EFFECTS OF ACUTE ETHANOL INTOXICATION ON [^{3}H]PALMITIC ACID TRANSPORT THROUGH HEPATOCYTE GOLGI APPARATUS

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1. Introduction

The role of rat hepatocyte Golgi apparatus in the synthesis and secretion of lipoproteins is well established [1–5]. During the lipoprotein synthesis cycle the rate of turnover of Golgi membranes seems to be directly proportional to the rate of synthesis and transport of the lipoprotein particles [6]. This appears to justify the implication of the Golgi apparatus in the pathogenesis of fatty livers where the morphological and functional modifications of this complex are related to an impaired lipoprotein metabolism [4].

In acute ethanol-intoxicated animals, electron microscopic observations revealed that the stacked cisternae, vacuoles, and vesicles of the Golgi apparatus increase both in number and in size and become loaded with very low density lipoproteins (VLDLs) [7,8]. Although the pathogenesis of acute ethanol-induced fatty liver is as yet undefined [9–11], the data suggest that the development of such disease could involve also an impairment of hepatocyte Golgi apparatus VLDLs synthetic and secretory mechanisms.

The object of the present study is to verify this possibility by analysing the triglyceride content and the chronological sequence of [^{3}H]palmitic acid transport in rat liver Golgi apparatus at different times after acute ethanol intoxication.

2. Materials and methods

Female Sprague Dawley rats (CD-COBS-Charles River), weighing 200 ± 25 g, were fed on a standard synthetic diet, devoid of antioxidants. Lots of 6 animals, fasted for 16 h, received by oral intubation either a single dose of ethanol (6 g/kg body wt as a 50%, w/v, solution) or isocaloric glucose. 104 μCi 5% albumin-bound [9,10-^{3}H]palmitate (Radiochemical Centre, Amersham; spec. act. 500 mCi/mmol) were injected intravenously into each rat. The animals were killed at different times after intubation (1.5, 3, 6, 12 h) and after [^{3}H]palmitic acid injection (5, 10, 20, 30 min).

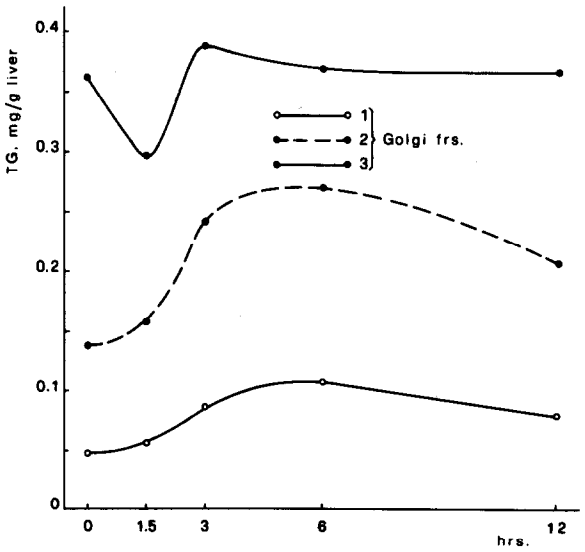

Fig.1. Triglyceride content of isolated fractions of rat liver Golgi apparatus at different times after acute ethanol intoxication (6 g/kg body wt).

Preparations of Golgi apparatus were made from pooled livers by discontinuous sucrose density-gradient ultracentrifugation, according to [7], slightly modified [12]. Three different fractions of purified Golgi membranes (F_1, F_2, F_3) were isolated. Four lots of animals were used for each experimental time. Triglyceride content was measured by an enzymatic method [13] after lipid extraction according to [14]. A known amount of protein and triglyceride from each Golgi fraction was processed for determination of specific radioactivity (cpm/mg protein or cpm/mg triglyceride), by addition of cold 1% phosphotungstic acid (PTA) in 20% trichloroacetic acid (TCA). PTA–TCA precipitates were washed twice with 5% TCA and once with distilled water. Aliquots of precipitates were immediately dissolved in Instagel (Packard). From other aliquots of PTA–TCA precipitates, lipids were extracted with a 2:1, v/v, mixture of chloroform/methanol, dried and dissolved in Instagel. Radioactivity was

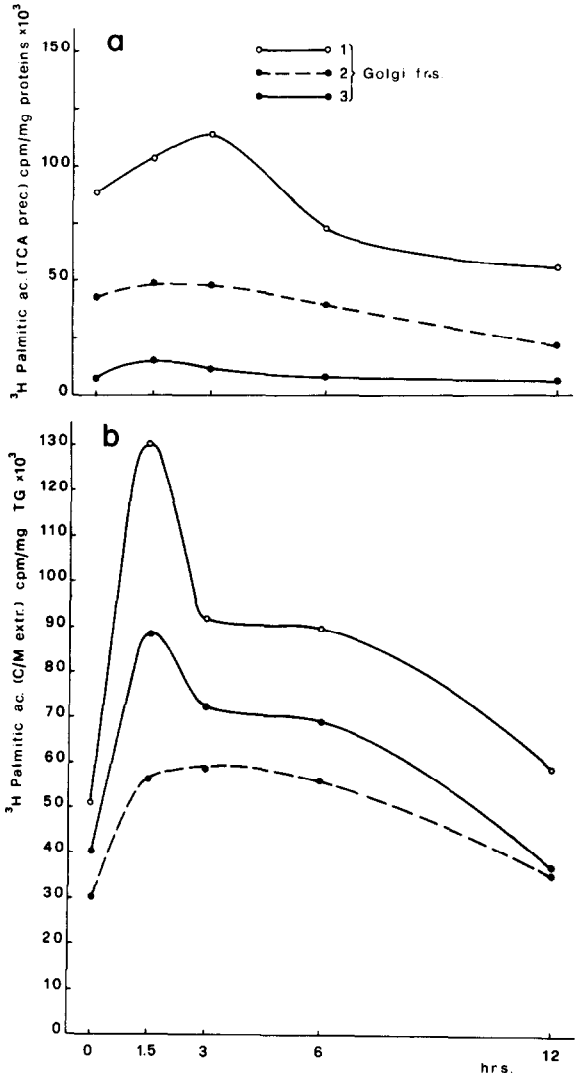

Fig.2. Radioactivity incorporated in liver Golgi apparatus 10 min after [^{3}H]palmitic acid intravenous injection in rats at different times after a single ethanol dose. The values represent the specific activity of the TCA-precipitates referred to the protein content (fig.2a) and of the chloroform/methanol extracts referred to the triglyceride content (fig.2b).

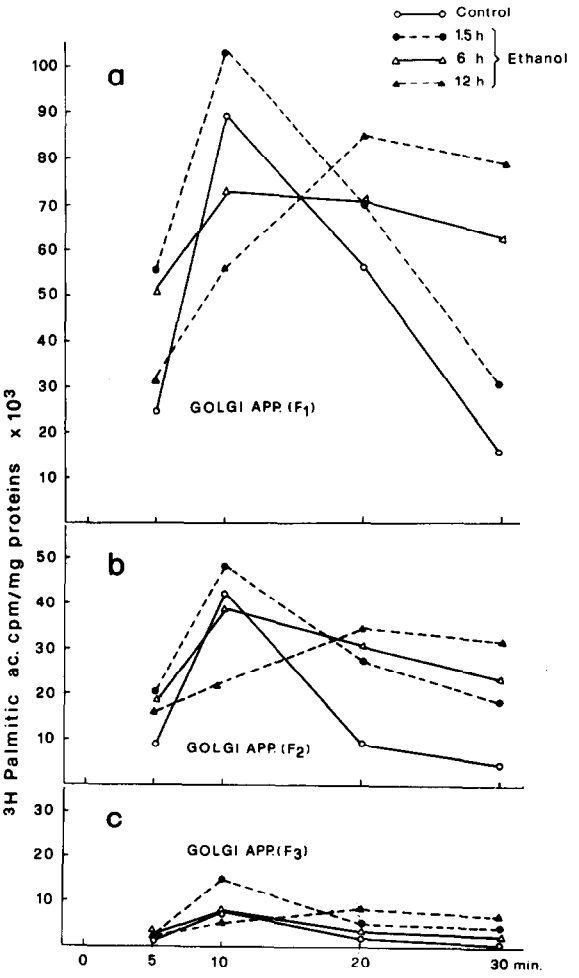

Fig.3. Transport of [^{3}H]palmitic acid (5–30 min after intravenous injection) into the liver Golgi apparatus fractions of ethanol-treated rats.

determined with a Tri-Carb liquid scintillation spectrometer, Packard model 3320.

Protein was estimated by the method [15] as modified [16].

3. Results and discussion

The triglyceride content of rat hepatocyte Golgi secretory membranes (F_1, F_2) significantly increases 3 h after acute ethanol intoxication, reaches a maximum at 6 h and shows a slight tendency to decrease only about 12 h later. On the other hand, in the Golgi apparatus formative membranes (F_3), the triglyceride content never significantly changes (fig.1). These results suggest an early implication of Golgi secretory membranes in lipoprotein storage during the initial stages of ethanol-induced fatty

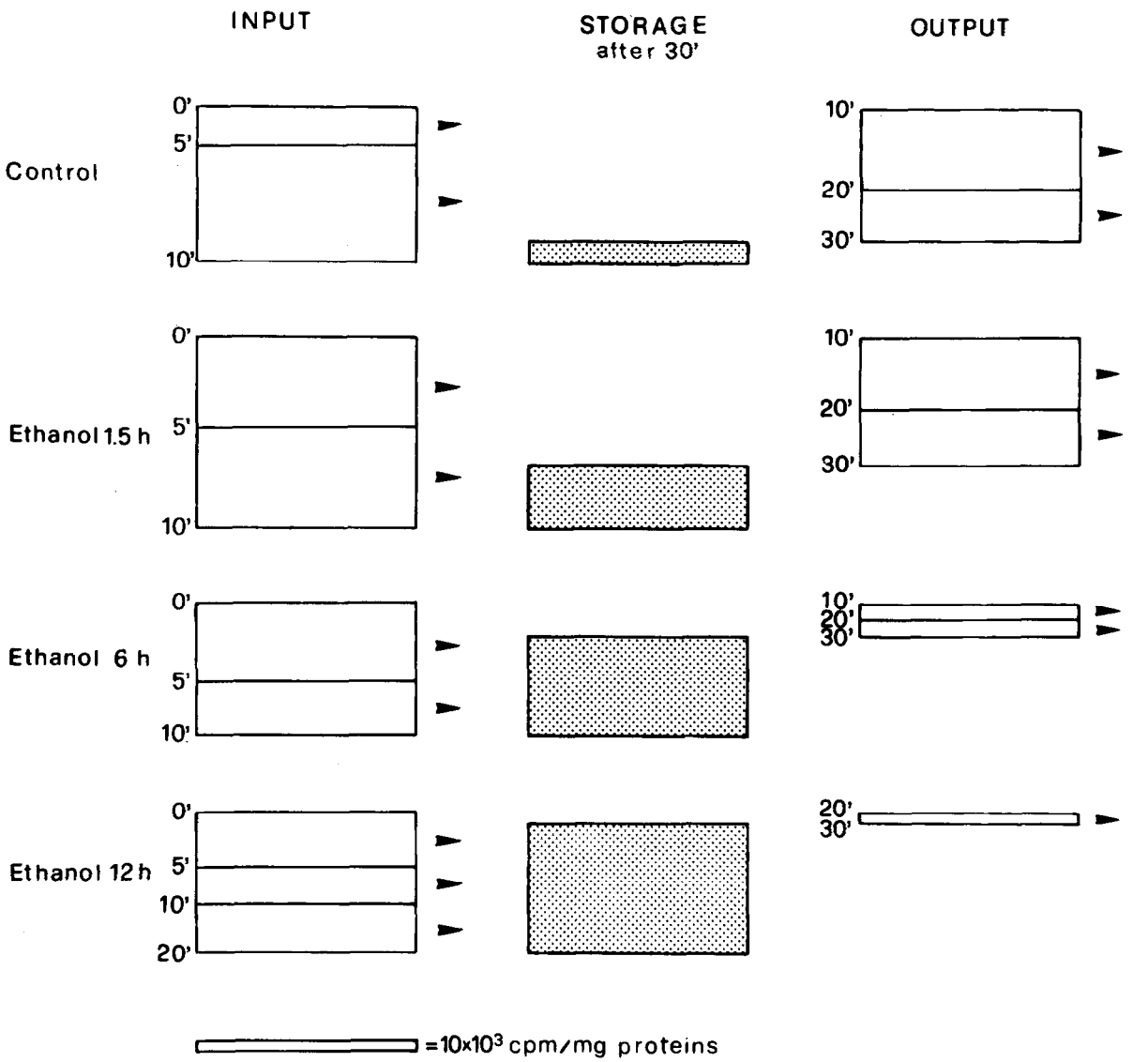

Fig.4. Diagram showing the various steps in the transport and storage of [^{3}H]palmitic acid through the combined F_1, F_2, F_3 fractions of Golgi apparatus.

liver. This could explain the electron microscopic observations of increasing numbers of osmiophilic particles in the cisternae and vesicles of Golgi apparatus after acute ethanol treatment [3,7,8].

Possible alterations of lipid movements into hepatocyte Golgi apparatus were studied 10 min after [^{3}H]palmitic acid injection. The radioactivity of isolated Golgi fractions (F_1, F_2, F_3), relative to the protein content is very high in the initial period after ethanol treatment. However, the specific activity, after a significant but short-lived increase only in F_3, decreases below normal values in all fractions within 6 h after ethanol intoxication (fig.2a). The chloroform/methanol extracted lipid radioactivity, as compared with the triglyceride content, shows a similar but more marked trend (fig.2b). These changes of lipid movement through the Golgi apparatus may be dependent on the ethanol-induced injury to the membranes responsible for intracellular VLDLs transport.

To determine whether the impairment of [^{3}H]-palmitic acid incorporation is due to changes of lipid material input into the Golgi apparatus or of lipoprotein output from the same complex, a time sequence of [^{3}H]palmitic acid distribution in the Golgi apparatus was determined in ethanol-treated rats injected with labelled lipid at different times through 5–30 min. The results are shown in fig.3. In each Golgi apparatus fraction either the [^{3}H]-palmitic acid input or output become lower. Such an impaired secretory mechanism is more evident 12 h after intoxication; at this time the accumulation of labelled lipid in the Golgi apparatus is significantly higher than in untreated rats (fig.4).

From these data it seems that, in ethanol-induced fatty liver, the Golgi apparatus is involved, at least temporarily, in an impaired hepatic lipoprotein secretion. This implication may be due either to an altered turnover of the membranes responsible for the entire process of liver lipoprotein synthesis, transport, and storage [6] or to damage to Golgi complex sites where VLDLs are elaborated by acquisition of new apoproteins and their progressive glycosylation [5,17].

Acknowledgements

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