Biochimica et Biophysica Acta 856 (1986) 571–577
Elsevier

BBA 73051

Ethanol-induced alterations of plasma membrane assembly in the liver

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(Received November 6th, 1985)

Key words: Plasma membrane; Glycoprotein incorporation; Ethanol effect; (Rat liver)

The effects of acute ethanol administration on the assembly of glycoproteins into the hepatic plasma membrane were studied in the rat. When [^{14}C]fucose and *N*-acetyl[^{3}H]mannosamine, a sialic acid precursor, were injected following an acute dose of ethanol, the incorporation of these precursors into the total pool of membrane glycoproteins was minimally affected. This finding indicated that ethanol treatment did not appreciably alter the glycosylation of proteins in the Golgi apparatus. However, the assembly of labeled fucoproteins and sialoproteins into the plasma membrane was markedly inhibited in the ethanol-treated animals. This inhibition of plasmalemmal glycoprotein assembly was accompanied by a corresponding accumulation of labeled glycoproteins in the cytosolic fraction of the hepatocyte. The content of labeled glycoproteins in the Golgi complex was not significantly altered by ethanol treatment. These results indicate that ethanol administration impairs the late stages of hepatic plasma membrane assembly and further suggest that ethanol administration interferes with the flow of membrane components from the Golgi apparatus to the surface membrane.

Introduction

The biosynthetic pathway of glycoprotein assembly into the plasma membrane has been shown to be similar, in many respects, to the secretory pathway of glycoproteins [1–5]. The various steps involved in plasmalemmal glycoprotein assembly have been described in a variety of systems including the liver [1,6–10]. The protein moiety of glycoproteins is synthesized on membrane-bound polysomes and is vectorially inserted into the rough endoplasmic reticulum where core-glycosylation takes place. The glycosylated protein is then transferred to the cis elements of the Golgi apparatus where the appropriate trimming of the oligosaccharide chain occurs. The distal sugars, including fucose and sialic acid, are then added in the trans region of the Golgi. The completed glycoproteins are packaged into vesicles and are transported for assembly into the plasma membrane.

Previous studies from our laboratory [11–15] have shown that both acute and chronic ethanol administration impairs the final steps of hepatic glycoprotein secretion, following the incorporation of the terminal sugars in the Golgi complex. This ethanol-induced secretory defect results in the retention of secretory glycoproteins in the liver. Recently, we have also shown that the assembly of labeled fucoproteins and sialoproteins into the plasma membrane was markedly inhibited in ethanol-treated rats [16]. Therefore, the purpose of this study was to further clarify the effects of acute ethanol administration on hepatic plasma membrane assembly. In this regard, we evaluated the

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influence of ethanol treatment on the translocation of fucose- and sialic acid-labeled glycoproteins from the Golgi apparatus to the plasma membrane.

Materials and Methods

Materials. L-[1-^{14}C]Fucose (59 mCi/mmol), *N*-acetyl-D-[6-^{3}H]mannosamine (27 Ci/mmol) and scintillation fluid, Aquasol, were purchased from New England Nuclear, Boston, MA.

Animal procedures. Male Sprague-Dawley rats were maintained on a standard laboratory diet with water ad libitum. The nonfasted rats (200–250 g) were given ethanol (6 g/kg body wt.) by gastric intubation, and the control animals received an isocaloric dose of glucose. Two hours after intubation, [^{14}C]fucose (10 μCi/100 g body wt.) and *N*-acetyl[^{3}H]mannosamine (50 μCi/100 g body wt.) were injected simultaneously into the dorsal vein of the penis. At various times following injection of the labels, the rats were killed by aortic exsanguination (under ether anesthesia) and blood samples were collected. Their livers were then perfused with ice-cold saline excised and weighed.

Isolation of Golgi, cytosol and plasma membrane fractions. The total homogenate, cytosol, and Golgi fractions were prepared by modifications of the methods of Ehrenreich et al. [17] and of Redman et al. [18] as previously described [15]. Briefly, weighed portions of the liver (about 5 g) were homogenized in 5 vol. ice-cold 0.25 M sucrose/10 mM Tris-HCl (pH 7.4). The homogenate was cleared of cell debris, nuclei, and mitochondria by centrifugation at 10000 $\times$ *g* for 10 min, and a total microsomal fraction was obtained by centrifuging the 10000 $\times$ *g* supernate at 105000 $\times$ *g* for 90 min. The resulting 105000 $\times$ *g* final supernate represented the cytosolic fraction. The microsomal pellet was resuspended and the concentration of sucrose was adjusted to 1.3 M. Aliquots of this suspension were used as a load fraction under layers of 1.10 M and 0.25 M sucrose with a bottom cushion of 2.0 M. Upon centrifugation in a swinging bucket rotor (Beckman SW27) for 3 h at 82000 $\times$ *g*, a band formed at the 0..25 M/1.10 M sucrose interface. This band was collected and centrifuged for 90 min at 105000 $\times$ *g*; the resultant pellet represented the fraction enriched in Golgi-derived elements.

Another weighed portion of the liver (about 4 g) was used to isolate plasma membranes according to the methods of Song et al. [19] and Yousef and Murray [20] as previously described [16].

Marker enzyme analysis. In order to ascertain the purity and recovery of the isolated membrane fractions, the activities of the following marker enzymes were determined in the whole homogenate and in the isolated fractions: canalicular plasma membrane, 5′-nucleotidase [21] and alkaline phosphodiesterase [21]; sinusoidal plasma membrane, glucagon-activated adenylate cyclase [22]; Golgi, galactosyltransferase [23]; endoplasmic reticulum, glucose-6-phosphatase [23]; lysosomes, acid phosphatase [24]; mitochondria, succinate dehydrogenase [25]; cytosol, alcohol dehydrogenase [26].

Determination of radiolabeled membrane glycoproteins. As previously described [16], aliquots of total liver homogenates and of suspensions of the isolated subcellular fractions were first treated with a multivalent rabbit antibody to rat serum in order to remove radiolabeled secretory glycoproteins. This treatment has been shown to effectively precipitate greater than 90% of radiolabeled secretory glycoproteins [13,15]. After removal of the interfering radiolabeled secretory glycoproteins from the samples, the remaining radiolabeled membrane glycoproteins were precipitated with trichloroacetic acid (10%)/phosphotungstic acid (1%) and radioactivity determined [16].

Other determinations. Protein was measured by the procedure of Lowry et al. [27], using bovine serum albumin as standard. Blood ethanol levels were determined as previously described [13]. The characterization and purity of the isolated membrane fractions were assessed by electron microscopy [23,28].

Statistics. The results are expressed as mean $\pm$ S.E. Comparisons were evaluated using the Student's *t*-test.

Results

The purity and recovery of the isolated membrane fractions as ascertained by marker enzyme analysis are indicated in Table I. The results show that the enrichment in specific activities and the distribution of total activities of the marker en-

TABLE I

MARKER ENZYME PROFILE OF ISOLATED LIVER MEMBRANE FRACTIONS

Subcellular fractions were obtained from rat livers and assayed as described under Materials and Methods. The relative specific activity is the ratio of specific activity (units of enzyme per mg protein) in any given fraction to that of the homogenate arbitrarily set at unity. Recoveries of enzyme activities are expressed as percentage of homogenate activities. The mean ± S.E. of eight separate preparations (obtained from four controls and four ethanol-treated rats) are shown in this table.

Enzymes	Golgi		Plasma membrane	
	Relative spec. act.	Recovery (%)	Relative spec. act.	Recovery (%)
5′-Nucleotidase	2.8 ± 0.8	1.1 ± 0.4	14.6 ± 1.6	17.6 ± 1.7
Phosphodiesterase	3.6 ± 1.0	1.4 ± 0.4	19.0 ± 1.3	22.1 ± 1.3
Galactosyltransferase	44.5 ± 3.3	21.1 ± 1.6	0.5 ± 0.05	0.5 ± 0.05
Glucose-6-phosphatase	1.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.02	0.6 ± 0.03
Acid phosphatase	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.05	0.8 ± 0.08
Succinate dehydrogenase	0.2 ± 0.05	< 0.1	0.5 ± 0.03	0.4 ± 0.03
Alcohol dehydrogenase	0.1 ± 0.04	< 0.1	0.08 ± 0.03	< 0.1

zymes of the Golgi apparatus and plasma membrane were as expected and were in good agreement to those reported in the literature [29]. The Golgi fraction showed nearly a 45-fold enrichment and a 21% recovery of galactosyltransferase activity. The plasma membrane fraction was significantly enriched in 5′-nucleotidase and alkaline phosphodiesterase activities, which serve as specific markers for the canalicular domain of the plasma membrane [22,30]. Recoveries of these markers ranged from nearly 18% for 5′-nucleotidase to 22% for phosphodiesterase. Analysis of the plasma membrane fraction for the sinusoidal marker, glucagon-activated adenylate cyclase [22,30,31], indicated a 6.4 ± 0.7-fold enrichment and a 12.1 ± 0.7% recovery of this enzyme. In addition, glucagon caused a 9.3 ± 1.3-fold stimulation of adenylate cyclase activity in the isolated plasma membrane fraction. In the cytosolic fraction, 79.1 ± 4.1% of the alcohol dehydrogenase activity was recovered, and the cytosol contained less than 3% activity of membrane-bound marker enzymes. Comparison of the specific activity and recovery of specific marker enzymes in the fractions isolated from control versus ethanol-treated rats indicated no major differences. For example, the recovery of galactosyltransferase in the Golgi and 5′-nucleotidase in the plasma membrane of the ethanol-treated rats (21.6 ± 2.9% and 17.5 ± 1.9%, respectively) was similar to that of the controls (20.6 ± 1.9% and 17.8 ± 3.1%, respectively). In addition, examination of the membrane fractions from both control and ethanol-treated animals by electron microscopy showed morphological features characteristic of the appropriate membrane fraction (not shown).

Blood ethanol concentrations reached a maximum of 40.7 ± 3.5 mM at 2 h after intragastric intubation and declined thereafter in a linear manner, reaching a level of 20.8 ± 4.5 mM at 4 h. Therefore, the effects of ethanol on hepatic plasma membrane assembly were studied over a time period of 2 to 4 h after ethanol or glucose administration.

Both fucose and *N*-acetylmannosamine, a specific precursor of sialic acid residues of glycoproteins [2], were rapidly incorporated into membrane glycoproteins in the liver. Maximum incorporation was achieved by 15 min after label injection, and this extent of labeling was maintained for 120 min (Fig. 1). Ethanol treatment caused a slight decrease in the incorporation of fucose at 15 min; however, the maximum level of incorporation was identical between the ethanol-treated rats and controls and no differences were observed at any of the other time periods (Fig. 1A). On the other hand, incorporation of sialic acid into total membrane glycoproteins was slightly reduced in the ethanol-treated animals (Fig. 1B).

Further experiments were carried out to ascertain the effects of ethanol on the labeling of membrane glycoproteins in specific membrane frac-

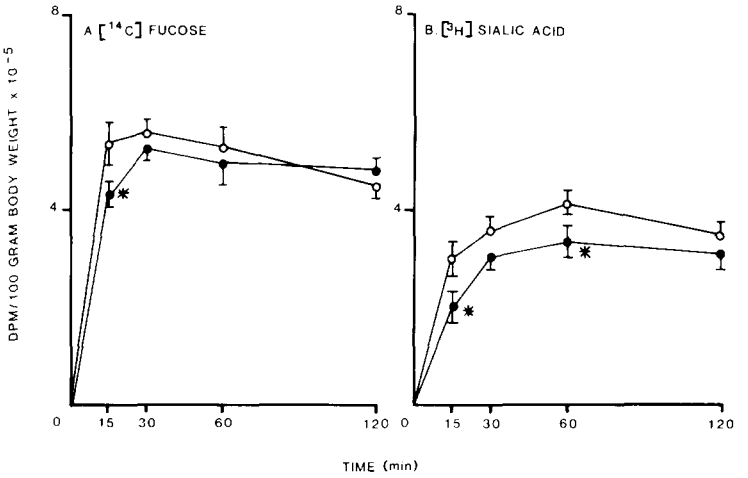

Fig. 1. Effect of ethanol administration on the incorporation of [^{14}C]fucose (A) and *N*-acetyl[^{3}H]mannosamine (B) into total membrane glycoproteins in the liver. Radiolabels were injected 2 h after ethanol or glucose treatment, and their incorporation into total membrane glycoproteins of liver homogenates was determined at the indicated time periods after injection. Each point represents the mean ± S.E. of 9–12 animals. ○, control; ●, ethanol-treated. Values that were significantly different from controls are indicated by the following symbol: *, $P < 0.05$.

tions; namely, the plasma membrane and Golgi apparatus. In these studies, the total content of radiolabeled membrane proteins in the plasma membrane, Golgi and cytosol was determined at different times after labeling. Since variable losses of membrane fractions occurred during isolation procedures, the total amount of radiolabeled membrane glycoproteins in a particular fraction was determined by measuring the amount of radiolabeled glycoproteins in each isolation fraction and correcting that value to 100% using the recovery of the marker enzyme for that fraction. The corrected value for the total radioactivity of membrane glycoproteins in a particular fraction was expressed as dpm/total liver per 100 g body wt. Such a correction is valid only if no appreciable changes in the total activity of the marker enzyme occurred during fractionation. In these studies, 90% of the alkaline phosphodiesterase activity (plasma membrane marker) and 87% of the galactosyltransferase activity (Golgi marker) present in the total homogenate was accounted for during the isolation procedures, indicating that the recoveries of these markers were reliable indicators of the recoveries of membrane fractions as a whole.

Despite minimal differences in the incorporation of fucose and sialic acid into the total pool of membrane glycoproteins (Fig. 1), the labeling of fucoproteins and sialoproteins in the plasma membrane was markedly inhibited in the ethanol-treated rats at all time periods tested (Fig. 2). Similar inhibitory effects of ethanol on the assem-

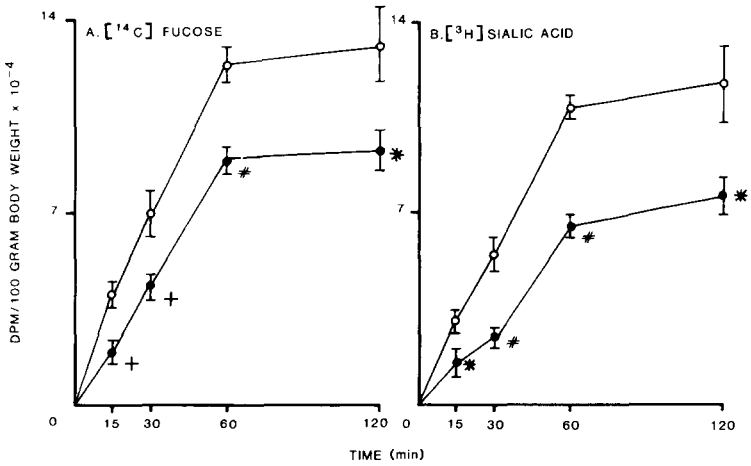

Fig. 2. Effect of ethanol administration on the hepatic assembly of ^{14}C-labeled fucoproteins (A) and ^{3}H-labeled sialoproteins (B) into the plasma membrane. Radiolabels were injected 2 h after ethanol or glucose treatment, and the appearance of labeled glycoproteins in the plasma membrane was determined at the indicated time periods after label injection. Each point represents the mean ± S.E. of 9–12 animals. ○, control; ●, ethanol-treated. Values that were significantly different from controls are indicated by the following symbols: *, $P < 0.05$; $^{+}$, $P < 0.01$; $^{\#}$, $p < 0.001$.

TABLE II

EFFECT OF ETHANOL ADMINISTRATION ON THE ASSEMBLY OF GLYCOPROTEINS INTO THE PLASMA MEMBRANE

Experimental conditions were the same as those described in Fig. 1 and 2. Results are expressed as percent plasma membrane assembly which was derived by calculating the ratio of the amount of labeled glycoproteins in the plasma membrane (dpm/total liver per 100 g body wt.) to the amount of labeled membrane glycoproteins in the total liver homogenate (dpm/total liver per 100 g body wt.); this value was multiplied by 100 and values expressed as mean ± S.E. for 9–12 animals. Time is indicated as minutes after label injection.

Time (min)	% Fucose labeling		% Sialic acid labeling	
	Control	Ethanol	Control	Ethanol
30	12.6 ± 1.5	8.2 ± 0.7 [b]	15.5 ± 1.4	8.1 ± 0.5 [b]
60	23.7 ± 1.3	18.0 ± 1.1 [b]	26.1 ± 1.1	19.8 ± 1.0 [a]
120	29.5 ± 2.9	19.7 ± 1.3 [a]	33.3 ± 2.7	25.1 ± 1.0 [a]

[a] $P < 0.01$ as compared to corresponding control.
[b] $P < 0.001$ as compared to corresponding control.

bly of fucoproteins and sialoproteins into the plasma membrane was also indicated when the results were expressed as dpm/mg protein (not shown). When the data on the effects of ethanol on plasma membrane assembly were expressed as a ratio of the amount of radiolabeled membrane glycoproteins present in the plasma membrane to the amount present in the total pool of membrane glycoproteins (in order to minimize the slight influence of ethanol on precursor incorporation), impaired assembly of fucoproteins and sialoproteins into the plasma membrane was also indicated (Table II). Significantly lower percentages of the total labeled membrane glycoproteins that were present in the plasma membrane were noted for both labels at all time periods tested in the ethanol-treated animals.

Since ethanol minimally affected the labeling of the total pool of membrane glycoproteins but markedly inhibited the labeling of the plasma membrane, the effects of ethanol on the subcellular distribution of labeled membrane glycoproteins in the plasma membrane, cytosol and Golgi fractions were examined. 30 min after fucose injection, approx. 65 to 75% of the total radiolabeled membrane glycoproteins were located in the Golgi complex and no difference was observed between the control and ethanol-treated animals (Table III). About 13% of the fucose-labeled membrane glycoproteins were present in the plasma membrane compartment in the control animals and this was significantly decreased in the ethanol-treated animals. The remaining radiolabeled glycoproteins were located in the cytosol, and a significant increase (nearly 2-fold) of membrane glycoproteins located in this compartment was observed in the ethanol-treated rats (Table III). A similar effect of ethanol on the distribution of membrane sialoproteins was also observed (Table III). Ethanol administration caused a significant increase in labeled sialoproteins in the cytosol, a significant decrease in the plasma membrane, and no significant difference in the Golgi fraction.

TABLE III

EFFECT OF ETHANOL ADMINISTRATION ON THE SUBCELLULAR DISTRIBUTION OF FUCOSE- AND SIALIC ACID-LABELED PLASMA MEMBRANE GLYCOPROTEINS IN THE LIVER

[^{14}C]Fucose and *N*-acetyl[^{3}H]mannosamine were injected 2 h following ethanol or glucose treatment. Labeled membrane glycoproteins in the total homogenate and subcellular fractions were determined at 30 min after injection of the labels. Results are expressed as 10^5 dpm/total liver per 100 g body wt. (mean ± S.E.) of 7–10 animals.

Fraction	Fucose-labeled		Sialic acid-labeled	
	Control	Ethanol	Control	Ethanol
Homogenate	5.34 ± 0.66	5.25 ± 0.42	3.56 ± 0.38	3.06 ± 0.38
Plasma membrane	0.70 ± 0.19	0.42 ± 0.09 [a]	0.56 ± 0.11	0.23 ± 0.09 [b]
Cytosol	1.08 ± 0.23	2.06 ± 0.28 [b]	0.68 ± 0.22	1.19 ± 0.16 [a]
Golgi	4.08 ± 0.60	3.56 ± 1.03	2.91 ± 0.53	2.34 ± 0.68

[a] $P < 0.01$ as compared to corresponding control.
[b] $P < 0.001$ as compared to corresponding control.

Discussion

The results of this study show that acute ethanol administration impairs plasma membrane assembly in the liver. Furthermore, it appears that the late stages of membrane assembly, following terminal glycosylation in the Golgi apparatus, are the primary sites of impairment; a finding consistent with our previous studies, showing that ethanol administration interferes with the late steps of hepatic glycoprotein secretion [11–15].

In these studies, radiolabeled fucose and *N*-acetylmannosamine, a specific precursor for sialic acid residues in glycoproteins [2,32], were used to label plasma membrane glycoproteins. Both sugars are incorporated into glycoproteins almost exclusively in the Golgi complex and label either secretory or plasma membrane integral proteins [2,4, 32–34]. Since secretory proteins were removed by immunoprecipitation in these experiments, the remaining fucose- and sialic acid-labeled glycoproteins that were measured in the total liver homogenates as well as in the various isolated subcellular fractions represented primarily plasma membrane glycoproteins. Therefore, the effect of ethanol on the labeling and fate of plasmalemmal glycoproteins could be followed from their site of synthesis in the Golgi to their final destination in the plasma membrane.

Ethanol administration minimally affected the incorporation of fucose and sialic acid into glycoproteins (Fig. 1), indicating that ethanol treatment does not appreciably affect the terminal glycosylation of proteins in the Golgi. However, a decreased assembly of labeled fucoproteins and sialoproteins into the plasma membrane was observed in the ethanol-treated rats. Since the isolated plasma membrane fraction was enriched in both canalicular markers (5′-nucleotidase and alkaline phosphodiesterase) and sinusoidal marker (glucagon-activated adenylate cyclase), it would appear that ethanol treatment affects the assembly of glycoproteins into both the canalicular and sinusoidal domains of the plasma membrane.

Since ethanol administration decreased the labeling of glycoproteins in the plasma membrane but did not appreciably affect the labeling of the total pool of membrane glycoproteins, one would expect that labeled membrane glycoproteins should be accumulating in some other subcellular compartment in the liver. Indeed, increased amounts of labeled membrane sialoproteins and fucoproteins were observed in the cytosol of the ethanol-treated rats. On the other hand, no significant differences in the amounts of labeled glycoproteins in the Golgi fraction were noted. The most likely explanation for these findings is that ethanol administration impairs the transport of membrane glycoproteins after they leave the Golgi complex on their way to the plasma membrane. Apparently, ethanol alters the transport of membrane glycoproteins through the cytosolic compartment and/or inhibits the insertion of glycoproteins into the plasma membrane.

Although no specific studies were conducted in order to explore the nature or mechanisms of the retained membrane glycoproteins in the cytosol, some comments deserve mentioning. It is possible that membrane glycoproteins located in the cytosol could have existed in light density transport vesicles, which are responsible for the translocation of glycoproteins from the Golgi to the plasma membrane [9,35], or could have leaked out of these transport vesicles, which are apparently quite fragile [36,37], during homogenization of the liver. However, the presence of labeled glycoproteins in the cytosolic fraction cannot be explained by contamination of the cytosol with Golgi elements or other membrane fractions since the activities of membrane-bound marker enzymes were very low in this fraction. Accumulation of nonsecreted glycoproteins has also been shown to occur in the hepatic cytosol of ethanol-treated rats [15]. Further studies on the nature of the membrane glycoprotein accumulating in the cytosol are currently in progress.

In conclusion, the results of this study show that acute ethanol administration inhibits the assembly of glycoproteins into the hepatic plasma membrane. The reduced ability of the ethanol-treated animals to assemble glycoproteins into their plasma membrane was accompanied by an increased amount of membrane glycoproteins accumulating in the cytosol. Therefore, it appears that ethanol administration interferes with the flow of membrane components from the Golgi apparatus to the surface membrane. Such alterations in vesicular traffic in the hepatocyte may play a role in

alcoholic liver injury; however, this remains to be established.

Acknowledgements

This investigation was supported by the Veterans Administration and by USPHS Grant No. AA04961. We are grateful to Sandy Kragskow and Marlene Kidder for their valuable technical assistance and to Judy VanLoh for typing this manuscript.

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