INFLUENCE OF ACUTE ETHANOL INTOXICATION ON RAT LIVER GOLGI APPARATUS GLYCOSYLATION ACTIVITIES

G. NANNI, M. A. PRONZATO, M. M. AVERAME, G. R. GAMBELLA, D. COTTALASSO and U. M. MARINARI

Istituto di Patologia Generale, Università di Genova, via L. B. Alberti 2, 16132 Genova, Italy

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1. Introduction

It has been established that plasma lipoproteins are also glycoproteins and that the liver is the major site of their synthesis [1–4]. Several studies have demonstrated that the glycosylation of VLDLs occurs in hepatocyte Golgi apparatus: labelled glucosamine is rapidly incorporated into lipoproteins isolated from the Golgi apparatus [4,5] and also sialic acid-containing peptides of plasma VLDL and HDL are present as a precursor pool within the Golgi apparatus [5,6]. In addition, purified asialo human apo-Ala (apo-C-III-1) can be glycosylated by isolated Golgi membranes [7] and nascent VLDLs are progressively glycosylated during the passage through the Golgi apparatus along the acquisition of apo-C-II and apo-C-III [8].

A specific multiglycosyltransferase system is required for the synthesis of each type of glycoprotein [9]. Most of these activities were recognized to be located in the Golgi membranes [10–12]; since they are highly active in transferring galactose from UDP-galactose to *N*-acetylglucosamine [9,13–16], it was suggested that this galactosyltransferase is a marker enzyme for Golgi apparatus [17,18].

Other transferase activities have also been shown to be associated with liver Golgi membranes, which are able to transfer various types of sugar both to protein [19,20] and lipoprotein acceptors [4,21].

On the basis of these data it seems clear that the hepatocyte Golgi apparatus plays a role not only in the concentration, packaging and transport of VLDLs [22,23] but particularly in their terminal enzymatic glycosylation before secretion from the liver into the blood stream [24]. Ultrastructural changes of the hepatocyte Golgi region were observed in acute ethanol-intoxicated animals [22,25,26].

Recently, evidence was presented that in such conditions there is an impairment of [^{3}H]palmitic acid transport through the Golgi apparatus clearly indicating a decrease, at least transient, of hepatic lipoprotein secretion [27].

The present studies are concerned with the possible implications of the liver Golgi apparatus glycosylation activities on the failure of VLDL transport. Therefore our object is the investigation of the patterns of [^{14}C]glucosamine incorporation into the hepatocyte-isolated Golgi apparatus and the behaviour of UDP-galactose:*N*-acetyl-glucosamine galactosyltransferase activity during acute ethanol intoxication.

2. Materials and methods

Female Sprague Dawley rats (CD-COBS-Charles River), 200 ± 25 g, fasted for 16 h, received by oral intubation either a single dose of ethanol (6 g/kg body wt) or isocaloric glucose.

D-[1-^{14}C]Glucosamine, 10 μCi (CEA-Sorin; spec. act. 57 mCi/mM) were injected intravenously into each rat. Lots of 6 animals were killed after 1.5 h, 3 h, 6 h and 12 h from ethanol treatment, and after 30 min, 45 min, 60 min and 90 min from [^{14}C]glucosamine injection. Four lots of animals were used for each experimental time.

Three different fractions (F_1, F_2, F_3) of liver Golgi

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apparatus were isolated as in [27]. Aliquots of PTA–trichloroacetic acid precipitates from each Golgi fraction were immediately dissolved in Instagel (Packard) and radioactivity was determined with a Tri-Carb liquid scintillation Spectrometer, Packard Model 3320.

UDP-galactose:*N*-acetyl-glucosamine galactosyltransferase activity was measured on aliquots of each Golgi fraction by the method in [28] as modified [13]. The assay mixture in sodium cacodylate buffer (pH 6.5) contained: UDP-[^{14}C]galactose, 0.15 μmol (The Radiochemical Centre Amersham; final spec. act. 2.3 × 10^6 dmp/μM) as carbohydrate donor; *N*-acetyl-glucosamine, 3 μmol (Calbiochem) as sugar acceptor and 40–80 μg protein equivalent of Golgi fraction. The blank contained all the mixture compounds except *N*-acetyl-glucosamine.

After 1 h incubation at 37°C the reaction was stopped with the addition of 0.3 M EDTA (pH 7.4) and the chilled samples and control tubes were processed by the method in [12].

Finally, the results obtained were expressed as nmol galactose transferred to *N*-acetyl-glucosamine/h/mg protein.

Protein was estimated by the method in [29] as modified [30].

3. Results and discussion

The incorporation of [^{14}C]glucosamine into the PTA–trichloroacetic acid precipitable proteins of rat hepatocyte Golgi secretory membranes was studied, at first, 45 min after intravenous injection of the labelled sugar.

The radioactivity starts to reduce 1.5 h after ethanol intoxication: within 3 h and 12 h the specific radioactivity (cpm/mg protein) decreases in a very significant and constant way. In the formative membranes (F_3) the incorporation of the label shows a marked reduction only later, about 12 h after ethanol intoxication (fig.1). Since these results suggest an impairment in the carbohydrate moiety attachment to lipoprotein in the Golgi apparatus during ethanol intoxication, further investigations have been undertaken to study the possible pathway of this failure.

A first approach to the problem was concerned with the chronological distribution of labelled sugar

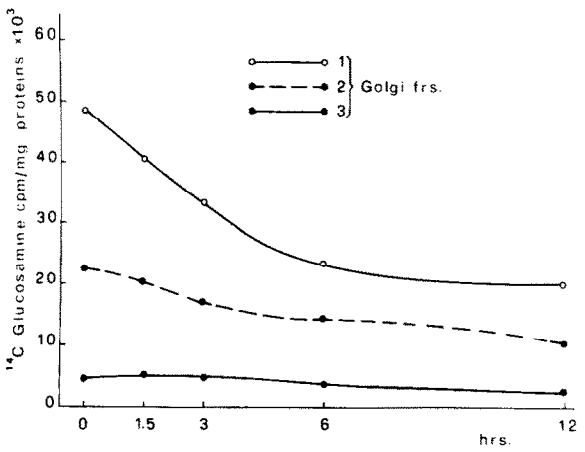

Fig.1. [^{14}C]Glucosamine incorporation in liver Golgi apparatus 45 min after intravenous injection in rats at different times after a single ethanol dose. The values represent the specific activity of PTA–trichloroacetic acid precipitates referred to the protein content.

by evaluating the time sequence of its uptake and the secretion from Golgi apparatus membranes.

The input of [^{14}C]glucosamine into the Golgi fractions of normal rats reaches a maximum 45 min after intravenous injection; at 60 min the output of radioactivity is completed, indicating a rapid transport of the carbohydrate through the glycosylating sites.

After acute ethanol intoxication, the uptake of radioisotope by the secretory fractions (F_1 and F_2) shows a precocious damage; in fact, at each intoxication time, but more significantly at 6 h and 12 h, the specific activity is lower with respect to normal values. Within 45 min and 90 min after [^{14}C]glucosamine injection, the output of the label decreases as the time from the ethanol intoxication increases: at 1.5 h the release of the radioisotope shows a significant slowing down and at 6 h and 12 h it is practically inhibited (fig.2a,b). In the Golgi apparatus formative membranes (F_3) the chronological sequence of the radioisotope incorporation never significantly changes (fig.2c).

These results suggest that in ethanol treated rats the Golgi apparatus secretory membranes are unable to develop their role in the formation of glycoproteins: consequently, the terminal glycosylation of VLDLs could be inhibited. An impairment of [^{14}C]-

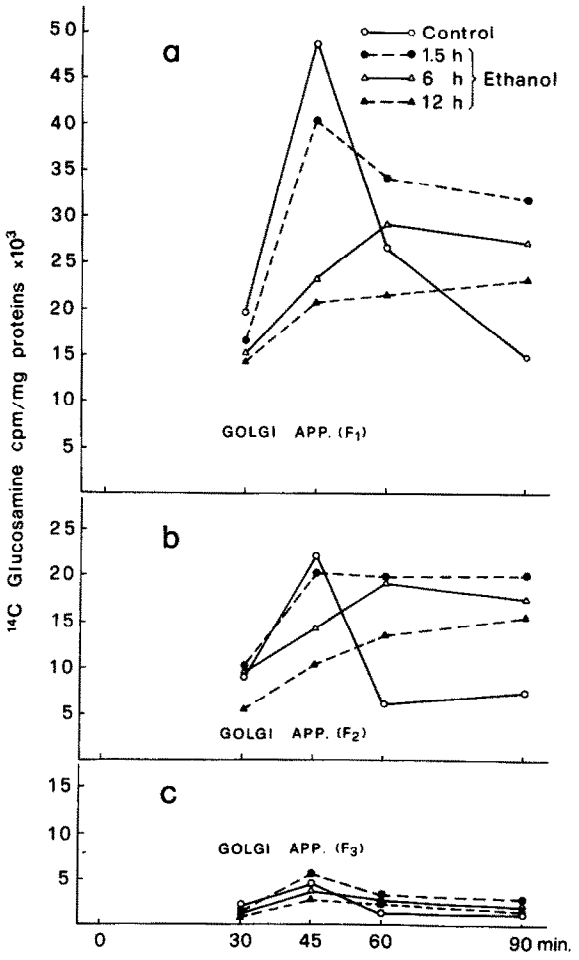

Fig.2. Transport of [^{14}C]glucosamine (30–90 min after intravenous injection) into the liver Golgi apparatus fractions of ethanol-treated rats.

glucosamine incorporation into the hepatocyte microsome proteins and a significant decrease of UDP-*N*-acetyl-hexosamine level in the liver of ethanol-intoxicated rats were shown [31]. Since recent studies demonstrated that some enzymatic activities implicated in the pathway of UDP sugar synthesis are located in the liver Golgi apparatus [32], the reduction of [^{14}C]glucosamine transport in acute ethanol-induced fatty liver may be dependent on a block in the synthesis of carbohydrate precursor pools responsible for the glycoprotein moiety formation of VLDLs. Besides, during the development of fatty liver due to dietary choline deficiency, the incorporation of [^{14}C]glucosamine into liver microsome proteins is inhibited, suggesting that the lipoprotein glycosylation is impaired also in these conditions [33–35].

In order to evaluate the behaviour of the glycosylating enzymes, a study of the UDP-galactose:*N*-acetyl-glucosamine galactosyltransferase activity has been undertaken in the three isolated Golgi fractions. After ethanol intoxication, the enzyme content in F_1 and F_2 significantly increases and at 1.5 h the specific activity reaches a peak with values which are doubled in comparison with the controls.

After this time the galactosyltransferase content shows an early reduction: at 6 h the specific activity drops to values significantly lower than the normal: only 12 h after intoxication the enzyme activity does normalize (fig.3). The enzyme content in normal F_3 is clearly low but its alterations after ethanol intoxica-

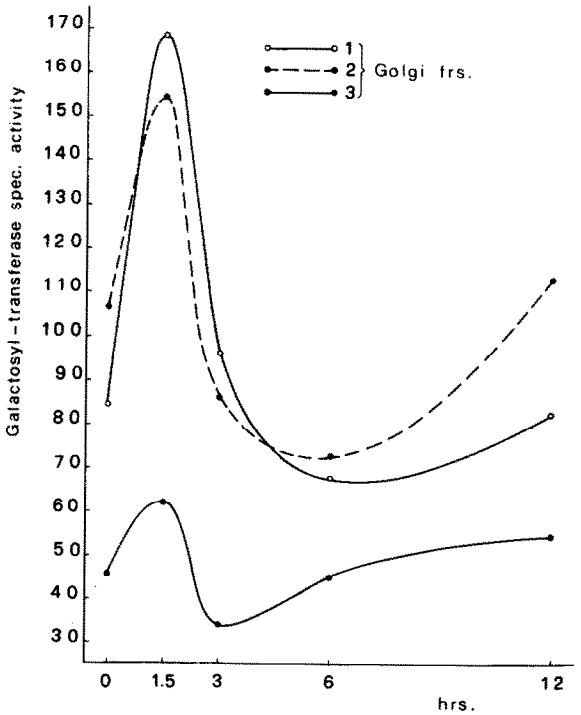

Fig.3. UDP-galactose:*N*-acetyl-glucosamine galactosyltransferase activity in rat liver Golgi apparatus fractions isolated at different times after acute ethanol intoxication. The values are expressed as nmol galactose transferred to *N*-acetyl-glucosamine/h/mg protein.

tion are similar to those observed in the other fractions: the specific activity shows an early rise at 1.5 h, quickly drops within 3 h and normalizes at 6 h (fig.3).

The increased enzyme activity observed at 1.5 h after ethanol intoxication agrees with the results obtained at the same time by [12,36] and may be dependent on the morphological changes induced by acute ethanol intoxication in hepatocyte Golgi apparatus vacuoles, saccules and vescicles, which increase both in size and in number and become loaded with VLDLs [25]. Therefore such behaviour of galactosyltransferase could provide a more rapid glycosylation of a pre-formed apo-VLDL pool whose presence has already been demonstrated [37,38] and, consequently, a fast secretion of VLDLs into the blood stream.

Unlike [39] however, at 3 h and 6 h after ethanol intoxication we found a marked reduction of the galactosyltransferase content in Golgi membranes. The enzyme failure is contemporaneous with the decrease of the [^{14}C]glucosamine incorporation into the Golgi apparatus: probably, ethanol and/or its metabolites may induce a block in the activity of the enzymes involved in the synthesis of UDP sugars into the Golgi apparatus. Furthermore the ethanol intoxication may affect the transfer of the UDP sugars to their acceptors by inhibiting the glycosyltransferase activities. From this point of view, the effect of ethanol may resemble that of puromycin which disrupts the galactosyltransferase activity by linking itself to liver Golgi membranes [40].

The alterations of [^{3}H]palmitic acid movements into hepatocyte Golgi apparatus during ethanol

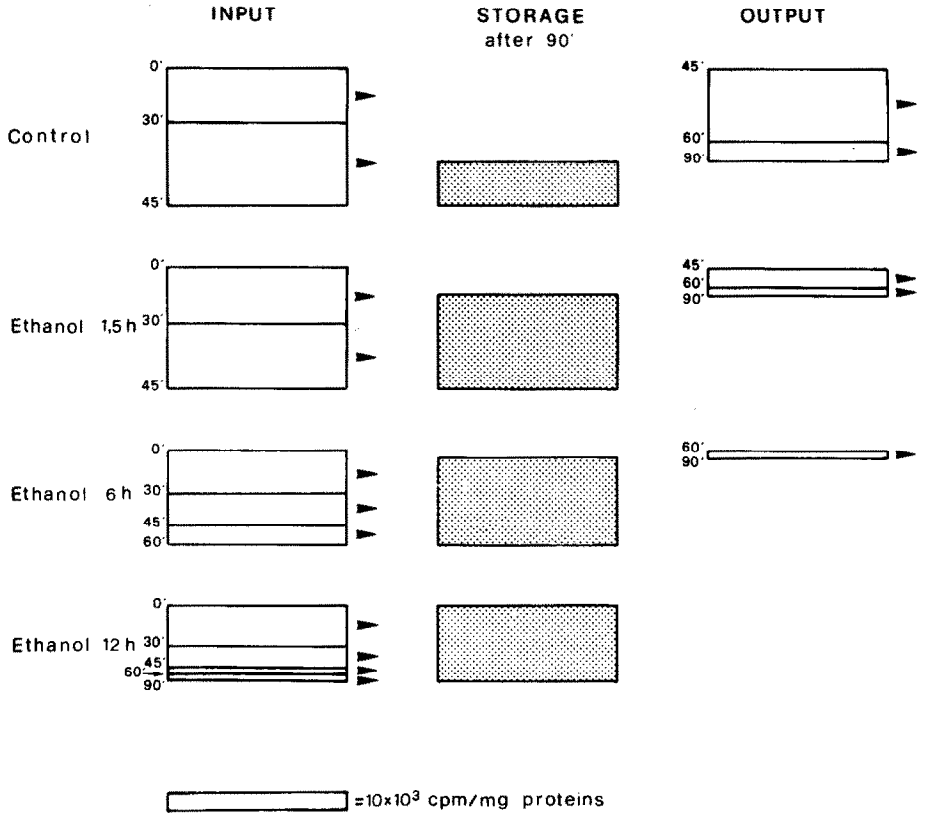

Fig.4. Diagram showing the various steps in the transport and storage of [^{14}C]glucosamine through the combined F_1, F_2 and F_3 fractions of Golgi apparatus.

intoxication were shown [27]. Such results, together with the actual data on the transport of labelled glucosamine through the Golgi apparatus, indicate that this complex of membranes is involved in the damage induced by ethanol intoxication on the synthetic and secretive mechanisms of VLDLs. Particularly, the [^{14}C]glucosamine input into the VLDLs and its output from the Golgi apparatus decrease very obviously (1.5 h after ethanol intoxication, fig.4) in comparison with the pathway of [^{3}H]palmitic acid incorporation and release into the blood stream which decrease at 6 h after ethanol intoxication at the same time of triglycerides accumulation [27].

In conclusion, the impaired hepatic lipoprotein secretion observed in ethanol-induced fatty liver may be due to altered mechanisms of progressive terminal glycosylation of VLDLs in the Golgi apparatus.

Acknowledgement

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