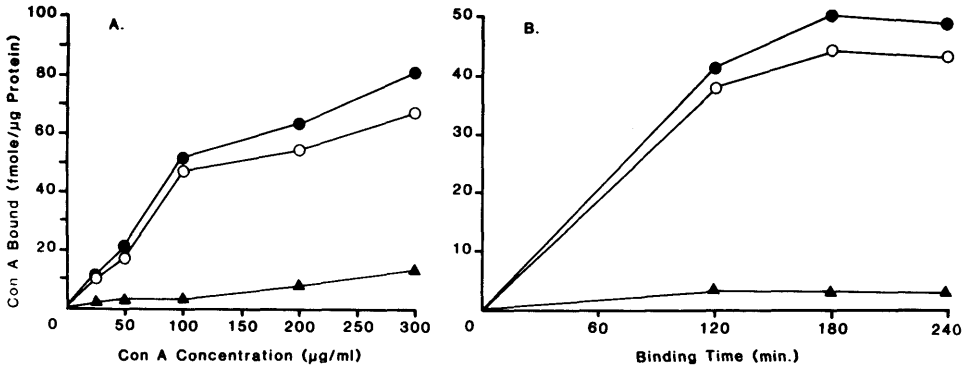

FIG. 1. Characterization of Con A binding to hepatocytes isolated from chow-fed rats. Binding to hepatocytes was determined (A) with varying concentrations of Con A at 0–4°C after 180 min of incubation, and (B) with a Con A concentration of 100 μg/ml at 0–4°C for varying time periods of incubation. Total binding (●) and nonspecific binding (▲) were determined in the absence and presence of α-methyl-mannoside, respectively, as described under Materials and Methods. Specific binding (○) was derived by subtracting the value of nonspecific binding from that of total binding.

time period (data not shown). The differences found in specific binding were very similar to those found in total binding in that specific binding at the high concentration of Con A was significantly lower in hepatocytes from the ethanol-fed rats than in the two control groups (Fig. 2B), whereas at the lower concentration of Con A there were no differences among the three groups (data not shown). Furthermore, there was no difference in total, nonspecific, or specific binding between the chow-fed and pair-fed control groups at either concentration or any binding time (Fig. 2). Identical effects of chronic ethanol on hepatic Con A binding were also evident when the binding data was expressed as femtomoles bound per microgram of DNA (data not shown).

To examine the acute effects of ethanol on

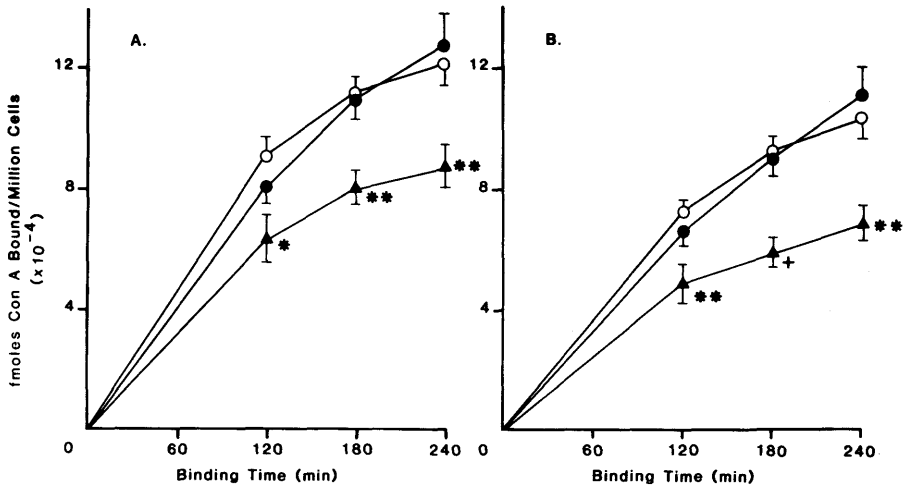

FIG. 2. Effect of chronic ethanol administration on total (A) and specific (B) Con A binding by the liver. Hepatocytes were isolated from ethanol-fed rats (▲), pair-fed controls (○), and chow-fed rats (●) and were incubated in the presence of Con A at a concentration of 100 μg/ml at 0–4°C. Con A binding (fmole/million cells) was expressed as mean ± SEM for seven to nine determinatons. Values significantly different from pair-fed controls are indicated: $*P < 0.05$; $**P < 0.01$; $+P < 0.001$.

Con A binding to hepatocytes, we added ethanol to a final concentration of 50 m*M* to the reaction flasks and measured binding after 180 and 240 min of incubation. No changes in total, nonspecific, or specific binding among hepatocytes from the three groups were observed as a result of the presence of 50 m*M* ethanol (data not shown).

Discussion. The initial studies of Con A binding to hepatocytes isolated from chow-fed controls showed results similar to those previously reported by others using isolated hepatic plasma membranes (22, 23). The results indicate that saturation of the binding process was not observed even at high concentrations of Con A and that binding was essentially completed after 180 min of binding (Fig. 1). From consideration of these initial binding experiments, the effects of chronic ethanol administration on Con A binding to isolated hepatocytes were studied, using both a high concentration (100 μg/ml) and a low concentration (1 μg/ml) of Con A in the incubation medium.

The results of this study demonstrate that hepatocytes isolated from chronically ethanol-fed rats have a decreased ability to bind the lectin, Con A, when compared to hepatocytes isolated from either pair-fed controls or chow-fed rats. Since Scatchard analysis of Con A binding is not appropriate as shown by others (22, 23), we were not able to directly distinguish whether altered binding reflected changes in affinity or binding site number. However, the observation that altered binding was noted at the high Con A concentration but not at the low concentration would suggest that the decreased number of binding sites is the most likely explanation for the reduced Con A binding by hepatocytes from ethanol-fed animals. This conclusion is supported by the studies of Henriquez *et al.* (22) who found that the decreased binding of Con A to hepatic plasma membrane isolated from rats fed a high fat diet correlatd with decreases in chemically determined plasma membrane-bound carbohydrates. Therefore, in our studies decreased Con A binding likely reflects a change in the composition of surface membrane glycoproteins in the livers of the ethanol-fed animals. Our previous studies (12, 24), indicating impaired plasma membrane assembly by the liver of ethanol-treated rats, are consistent with this conclusion. Decreased assembly or incorporation of glycoproteins into the plasma membrane, as reported in our previous studies (12, 24), should result in less glycoproteins in the plasma membrane, and thus, fewer carbohydrate residues would be available on the exterior surface for lectin binding.

The failure to observe effects on Con A binding in the presence of ethanol (50 m*M*) in the incubation medium of hepatocytes isolated from either ethanol-fed rats, pair-fed controls, or chow-fed animals would indicate that ethanol per se does not directly alter Con A binding. Instead, it appears that long-term exposure of ethanol (chronic ethanol feeding) is required to impair hepatic Con A binding. This finding is not surprising since one would expect that a decrease in cell surface glycoproteins would be a time-requiring process.

The possible implications of these studies are important and potentially relevant in respect to ethanol-induced changes in hepatic structure and function. Changes in hepatic surface membrane characteristics induced by chronic ethanol feeding, as manifested by decreased binding of Con A in these studies, could be damaging to the liver. Changes in the glycoprotein composition of the surface membrane could result in alterations in cell-to-cell associations, cellular integrity, and receptor-mediated events.

This investigation was supported by the Veterans Administration and by Grant AA04961 from the National Institute on Alcohol Abuse and Alcoholism. We gratefully acknowledge the excellent technical assistance of Sandy Kragskow and thank MaryAnne Nowicki for skillful preparation of the manuscript.

1. Rosa J, Rubin E. Effect of ethanol on amino acid uptake by rat liver cells. Lab Invest **43**:366–372, 1980.
2. Baraona E, Lieber CS. Effects of ethanol on lipid metabolism. J Lipid Res **20**:289–315, 1979.
3. Rubin E, Lieber CS. Hepatic microsomal enzymes in man and rat: Induction and inhibition by ethanol. Science **162**:690–691, 1968.
4. Rubin E, Gang H, Misra PS, Lieber CS. Inhibition of drug metabolism by acute ethanol intoxication: A hepatic microsomal mechanism. Amer J Med **49**:801–806, 1970.
5. Jeejeebhoy KN, Phillips MJ, Bruce-Robertson A, Ho J, Sodtke U. The acute effect of ethanol on albumin, fibrinogen, and transferrin synthesis in the rat. Biochem J **126**:1111–1126, 1972.

6. Baraona E, Leo MA, Borowski SA, Lieber CS. Pathogenesis of alcohol-induced accumulation of protein in the liver. J Clin Invest **60**:546–554, 1977.
7. Sorrell MF, Tuma DJ, Schafer EC, Barak AJ. Role of acetaldehyde in the ethanol-induced impairment of glycoprotein metabolism in rat liver slices. Gastroenterology **73**:137–144, 1977.
8. Sorrell MF, Tuma DJ. Selective impairment of glycoprotein metabolism by ethanol and acetaldehyde in rat liver slices. Gastroenterology **75**:200–205, 1978.
9. Tuma DJ, Jennett RB, Sorrell MF. Effect of ethanol on the synthesis and secretion of hepatic secretory glycoproteins and albumin. Hepatology **1**:590–598, 1981.
10. Tuma DJ, Sorrell MF. Effects of ethanol on glycoprotein synthesis and secretion during inflammation-induced stimulation of hepatic glycoprotein secretion. Toxicol Appl Pharmacol **63**:303–311, 1982.
11. Sorrell MF, Nauss JM, Donohue TM, Tuma DJ. Effects of chronic ethanol administration on hepatic glycoprotein secretion in the rat. Gastroenterology **84**: 580–586, 1983.
12. Malliard ME, Sorrell MF, Volentine GD, Tuma DJ. Impaired plasma membrane glycoprotein assembly in the liver following acute ethanol administration. Biochem Biophys Res Commun **123**:951–958, 1984.
13. Chronholm T, Eriksson H. Effects of prolonged ethanol administration on the hepatic estrogen receptor in the rat. FEBS Lett **133**:272–274, 1981.
14. Lee H, Hosein EA. Chronic alcohol feeding and its withdrawal on the structure and function of the rat liver plasma membrane: A study with ^{125}I-labeled glucagon binding as a metabolic probe. Canad J Physiol Pharmacol **60**:1171–1176, 1982.
15. Rifkin RM, Todd WW, Toothaker DR, Sussman A, Trowbridge M, Draznin B. Effects of in vivo and in vitro alcohol administration on insulin binding and glycogenesis in isolated rat hepatocytes. Ann Nutr Metab **27**:313–319, 1983.
16. Alroy J, Ucci AA, Pereira MEA. Lectins: Histochemical probes for specific carbohydrate residues. In: Delellis RA, Ed. Advances in Immunochemistry. New York, Masson, pp67–88, 1984.
17. Lieber CS, De Carli LM. The feeding of alcohol in liquid diets: Two decades of applications and 1982 update. Alcoholism: Clin Exp Res **6**:523–531, 1982.
18. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol **13**:29–83, 1976.
19. Brewster DW, Madhukar BV, Matsumura F. Influence of 2,3,7,8,-TCDD on the protein composition of the plasma membrane of hepatic cells from the rat. Biochem Biophys Res Commun **107**:68–74, 1982.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
21. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J **62**:315–323, 1956.
22. Henriques DS, Tepperman HM, Tepperman J. Effects of high-glucose and high-fat diets on concanavalin A binding to rat liver plasma membranes and on the amount and pattern of their glycoprotein carbohydrates. J Lipid Res **20**:624–630, 1979.
23. Chandramouli V, Stanford W, Marshall JS, Carter J. Cell surface changes in diabetic rats: Studies of lectin binding to liver cell plasma membranes. Biochim Biophys Acta **465**:19–33, 1977.
24. Tuma DJ, Mailliard ME, Casey CA, Volentine GD, Sorrell MF. Ethanol-induced alterations of plasma membrane assembly in the liver. Biochim Biophys Acta **856**:571–577, 1986.

Received September 2, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted December 30, 1986.