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# EFFECTS ON GLYCOSYLATION ENZYMES FROM MEMBRANE FRACTIONS, INDUCED BY CHRONIC ETHANOL ADMINISTRATION

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**Abstract**—To study the effect of chronic ethanol administration on the enzyme activities involved in the glycosylation processes (glycosidases and glycosyltransferases), we used 6-week ethanol-treated female Wistar rats and pair-control rats. Biological material was membrane fractions of microsomes and plasma membrane obtained by subcellular fractionation technique. Ethanol treatment increased with statistical significance the $V_{max}$ of *N*-acetyl-β-D-glucosaminidase ($P < 0.10$), β-D-glucuronidase and α-D-mannosidase ($P < 0.001$) activities from microsomal fractions and likewise it increased the $K_m$ value of β-D-glucuronidase ($P < 0.001$). *In vitro* doses of ethanol (0.1–1.7 M) were added to delucidate hypothetical membrane tolerance responses. However *in vitro* addition of ethanol provoked no differential effects in treated and untreated rats. Glycosyltransferase assays were carried out using [$^{14}$C]sugar derivatives. We detected several glycosyltransferase activities in both microsome and plasma membrane fractions. Chronic ethanol exposure appeared to affect *N*-acetyl-neuraminyltransferase activity from both membrane fractions, producing a greatly increased incorporation in the presence of asialofetuin. Glucosyl and mannosyltransferase activities from plasma membrane fractions were also altered by ethanol treatment, producing an increased enzyme activity when reaction was performed in the presence of phosphatidylcholine + dolichol-phosphate liposomes.

A variety of mainly recent studies have shown that ethanol, in both acute and chronic administration, may lead to structural [1–3] and/or molecular [4–7] alterations in biological membranes. Changes produced by ethanol in the biophysical and biochemical characteristics of biological membranes have been emphasized in the mechanism of ethanol toxicity [7].

Structural modifications have been intensively studied in different subcellular organelles (mitochondria, endoplasmic reticulum, plasma membrane, Golgi apparatus, etc.) obtained by techniques of subcellular fractionation [8].

It has been extensively reported that the most obvious effect of ethanol, especially in acute doses or *in vitro*, is the derangement of the membrane, producing an increase in its fluidity [1, 2, 9]. However, chronic exposure to ethanol may lead to cellular responses of tolerance [10].

The greatest efforts are currently being focussed on analysing the molecular disposition within the membranes by physical chemistry techniques of fluorescence polarization and electron paramagnetic resonance, both of which afford great resolution [1, 2].

The molecular alterations produced by ethanol in biological membranes have been analysed in two directions: changes produced in membrane-bound enzymes [4, 7, 11–13], and the influence of their hydrophobic environment (phospholipid bilayer) on enzyme activity, in both native membrane and liposomes of controlled lipid composition [13–16].

The present study describes our results concerning the effect of chronic ethanol administration on the activities of some liver membrane-bound enzymes belonging to the enzyme systems involved in glycosylation processes: glycosidases and glycosyltransferases. We have made a correlated enzyme study of these systems in samples of microsomal and plasma membrane fractions from chronically ethanol-treated rats and controls. The alteration of the lipid environment of membrane-bound enzymes has thus been analysed.

Moreover, ethanol was added *in vitro* to membrane preparations in order to specify the adaptative changes proposed for chronic ethanol ingestion.

## MATERIALS AND METHODS

*Biological material and alcohol treatment.* After weaning, 28-day-old female Wistar rats weighing 45–50 g were kept in a room at a constant temperature (20–22°) on a 12-hr light/dark cycle, fed rat chow A-04 from Panlab (Barcelona, Spain) containing 17% protein, 3% lipids and 59% carbohydrates, and given progressively an aqueous ethanol solution (5–20%) (from ethyl alcohol absolute, reagent grade, Farmitalia Carlo Erba, Milano, Italy) for 14 days, followed by an additional period of 40 days using a 20% ethanol solution. Control rats, belonging to the same litter as the ethanol-treated ones, were given a similar diet for the same period, but without ethanol.

Rats were decapitated and livers were removed and rapidly placed in an ice-cold medium. Homogenization processes were performed in glass/Teflon homogenizers.

*Preparation and purification of subcellular fractions.* Microsomal fractions were obtained by the homogenization of livers in a solution containing 250 mM sucrose and 10 mM Tris–HCl buffer

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Table 1. Glycosidase activities in microsomal fractions purified from liver of alcoholic and control rats

| Substrates used | sp. act. × $10^3$ (μmol) pNP/mg/30 min | | |
|---|---|---|---|
| | Control | Ethanol | P |
| pNP-β-D-glucopyranoside | 21.58 ± 4.32 (5) | 26.26 + 7.21 (5) | NS |
| pNP-*N*-acetyl-β-D-glucosaminide | 114.82 ± 3.16 (4) | 136.21 ± 11.28 (4) | <0.10 |
| pNP-β-D-mannopyranoside | 13.76 ± 3.05 (5) | 12.74 ± 2.34 (5) | NS |
| pNP-β-D-glucuronide | 256.85 ± 5.32 (4) | 230.12 ± 13.35 (4) | <0.10 |
| pNP-α-D-glucopyranoside | 22.64 ± 6.98 (5) | 24.42 ± 7.58 (5) | NS |
| pNP-α-D-mannopyranoside | 48.22 ± 4.70 (5) | 57.76 ± 4.52 (5) | <0.10 |
| pNP-α-L-fucopyranoside | 17.22 ± 2.05 (5) | 22.40 ± 4.69 (5) | NS |
| pNP-α-D-galactopyranoside | 11.62 ± 4.52 (5) | 8.56 ± 2.98 (5) | NS |
| pNP-β-D-galactopyranoside | 17.68 ± 7.31 (5) | 11.38 ± 3.12 (5) | NS |
| pNP-*N*-acetyl-β-D-galactopyranoside | 16.94 ± 6.64 (5) | 8.48 ± 5.25 (5) | NS |

Enzyme assays were carried out as described in Materials and Methods, using 1.6 mM substrate concentration and 30 min incubation.

Results are expressed as specific activity (sp. act.) ± standard error (SE).

The number of experiments is given in parentheses.

NS: not significant.

$P < 0.10$: 90% of significance.

(pH 7.4) at 4°. The homogenate was fractionated by differential centrifugation according to Bador *et al.* [17]. Plasma membrane fractions were isolated by homogenization and differential centrifugations of the livers in a solution containing 1 mM $NaHCO_3$, 1 mM $CaCl_2$, 5 mM 2-mercaptoethanol, pH 7.5. Purification of the plasma membrane was achieved on a sucrose discontinuous gradient as described previously [18].

The purity of fractions was assessed by the determination of several specific marker-enzymes: alkaline *p*-nitrophenyl phosphatase (EC 3.1.3.1) according to Ray [19] as a marker of plasma membrane, monoamine oxidase (EC 1.4.3.4) according to Tabor *et al.* [20] for mitochondrial fraction, glucose-6-phosphatase (EC 3.1.3.9) according to Jeffrey and Brown [21] for microsomal fraction, and acid-phosphatase (EC 3.1.3.2) according to Walter and Schütt [22] for lysosomes.

*Protein measure.* Protein concentration was determined using Gornall's procedure [23].

*Measurement of glycosidase activities.* Assays of glycosidase activities were carried out using the method described by Calvo *et al.* [24] based on the spectrophotometrical determination of enzymatic release of *p*-nitrophenol from *p*-nitrophenyl-derivative substrates. Incubation mixtures contained, in 1 mL, the following components: 800 mM citrate buffer pH 5.0, 0.1–5.0 mM *p*-nitrophenyl-glycoside substrate and 0.4 mg protein of membrane preparation. Reactions were developed at 37° and stopped by the addition of 1 mL of cold 500 mM $Na_2CO_3$.

Enzyme activity was expressed as μmol of *p*-nitrophenol released per mg of protein per incubation time (min).

Kinetic parameters ($K_m$ and $V_{max}$) were calculated by the nonlinear regression data analysis program "Enzffiter" (Elsevier) for IBM PC computer and compatible systems.

Results were given as means and standard error (SE). The significance of the differences was assessed by Student's *t*-test.

*Glycosyltransferase activities measurement.* The reaction mixture used for the study of the incorporation of [$^{14}$C]mannose, [$^{14}$C]glucose and [$^{14}$C]*N*-acetylglucosamine into lipidic acceptors of subcellular fractions contained (in 1 mL): 0.8 mg protein of membrane preparation, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 4 mM NaF, 5 mM 2-mercaptoethanol, 10 mM Tris–HCl buffer pH 7.4, 0.8 μM of labeled substrate and 7.2 μM of cold substrate. When the glycosylation reaction was performed in the presence of exogenous dolichol-phosphate (DolP)* and/or phospholipid effectors, these were added at standard concentrations of 17 μM and 380 μM, respectively, to be subsequently evaporated under a stream of nitrogen, and the samples sonicated in an ultrasonic water bath according to Mitranic *et al.* [25]. Incubations were performed at 37° and stopped by the addition of 10 vol. of chloroform/methanol (2:1, v/v) [26]. Extraction of lipid products was performed according to the method described by Gateau *et al.* [27]. When the reaction of neuraminyltransferase activity was carried out in the presence of an exogenous protein acceptor, such as asialofetuin, the reaction mixture was supplemented with 80 μmol of a 20 mg/mL asialofetuin solution, and a concentration of up to 0.1% of Triton X-100. The incubation was performed at 37° and stopped by addition of 20 vol. of 20% TCA (w/v). Precipitates were collected in glass microfibre filters (Whatman GF/B), washed and dried.

Radioactivity was measured in a Beckman LS 1800 liquid scintillation counting system, using butyl-PBD/toluol (5 g/L) as a scintillation counting mixture.

In all cases enzyme activity was expressed as picomol of [$^{14}$C]monosaccharide incorporated per mg of protein per incubation time.

*Reagents.* UDP-[$^{14}$C]galactose (333 mCi/mmol),

* Abbreviations: UDP, uridinediphosphate; CMP, cytidinemonophosphate; SDS, sodium sulphate; DolP, dolichol-phosphate; PC, phosphatidylcholine; pNP, *p*-nitrophenyl-phosphate; TCA, trichloroacetic acid.

Table 2. Comparison between kinetic parameters of glycosidase activities detected in microsomal fraction purified from liver of alcoholic and control rats

| Enzyme activity | Kinetic parameter | Control | Ethanol | P |
|---|---|---|---|---|
| *N*-Acetyl-β-D-glucosaminidase | $V_{max}$ | 178.52 ± 16.95 (9) | 226.14 ± 21.83 (9) | <0.10 |
| | $K_m$ | 0.27 ± 0.02 (9) | 0.27 ± 0.03 (9) | NS |
| β-D-glucuronidase | $V_{max}$ | 467.17 ± 9.68 (6) | 548.17 ± 10.49 (6) | <0.001 |
| | $K_m$ | 0.78 ± 0.13 (6) | 1.50 ± 0.05 (6) | <0.001 |
| α-D-mannosidase | $V_{max}$ | 159.75 ± 3.23 (4) | 239.25 ± 2.74 (4) | <0.001 |
| | $K_m$ | 2.73 ± 0.38 (4) | 2.69 ± 0.13 (4) | NS |

Experiments were carried out as described in Materials and Methods, using substrate concentrations from 0.01 mM to 5.00 mM and 30 min incubation.

Results are expressed as mM ($K_m$) and μmol pNP/mg/30 min ($V_{max}$) ± standard error (SE).

The number of experiments is given in parentheses.

NS: not significant.

$P < 0.10$: 90% of significance.

$P < 0.001$: 99.9% of significance.

UDP-[$^{14}$C]glucose (322 mCi/mmol), GDP-[$^{14}$C]-mannose (282 mCi/mmol), UDP-[$^{14}$C]*N*-acetyl-glucosamine (238.8 mCi/mmol) and CMP-[$^{14}$C]sialic acid (1.8 mCi/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Desialylated fetuin, dolichol monophosphate (grade III), phospholipids and *p*-nitrophenyl-glycosides were supplied by the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

All the other reagents were of the highest purity commercially available.

## RESULTS

### *Alterations produced by chronic ethanol consumption in glycosidase activities associated with microsomal and plasma membrane fractions*

In preliminary studies of ethanol effects on glycosidase activities, we tested various glycosidase substrates (*p*-nitrophenyl derivatives) (Table 1). The the most active glycosidases in microsomal fractions were: *N*-acetyl-β-D-glucosaminidase activity, β-D-glucuronidase activity and α-mannosidase activity. Their high specific activity rendered them suitable for a detailed kinetic study. Moreover, they were linear with time (data not shown), sufficiently reproducible, and differed significantly in treated and control rats. As shown in Table 2, $V_{max}$ values were always increased on chronic ethanol treatment, the statistical significance being $P < 0.10$ for *N*-acetyl-β-D-glucosaminidase, and $P < 0.001$ for β-D-glucuronidase and α-D-mannosidase activities, whereas only the $K_m$ of β-D-glucuronidase activity was significantly affected ($P < 0.001$).

In the same way, we have also studied the glycosidase systems of plasma membrane fractions. Table 3 shows the specific activities obtained from several screening studies. According to these analyses, from the most active and stable activities (data not shown), we have chosen *N*-acetyl-β-D-glucosaminidase activity for the calculation of kinetic parameters (see Table 4), which, however, were not affected by chronic ethanol exposure.

### *Effect of several modulators on the β-D-glucuronidase (microsomal fractions) and N-acetyl-β-D-glucosaminidase (plasma membrane fractions) activities*

We have analysed five kinds of effectors capable of modifying glycosidase activities: sugars, lactones, cations, detergents and stabilizing agents.

Statistically significant differences produced by ethanol treatment on glucuronidase activity were obtained only in the presence of detergents (1%) or stabilizing agents (data not shown). Thus, the strong inhibition provoked by sodium cholate or the slow activation produced by Triton X-100 were significantly affected by ethanol ($P < 0.10$ and $P < 0.05$, respectively) while the almost total inhibition in the presence of SDS was not. Similarly, the effects of ethylenglycol (10%) and 2-mercaptoethanol (100 mM) were different on glucuronidase activity from control and alcohol-treated rats ($P < 0.05$).

*N*-Acetyl-β-D-glucosaminidase activity from plasma membrane fractions only appears significantly affected ($P < 0.05$) after ethanol administration by *N*-acetyl-glucosamine or *N*-acetyl-galactosamine (100 mM); moreover, these sugars (as other sugars) strongly inhibited enzyme activity (80–93%) (data not shown).

### *Effects of single doses* in vitro *of ethanol on glycosidase activities of microsomal fractions from the liver of alcoholic and control rats*

In order to ascertain the possible alcohol tolerance induced by chronic ethanol administration we have analysed the effect *in vitro* of several ethanol concentrations (0.1–1.7 M) on the three glycosidase activities detected in microsomal fractions isolated from treated and untreated rats.

*N*-Acetyl-β-D-glucosaminidase activity was unaffected by the addition of ethanol *in vitro* for all concentrations tested (data not shown). Figure 1 shows the results obtained for β-D-glucuronidase and α-D-mannosidase activities. It can be seen that glucuronidase activity decreased gradually for increasing ethanol concentrations (from 92–94% at 0.1 M ethanol to 75% at 1.7 M); furthermore, the decrease

Table 3. Glycosidase activities in plasma membrane fractions purified from liver of alcoholic and control rats

| Substrates used | sp. act. × $10^3$ (μmol) pNP/mg/30 min | | P |
|---|---|---|---|
| | Control | Ethanol | |
| pNP-β-D-glucopyranoside | 11.06 ± 1.43 (3) | 4.93 ± 1.90 (3) | <0.01 |
| pNP-*N*-acetyl-β-D-glucosaminide | 295.82 ± 28.50 (3) | 214.75 ± 12.94 (3) | <0.01 |
| pNP-β-D-mannopyranoside | 6.63 ± 4.70 (3) | 10.54 ± 2.46 (3) | NS |
| pNP-β-D-glucuronide | 44.90 ± 1.84 (3) | 49.75 ± 11.35 (3) | NS |
| pNP-α-D-glucopyranoside | 0.89 ± 0.63 (3) | 0.28 ± 0.20 (3) | NS |
| pNP-α-D-mannopyranoside | 19.84 ± 10.47 (3) | 9.25 ± 6.56 (3) | NS |
| pNP-α-L-fucopyranoside | ND | ND | — |
| pNP-α-D-galactopyranoside | ND | ND | — |
| pNP-β-D-galactopyranoside | ND | ND | — |
| pNP-*N*-acetyl-β-D-galactopyranoside | 23.06 ± 5.45 (3) | 12.87 ± 0.62 (3) | <0.05 |

Enzymatic assays were carried out as described in Materials and Methods, using 1.6 mM substrate concentration and 30 min incubation.
Results are expressed as specific activity (sp. act.) ± standard error (SE).
The number of experiments is given in parentheses.
ND: not detected.
NS: not significant.
$P < 0.01$: 99% of signficance.
$P < 0.05$: 95% of significance.

Table 4. Comparison between kinetic parameters of *N*-acetyl-β-D-glucosaminidase activity detected in plasma membrane fractions purified from liver of alcoholic and control rats

| Enzyme activity | Kinetic parameter | Control | Ethanol | P |
|---|---|---|---|---|
| *N*-Acetyl-β-D-glucosaminidase | $V_{max}$ | 470.99 ± 156.30 (5) | 502.03 ± 164.44 (5) | NS |
| | $K_m$ | 0.36 ± 0.08 (5) | 0.38 ± 0.08 (5) | NS |

Experiments were carried out as described in Materials and Methods, employing substrate concentrations from 0.1 mM to 6.0 mM and 90 min incubation.
Results are expressed as mM ($K_m$) and μmol pNP/mg/30 min ($V_{max}$) ± standard error (SE).
The number of experiments is given in parentheses.
NS: not significant.

in activity was parallel for microsomal fractions from treated and untreated rats (Fig. 1a). Similar results were obtained for mannosidase activity; however, mannosidase showed higher inhibition caused by ethanol *in vitro* (from 90% at 0.1 M ethanol to 34–39% at 1.7 M). This inhibitory effect tended to be greater in microsomal fractions of treated animals (Fig. 1b).

*Glycosyltransferase activities from microsomal fractions: modulation by phospholipidic effectors and alteration by chronic ethanol administration*

We have tested the microsomal glucosyltransferase, mannosyltransferase and *N*-acetyl-glucosaminyltransferase activities on lipidic endogenous acceptors in the absence or presence of DolP plus PC. PC was employed as a tool to ascertain the influence of the lipidic environment on enzyme activities, and DolP was used as a polyisoprenoid lipid intermediate in the transfer of sugars from their respective sugar nucleotides to suitable acceptors or as a lipidic modulator of the activity. All determinations were carried out as described in Materials and Methods.

As shown in Table 5, glucose incorporation into a lipidic acceptor was not significantly modified by DolP + PC liposomes. Ethanol had no effect either. Mannosyltransferase and *N*-acetylglucosaminyltransferase activities showed different behaviour under these conditions; for the former the activity on lipidic acceptors was found to be two or three times greater when DolP + PC were present. Again, chronic ethanol exposure had no additional effect.

Table 5 also shows the results obtained from the analysis of *N*-acetylneuraminyltransferase activity on endogenous or exogenous protein acceptors (such as asialofetuin). Asialofetuin was used as a tool to determine whether there was a lack of enzyme activity or a saturation of endogenous acceptors. The data obtained show that the incorporation into endogenous acceptors was similar in treated and control rats; however, when asialofetuin was present in the reaction mixture a very strong increase in activity was found, which was, moreover, significantly greater in the alcohol-treated rats.

*Glycosyltransferase activities from plasma membrane fractions: modulation by phospholipidic effectors and alteration by chronic ethanol administration*

The experiments on glycosyltransferase activities

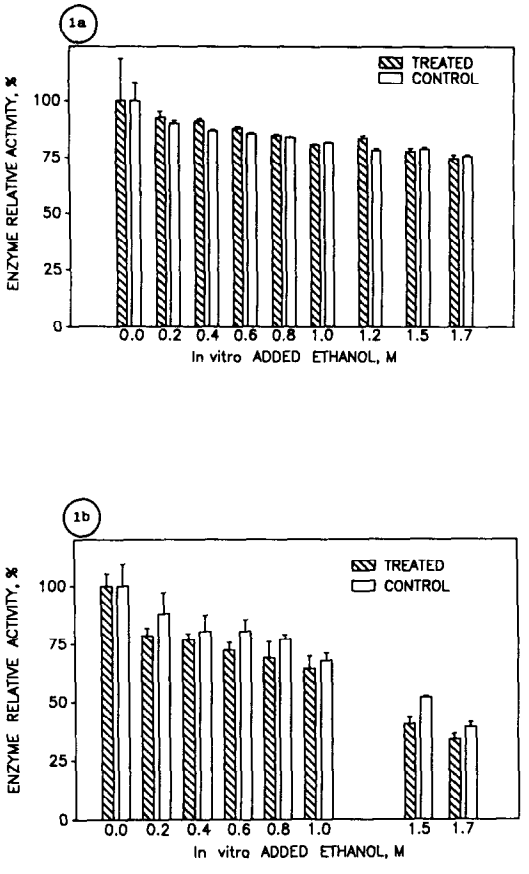


Fig. 1. Effects of ethanol *in vitro* on microsomal glycosidase activities from chronically treated and control rats: (a) β-D-glucuronidase activity and (b) α-D-mannosidase activity. Results are expressed as percentages of activity in comparison with those obtained without ethanol addition and are means of four experiments ± SE (standard error).

associated with plasma membrane fractions were performed as stated for microsomal fractions. The results obtained are shown in Table 6. As shown, the incorporation of glucose (from UDP-glucose) into lipidic endogenous acceptors was strongly enhanced by DolP + PC liposomes and chronic ethanol ingestion increased this activity. *N*-Acetyl-glucosaminyltransferase activity was also positively modulated by DolP + PC but no effect was found after ethanol consumption. Ethanol treatment, however, may result in some alterations of mannosyltransferase activity.

Table 6 also shows the results obtained for *N*-acetylneuraminyltransferase activity on protein acceptors. This activity is differentially modified by ethanol in the presence or absence of asialofetuin (exogenous protein acceptor); thus, in control rats, incorporation into endogenous acceptors was greater than in ethanol-treated rats (33.33 and 16.65 pmol/mg, respectively), but in the presence of asialofetuin the effect was inverted (123.41 and 217.48 pmol/mg, respectively).

## DISCUSSION

Our most important aim in the present work was to study enzymes involved in the processes of glycosylation and to analyse alterations caused by ethanol consumption.

Since ethanol can alter physically the fluidity of the biological membranes and their molecular organization [1–3] we were very interested in alterations that ethanol could produce in membrane-bound glycosidases and glycosyltransferases of microsomes and plasma membrane fractions. First we purified plasma membrane and microsomal fractions according respectively to a recent variation of Ray's method [18] and to the method of Bador *et al.* [17], thus obtaining suitable biological material for our subsequent studies.

Table 5. Glycosyltransferase activities in microsomal fractions obtained from liver of alcoholic and control rats

| | | Sugar incorporation into lipid endogenous acceptors | | Sugar incorporation into protein acceptors | |
|---|---|---|---|---|---|
| Enzyme activity | Material | Without exogenous addition | In presence of PC + DolP | Without exogenous addition | In presence of asialofetuin |
| Glucosyltransferase | Control | 66.67 ± 7.20 | 71.66 ± 6.96 | — | — |
| | Ethanol | 76.33 ± 5.82 | 88.33 ± 9.80 | — | — |
| *N*-Acetylglucosaminyltransferase | Control | 33.33 ± 0.67 | 71.67 ± 9.32 | — | — |
| | Ethanol | 33.35 ± 0.67 | 86.67 ± 7.52 | — | — |
| Mannosyltransferase | Control | 13.33 ± 2.67 | 32.00 ± 2.00 | — | — |
| | Ethanol | 10.57 ± 0.25 | 30.85 ± 0.15 | — | — |
| *N*-Acetylneuraminyltransferase | Control | — | — | 42.25 ± 6.32 | 2779.00 ± 29.91 |
| | Ethanol | — | — | 40.80 ± 7.29 | 5235.00 ± 608.87 |

Enzyme assays were carried out for 10 min (5 min for glucosyltransferase activity), as described in Materials and Methods.

DolP: Dolicholphosphate. PC: Phosphatidylcholine. DolP and PC were added at 17 μM and 380 μM concentrations, respectively. Asialofetuin solution (80 μL of a 20 mg/mL) was added to incubation mixture (1 mL final volume).

Results, mean of two duplicate experiments, are expressed as pmol of sugar incorporation in lipid or protein acceptors per mg protein of microsomal preparation.

Table 6. Glycosyltransferase activities in plasma membrane fractions obtained from liver of alcoholic and control rats

| Enzyme activity | Material | Sugar incorporation into lipid endogenous acceptors | | Sugar incorporation into protein acceptors | |
|---|---|---|---|---|---|
| | | Without exogenous addition | In presence of PC + DolP | Without exogenous addition | In presence of asialofetuin |
| Glucosyltransferase | Control | 4.37 ± 1.98 | 96.66 ± 6.65 | — | — |
| | Ethanol | 6.00 ± 1.63 | 163.00 ± 8.90 | — | — |
| *N*-Acetylglucosaminyl-transferase | Control | 7.00 ± 1.73 | 57.00 ± 7.00 | — | — |
| | Ethanol | 8.90 ± 2.61 | 58.50 ± 1.50 | — | — |
| Mannosyltransferase | Control | ND | 91.60 ± 7.32 | — | — |
| | Ethanol | ND | 114.10 ± 10.48 | — | — |
| *N*-Acetylneuraminyl-transferase | Control | — | — | 33.33 ± 1.67 | 123.41 ± 12.59 |
| | Ethanol | — | — | 16.65 ± 3.35 | 217.48 ± 17.49 |

Enzyme assays were carried out for 8 min (10 min for *N*-acetylneuraminyltransferase activity), as described in Materials and Methods.

DolP: Dolicholphosphate. PC: Phosphatidylcholine. DolP and PC were added at 17 μM and 380 μM concentrations, respectively. Asialofetuin solution (80 μL of a 20 mg/mL) was added to incubation mixture (1 mL final volume).

Results, the mean of two duplicate experiments, are expressed as pmol of sugar incorporated on lipid or protein acceptors per mg protein of plasma membrane preparation.

ND: not detected.

Glycosidases and glycosyltransferases carry out the glycosylation processes and, consequently, they are a key to determining the molecular mechanism of glycosylation as well as its hypothetical modifications under specific conditions (ethanol treatment, for example). The treatment chosen was chronic ethanol administration, since biochemical and biophysical consequences are greater than those produced by acute, short-term or *in vitro* doses. In addition, after chronic ethanol exposure, it is sometimes possible to observe enzyme adaptation processes [12].

In the conditions of this study, the daily dose of ethanol ingested was 32.5 g of alcohol/kg body weight (when using 20% ethanol). This result is of the same order as that obtained in mice [13] and twice that obtained in rats [7]. These different doses of ethanol ingested could produce differential results and render difficult the comparison of the findings obtained from each study.

Glycosidases have been extensively described from many invertebrates, mammalian tissues and serum, in soluble fractions as well as in different subcellular organelles [28]. They have also been studied in relation to different diseases [28]. Changes in these enzymes occurring after ethanol treatment indicated in general a decrease in activity although the most common finding is the absence of any effect on either brain or liver tissues [5, 29].

In this regard we have found both activatory and inhibitory effects of ethanol on the specific activity of some glycosidases of membrane fractions. Thus, in the microsomal fractions chronic ethanol administration seems to produce statistically significant alterations of *N*-acetyl-β-D-glucosaminidase, β-D-glucuronidase and α-D-mannosidase activities. Throughout, $V_{max}$ values increased whereas only for β-D-glucuronidase activity did the $K_m$ also significantly increase, indicating a decreased affinity for the substrate of the enzyme from alcohol-treated rats.

On the other hand, *N*-acetyl-β-D-glucosaminidase activity from plasma membrane fractions was affected by ethanol in its specific activity under the assayed conditions, but no changes in kinetic parameters were noticed.

Another important aspect of disturbances induced by ethanol on biological systems is the treatment as a single (acute), short-term and/or *in vitro* dose allowing us to ascertain hypothetical tolerance responses [7, 9]. Many enzyme changes of membrane-bound enzymes have been linked to these treatments by several authors [4, 7, 11]. It has also been determined that ethanol exerts *in vitro* effects when used at high concentrations (>100 mM) [7]. At up to 1.7 M, we found no statistically significant changes in the activities of three glycosidases of microsomal fractions assayed. Therefore, no tolerance effects have been found, since *in vitro* doses affected alcohol samples more intensively than controls.

The noteworthy role that glycosyltransferases play in glycoprotein biosynthesis led to a detailed study of them. In the present work we have studied the influence of a 6-week chronic ethanol administration on microsomal and plasma membrane glycosyltransferases. Thus, Jensen and Schutzbach [30] noted that dolichol and/or dolichol derivatives added to liposomes of different phospholipids had a much greater enhancer effect on glycosyltransferases. Also evident was the influence that the nature of lipid headgroups had on the activation phenomenon of glycosyltransferase enzymes [13, 25, 26, 31–33].

In this way, previous works indicated that PC and PC + DolP liposomes were good glycosyltransferase enhancers [26]. To this end, we used these liposomes as a tool to acquire suitable incorporation levels

to facilitate the discrimination of ethanol effects. Results obtained from our studies have emphasized this effect of PC + DolP on glycosyltransferase activities from both microsomal and plasma membrane fractions, except on microsomal glucosyltransferase activity. A similar observation is valid for asialofetuin, used as an exogenous protein acceptor. In both sets of membrane fractions it has been a successful enhancer of *N*-acetyl-neuraminyltransferase activity. These data would appear to be consistent with the findings obtained for mouse plasma membrane glucosyl- and galactosyltransferase activities [26, 34].

Stimulatory effects of chronic ethanol administration have only been found for *N*-acetyl-neuraminyltransferase activity from microsomal fractions and from the plasma membrane fractions.

It has been stated that plasma membrane is the cellular structure most sensitive to ethanol exposure [2, 3]. Thus, we have obtained marked differences in *N*-acetyl-neuraminyl-, glucosyl- and mannosyltransferase activities. In the last two cases, enzyme activity from treated rats showed an increased level of sugar incorporation in the presence of PC + DolP liposomes with regard to control rats.

The present study suggests that chronic ethanol administration affects some membrane-bound enzymes involved in the glycosylation processes. These experimental observations seem to be a consequence of membrane disturbances induced by ethanol, as a direct effect on membrane-bound proteins or as an effect on physical characteristics of membranes. Ethanol actions on membranes can be exercised by alterations on lipid structure, and the activity of membrane enzymes is dependent on a suitable lipid environment. Thus, it is not surprising that ethanol can produce differential changes in the specific membrane domains in which enzymes are located. These considerations are in agreement with our results, as well as with findings reported by other authors [4–7, 12, 13], though the effects of ethanol on membrane components are far from being completely understood, and more studies on this topic are necessary.

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