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INHIBITION OF GLYCOPROTEIN SECRETION BY ETHANOL AND ACETALDEHYDE IN RAT LIVER SLICES

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Abstract—The effects of ethanol and its metabolites on glycoprotein secretion were investigated in rat liver slices. A pulse-chase system was used to study the influence of these agents on glycoprotein secretion independently of their effects on synthesis. When ethanol (10 mM) was present in the incubation medium, the secretion of [^{14}C]leucine- and [^{14}C]glucosamine-prelabeled proteins into the medium was inhibited. The secretion of glycoproteins, that were prelabeled in the terminal positions of the oligosaccharide side chain with [^{14}C]fucose or [^{14}C]galactose, was also inhibited by ethanol. When low concentrations of acetaldehyde, similar to levels generated during *in vivo* ethanol oxidation, were maintained in the medium by infusion, the secretion of [^{14}C]leucine-, [^{14}C]glucosamine- and [^{14}C]fucose-prelabeled glycoproteins was impaired. Acetate (1 and 5 mM) did not affect the secretion of prelabeled glycoproteins. Colchicine (50 μM), an agent known to block the secretion of completed glycoproteins, behaved in a manner similar to ethanol in inhibiting the secretion of glycoproteins in our system. These results indicate that ethanol via its metabolite, acetaldehyde, impairs the secretion of glycoproteins by the liver and suggest that this impairment follows the attachment of the terminal monosaccharides in the Golgi membrane.

The majority of plasma proteins, with the exception of albumin, are synthesized and secreted by the liver in the form of glycoproteins [1, 2]. The protein backbone of glycoproteins is synthesized on polysomes attached to the rough endoplasmic reticulum [3–5]. The carbohydrate moieties are subsequently added as the secretory proteins traverse the channels of the rough and smooth endoplasmic reticulum with terminal glycosylation occurring in the Golgi apparatus [2, 6–8]. The completed glycoproteins are packaged into secretory vesicles which fuse with the plasma membrane, and these macromolecules are then discharged into the circulation [9–11].

Many studies have demonstrated that acute ethanol administration impairs general protein synthesis [12–16]; however, we have recently reported that ethanol can also modify secretory glycoprotein metabolism at sites beyond the synthesis of the protein backbone [12, 17]. The presence of ethanol or its metabolite, acetaldehyde, in a liver slice system impairs the release of [^{14}C]leucine- and [^{14}C]glucosamine-prelabeled glycoproteins into the medium, suggesting a post-ribosomal modification of glycoprotein metabolism that follows the incorporation of glucosamine into the macromolecules [17]. This impairment could include further attachment of terminal monosaccharide units to the growing oligosaccharide chain at the Golgi membrane or could involve the final steps of secretion of the completed glycoproteins [17]. The purpose of this study was to investigate whether ethanol and its metabolites, acetaldehyde and acetate, interfere with the final steps of secretion which follow the attachment of the terminal sugars to the glycoproteins at the Golgi region.

METHODS

Materials. Cycloheximide, D-glucosamine hydrochloride, α-L-fucose, D-galactose and colchicine were obtained from the Sigma Chemical Co., St. Louis, MO. D-[1-^{14}C]Glucosamine hydrochloride (8–10 mCi/mmole), L-[U-^{14}C]leucine (300–325 mCi/mmole), D-[1-^{14}C]galactose (5–10 mCi/mmole), L-[1-^{14}C]fucose (40–55 mCi/mmole) and scintillation fluid, Aquasol, were purchased from New England Nuclear, Boston, MA. Acetaldehyde was purchased from the Eastman Kodak Co., Rochester, NY. Gas chromatographic analysis of acetaldehyde [18] showed a purity of greater than 99.98 per cent with the only detectable impurity being acetic acid. All other chemicals used were of reagent grade quality.

Measurement of protein and glycoprotein secretion by liver slices. A pulse-chase system was used, as described previously in detail [17, 19], to determine macromolecular secretion which was independent of synthesis. Liver slices were prepared from nonfasted male, Sprague–Dawley rats (250–300 g). The incubation medium consisted of 2.5 ml of Krebs–Ringer phosphate buffer (pH 7.4) containing 0.54 mM calcium. All incubations were conducted at 37° in the presence of 95% O_2/5% CO_2. Slices were first incubated for either 15 or 30 min with the appropriate radioactive monosaccharide precursor in the medium. After this initial prelabeling period, the slices were removed, rinsed with buffer, and placed in a fresh medium containing the corresponding unlabeled monosaccharide (20 mM) and no radioactive label. The diluent effect of the non-radioactive monosaccharide minimized further labeling of gly-

coproteins in the chase medium. The secretion of the prelabeled glycoproteins was then determined by precipitating the medium proteins with trichloroacetic acid at various time periods during incubation in the chase medium. The resulting trichloroacetic acid precipitates were washed three times with cold trichloroacetic acid and delipidized with three washes of methanol–ether–chloroform (1:1:1). The purified precipitates were then dissolved in 1 N NaOH and aliquots were taken for radioactive and protein measurements so that specific activities (d.p.m./mg of protein) could be determined. The secretion of prelabeled proteins was studied in the same manner as glycoproteins except that the slices were first pulsed with [^{14}C]leucine and then transferred to a chase medium containing cycloheximide (1 mM). Cycloheximide, by inhibiting protein synthesis [20], minimized further labeling of proteins in the chase medium.

Additions to the liver slice medium. Acetaldehyde was added to the medium by constant infusion (0.25 μmole/min), employing a Harvard model 935 infusion pump (Harvard Apparatus, Millis, MA). The infusions of acetaldehyde, as well as the corresponding control infusions (Krebs–Ringer phosphate buffer), were conducted in the presence of pyrazole (4 mM) in order to block the reduction of acetaldehyde to ethanol [21]. Initial test experiments showed that pyrazole had no effect on either protein or glycoprotein synthesis or secretion. All other test substances (e.g. ethanol, acetate and colchicine) were added directly to the chase medium prior to incubation. Slices from the same liver were used for each appropriate set of experimental conditions.

Analytical methods. Protein was determined by the method of Lowry *et al.* [22]. Whenever acetaldehyde concentrations were determined in the total incubation system, the incubations were terminated by the addition of ice-cold perchloric acid containing thiourea (25 mM). Acetaldehyde was then determined in the perchloric acid extracts by headspace gas chromatography by the method of Eriksson *et al.* [23]. When acetaldehyde levels were determined in the liver slices, the slices were quickly removed from the medium and freeze-clamped with aluminium tongs precooled in liquid nitrogen. After pulverization and perchloric acid extraction, acetaldehyde was determined as indicated above.

Statistics. The results are expressed as means ± S.E. Comparisons were evaluated by using Student's *t*-test.

RESULTS

Since ethanol administration is known to affect hepatic protein synthesis [12–16], a pulse-chase system was employed to study glycoprotein secretion independent of synthesis. In this way, it was possible to focus on the post-ribosomal effects of ethanol and its metabolites on the secretion of glycoproteins by the liver. In accordance with our previous findings [17], the presence of ethanol (10 mM) in the chase medium markedly inhibited the release of both [^{14}C]leucine- and [^{14}C]glucosamine-prelabeled proteins into the medium (Fig. 1). To determine whether ethanol blocked the secretion of glycoproteins following the addition of the terminal sugars in the Golgi complex, we tested the effects of ethanol on the secretion of glycoproteins which were prelabeled with [^{14}C]galactose and [^{14}C]fucose, the pentultimate and terminal sugars, respectively, of the oligosaccharide side chains of secretory proteins. In this case, ethanol also impaired the secretion of these terminally prelabeled glycoproteins (Fig. 2).

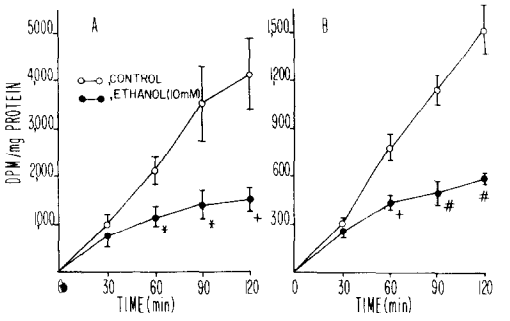

Fig. 1. Effects of ethanol on protein and glycoprotein secretion by rat liver slices. Slices were first pulse labeled for 30 min with [^{14}C]leucine (A) or [^{14}C]glucosamine (B) and then incubated in the corresponding chase medium containing 1 mM cycloheximide (A) or 20 mM glucosamine (B). During a 2-hr incubation in the chase medium with or without ethanol, the specific activities of the medium proteins (d.p.m./mg of protein) were determined. Results of four to six sets of such experiments have been averaged (±S.E.). Values significantly different from controls are indicated by the following symbols: (*) $P < 0.05$; (+) $P < 0.02$; and (#) $P < 0.01$.

Since it has been demonstrated that colchicine inhibits the secretion of proteins by rat liver after the addition of the terminal sugars to the secretory proteins has taken place in the Golgi [24], the effect of colchicine on glycoprotein secretion with our system was investigated. The presence of colchicine in

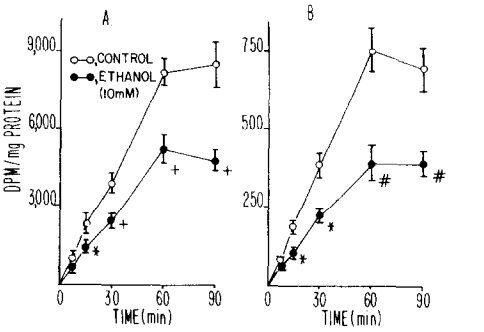

Fig. 2. Effects of ethanol on glycoprotein secretion by rat liver slices. Slices were first pulse labeled for 30 min with [^{14}C]fucose (A) or for 15 min with [^{14}C]galactose (B) and then incubated in the corresponding chase medium containing 20 mM fucose (A) or 20 mM galactose (B). During a 90-min incubation in the chase medium with or without ethanol, the specific activities of the medium proteins (d.p.m./mg of protein) were determined. Results of five to eight sets of such experiments have been averaged (±S.E.). Values significantly different from controls are indicated by the following symbols: (*) $P < 0.05$; (+) $P < 0.01$; and (#) $P < 0.001$.

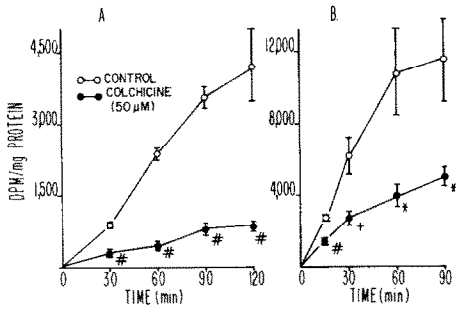

Fig. 3. Effects of colchicine on protein and glycoprotein secretion by rat liver slices. Slices were first pulse labeled for 30 min with [^{14}C]leucine (A) or [^{14}C]fucose (B) and then incubated in the corresponding chase medium containing 1 mM cycloheximide (A) or 20 mM fucose (B). During a 90–120 min incubation in the chase medium with or without colchicine, the specific activities of the medium proteins (d.p.m./mg of protein) were determined. Results of four to seven sets of such experiments have been averaged (±S.E.). Values significantly different from controls are indicated by the following symbols: (*) $P < 0.02$; (+) $P < 0.01$; and (#) $P < 0.001$.

the chase medium markedly impaired the release of both [^{14}C]leucine- and [^{14}C]fucose-prelabeled proteins into the medium (Fig. 3), indicating that colchicine and ethanol similarly decreased the secretion of glycoproteins.

Our previous studies [12, 13, 17] have shown that the alterations of glycoprotein metabolism induced by ethanol were a consequence of ethanol metabolism and may be attributable to acetaldehyde formation. In view of this, the effects of the immediate metabolites of ethanol oxidation, acetaldehyde and acetate, on glycoprotein secretion were examined. In experiments identical to those conducted to investigate the effects of ethanol, the presence of acetate (1 mM and 5 mM) in the medium did not alter significantly the secretion of either [^{14}C]leucine-, [^{14}C]glucosamine- or [^{14}C]fucose-prelabeled proteins (data not shown). These results would appear to indicate that the impairment of glycoprotein secretion by ethanol is not due to acetate formation. Acetaldehyde was added to the chase medium by infusion to avoid exposure of the liver to initial high aldehyde concentrations and to maintain levels comparable to those generated during ethanol oxidation [21, 25, 26]. As was the case with ethanol, the infusion of acetaldehyde (0.25 μmole/min) also impaired the secretion of [^{14}C]leucine-, [^{14}C]glucosamine- and [^{14}]fucose-prelabeled glycoproteins (Fig. 4). The concentration of acetaldehyde in the total incubation medium ranged from 0.34 ± 0.05 to 0.58 ± 0.06 μmole/ml when samples were analyzed at 15-min intervals during the infusion; however, when acetaldehyde levels were determined in the liver slice alone, the levels were significantly lower, ranging from 0.068 ± 0.004 to 0.085 ± 0.005 μmole/g ($P < 0.001$). On the other hand, when acetaldehyde was measured during ethanol (10 mM) oxidation by liver slices, the levels were slightly higher in liver, ranging from 0.021 ± 0.002 to 0.023 ± 0.002 μmole/g compared to 0.015 ± 0.001 to 0.016 ± 0.001 μmole/ml in the total medium ($P < 0.05$).

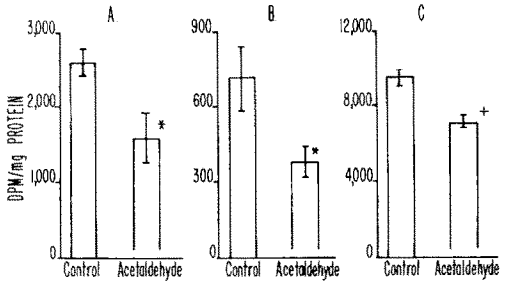

Fig. 4. Effects of acetaldehyde infusion on the secretion of proteins and glycoproteins by rat liver slices. Slices were first pulse labeled for 30 min with [^{14}C]leucine (A), [^{14}C]glucosamine (B) or [^{14}C]fucose (C) and then incubated in the corresponding chase mediums containing 1 mM cycloheximide (A), 20 mM glucosamine (B) or 20 mM fucose (C). Acetaldehyde was infused into the chase medium at a rate of 0.25 μmole/min for 1 hr. Controls were treated identically except that an equivalent volume of buffer was infused. Specific activities of the medium proteins were determined after a 1-hr incubation in the chase medium. The results are expressed as means ± S.E. for five to eight sets of experiments. Values that were significantly different from appropriate controls are indicated by the following symbols: (*) $P < 0.05$; and (+) $P < 0.01$.

DISCUSSION

It is evident from this study that ethanol administration profoundly influences the metabolism of plasma glycoproteins. Previous studies [12–16] have demonstrated that acute alcohol administration inhibits general peptide synthesis including the formation of the protein moiety of secretory proteins. The results of this study, as well as our previous work [17], show that ethanol, in addition, also impairs later events in the complex sequence of glycoprotein synthesis and secretion. The ability of ethanol to inhibit the release of galactose- and fucose-prelabeled glycoproteins into the incubation medium by liver slices (Fig. 2) indicates that ethanol interferes with the final steps of secretion which occur following the attachment of the terminal sugars to glycoproteins. Furthermore, the observation that colchicine, an agent known to block the secretion of completed glycoproteins [24], behaved in a manner similar to ethanol in blocking the secretion of prelabeled glycoproteins in our system (Fig. 3) further supports this conclusion. Thus, it appears that ethanol can impair the process of glycoprotein synthesis and secretion in at least two separate and distinctive ways. The first is at the level of the synthesis of the protein moiety, and the second involves the final stages of secretion of the completed glycoproteins.

Our previous studies [12] have shown that the ethanol-induced alterations in glycoprotein metabolism were the result of the oxidation of ethanol; therefore, the effects of the metabolites of alcohol, acetate and acetaldehyde, were investigated. Acetate concentrations of 1 and 5 mM, which are within the range of values reported to occur during ethanol oxidation [27], had no effect on the secretion of prelabeled glycoproteins. Acetaldehyde, however, did mimic the effects of ethanol in blocking glycoprotein secretion (Fig. 4). Acetaldehyde was added

to the liver slice system by infusion at a rate (0.25 μmole/min) which approximated the rate that ethanol was oxidized per flask of liver slices. This infusion rate resulted in concentrations of acetaldehyde in the total system (incubation medium plus slices) of about 300–600 μM; however, acetaldehyde levels in the liver slices alone were much lower, ranging from 70 to 85 μM. This difference in acetaldehyde levels would indicate that the rate of acetaldehyde oxidation in the liver exceeded its rate of entry into the liver. A contributing factor to this occurring is that at 37° acetaldehyde, because of its volatility, probably exists in the vapor phase which would limit its availability to the liver. On the other hand, acetaldehyde levels generated during ethanol oxidation by the liver slices were somewhat lower. In the liver the levels were slightly over 20 μM, whereas in the total system they were about 15 μM. Hepatic levels of acetaldehyde during ethanol oxidation, reported in the literature, vary considerably, ranging from about 3 to 200 μM [21, 26, 28], and are influenced by many factors including methods of analysis and composition of the diet [29–31].

Several implications of impaired secretion of plasma glycoproteins are evident. Plasma glycoproteins carry out numerous physiological functions including lipid transport, hemoglobin binding, hormone transport and blood coagulation [1]. Therefore, many homeostatic mechanisms of the organism could be perturbed as a result of an ethanol-induced inhibition of glycoprotein secretion. In addition, retained secretory proteins may be a contributing factor to liver enlargement observed in alcoholics as well as in rats chronically fed ethanol [32]. Baraona *et al.* [32] have suggested that the hepatic retention of export proteins attributable to faulty secretion could contribute to the hepatomegaly observed in rats chronically fed alcohol. These authors further suggest that impaired microtubule formation may be responsible for this decrease in secretion. However, the mechanism of the ethanol-induced inhibition of glycoprotein secretion still remains to be established. Future goals of our laboratory are to investigate the mechanism by which acute ethanol administration impairs glycoprotein secretion and to determine whether similar mechanisms of impaired secretion are operative in animals treated chronically and acutely with ethanol.

In conclusion, the results of this study indicate that ethanol via its metabolite, acetaldehyde, impairs the secretion of glycoproteins by the liver and suggest that this impairment follows the attachment of the terminal sugars in the Golgi region.

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