ON THE MECHANISM OF PROTECTIVE ACTION OF COLD ACCLIMATIZATION AGAINST CARBON TETRACHLORIDE- AND ETHIONINE-INDUCED FATTY LIVER

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In this study the hepatic lipoprotein lipase (LPL) activity was evaluated in adult female mice acclimatized at 5 °C and submitted to carbon tetrachloride (CCl_4) or ethionine, in order to determine the possible role of this enzyme in the fatty liver. The results were compared with those obtained in mice kept at room temperature (27 °C) that received the same hepatoesteatosis inducing agent. In contrast to animals kept at room temperature, in cold acclimatized mice neither the enhancement of the LPL-liver activity by the action of CCl_4 or ethionine occurred nor the development of fatty infiltration in the liver was observed. We conclude that the low temperature induced a protective effect against CCl_4- or ethionine-induced fatty liver that was correlated with the no-increase of the hepatic LPL activity.

The liver plays a central role in the synthesis and degradation of plasma lipoproteins, having specific receptors for remnant chylomicrons and low density lipoproteins (LDL) internalization (Brown & Goldstein, 1983). Moreover, heparin induces hepatic release of lipolytic-enzymes to the bloodstream, so the liver contributes with 63% of the total triacylglycerol lipase (TGL) (Jansen & Hulsman, 1974). *In vitro* this liver's lipolytic activity degradates to chylomicrons and in lesser extent to very low density lipoproteins (VLDL) (Krauss et al., 1973). *In vivo*, the liver removes 20 to 30% of the triglycerides (TG) contained in the circulating chylomicrons (Bergman et al., 1971). Clearance by the other tissues is carried out by lipoprotein lipase (LPL, E.C. 3.1.1.3.4), enzyme which was considered of extrahepatic origin, but now it has been demonstrated that is present in the liver of a variety of adult animals including sheep (Bergman et al., 1971), fish, chicken and mouse (Reina et al., 1987), rat (Felts & Mayes, 1967), dog and man (Condon et al., 1965). It is also

found in fetus and newborn animals (Reina et al., 1987). Another lipolytic enzyme, the lipase hepatic (LH), is also liberated from liver by the effect of heparin. LH has different behavior in response to activators and inhibitors of LPL and its function is related with high density lipoprotein (HDL) degradation (Norum et al., 1983).

In hepatosteatosis there is an imbalance in the input and output of liver lipids. In some cases, lipoprotein synthesis in the hepatocyte decreases (Truswell et al., 1969); in others, the fatty acids influx exceeds the liver lipoproteins exporter capacity (Feigelson et al., 1981). Participation of hepatic LPL in the generation of fatty liver is possible because its action provides to hepatocyte the triglyceride-components, these eventually will be deposited in liver tissue. Previously, Fernández et al. (1970) have demonstrated that LPL-activity increases in aortic tissue of rabbits with experimental atherosclerosis and Zilversmit (1973) has indicated that this activity enhancement induces higher offer to arterial wall of cholesterol-rich lipoproteic particles, contributing to tissue lipid deposits.

The cold-acclimatization modified the lipid metabolism. Seller & You (1952) have observed non liver-triglyceride accumulation in rats submitted to a choline poor diet and main-

tained at low temperature. In our laboratory, this result was confirmed in cold-acclimatized mice receiving either carbon tetrachloride (CCl_4) or ethionine (Carmona et al., 1981) two fatty liver inducers. It is unknown the mechanism by which cold protects against CCl_4 or ethionine-induced hepatosteatosis. Some modifications of lipoprotein lipase activity in tissues of cold-adapted rats have been reported (Radomski & Orme, 1971). It is possible that the lipotropic effect developed in animals submitted to low temperature, may be related with liver LPL-activity.

The present study was undertaken to measure the activity of hepatic lipoprotein lipase in acclimatized-mice (5 °C) submitted to CCl_4 or ethionine, in order to determine the possible role of this enzyme in the fatty liver.

MATERIALS AND METHODS

Adults albino female mice weighing 25-30 g were housed in individuals cages and allowed free access of water and mouse laboratory pellets (Protinal, Venezuela) and the light on from 7 to 19 hours.

Mice were maintained at either 5 ± 1 °C or 27 ± 2 °C for three weeks. At the end of this adaptation period the animals were treated with the fatty liver inducer. A group of mice from each temperature (27 °C and 5 °C) received CCl_4 (2 ml/kg in oil vehicle) by gastric intubation. A group from each temperature was injected i.p. with DL-ethionine (7.5 mg/20 g in saline solution). In control mice (27 °C and 5 °C) the same volume of vehicle was given. After treatment all animals were fasted for 48 hours at room temperature before sacrifice by decapitation.

The livers were excised and weighed. The amount of total lipids was measured gravimetrically after fat extraction using Folch's method. The results were expressed in g/100 g of fresh tissue. Samples of each liver were histologically analyzed stained with hematoxilin-eosin and Sudan IV.

Lipoprotein lipase assays – The livers were perfused *in situ* through the porta vein using a cold Tyrode's solution to wash out any residual blood. The livers were removed, weighed and homogenized in 0.005 M buffer veronal pH 7 for 1 min using an Ultra Turrax (JK-IKA-Werk, Staunfen, Germany). The final

concentration of tissue homogenates was 10% w/v. Homogenates were immediately centrifuged for 20 min at 1000 g in a refrigerated centrifuge (International Equip. Co., Needham HTS, Mass., USA). All operations were conducted at 4 °C.

Protein determinations in the supernatants were carried out using Kjendahl's method and the activity of lipoprotein lipase was determined by the procedure described by Patten and Hollenberg, but improvement of the incubation medium was done according to Corey & Zilversmit (1977).

The composition of assay medium was: 2 vol of working substrate and 1 vol of 0.62 M Tris-HCl buffer pH 8.6. A fresh working substrate was prepared daily diluting 2 vol of stock substrate with 2 vol of 5% (w/v) bovine serum albumin in 0.62 M Tris-HCl buffer pH 8.6 and 1 vol of rat plasma, previously heated for 60 min at 56 °C in order to inactivate some endogenous plasma lipolytic activity.

The stock substrate was prepared homogenizing 553.43 mg of trioleine and 27.07 mg of yolk lecithin in 10 ml of glycerol using an Ultra Turrax.

Assays were carried out by incubating 0.6 ml of the medium in a shaking water bath (Lab-line Instruments Inc., Melrose Park, Ill, USA) at 30 °C with 0.4 ml of supernatant tissue homogenate. Tubes were taken out in pairs, after 0 and 1 hr. The release of fatty acids was determined according to the microtechnique of Goss & Lein (1967) using palmitic acid as standard. Control assays without homogenate were performed in order to substract fatty acids release by spontaneous medium's lipolysis. The enzymatic activity was expressed in µEq of free fatty acids release/ hour per mg of protein. The results were analyzed using the Student's "t" test.

Chemicals – DL-Ethionine was from BDH Chemicals Ltd. (Poole, England) and all others chemicals were obtained from Merck (Darmstadt, Germany).

RESULTS

Large changes were found in the hepatic lipoprotein lipase activity. It was significantly increased in the 27 °C adapted mice that received CCl_4 compared with the control animals (Table I). In this condition was observed

a liver-lipid content (15.20 ± 4.36 g%) two fold higher than of the control value (7.69 ± 1.38 g%) and severe histological lesion with extensive necrosis in the centrilobular region and hepatic fatty changes.

TABLE I

Effect of carbon tetrachloride and ethionine on hepatic lipoprotein lipase activity of acclimatized mice

	Lipoprotein μEq/h/mg protein
27 °C adapted mice	
A. Control	0.104 ± 0.076 (7)
B. With CCl4	0.259 ± 0.060 (13)[a]
C. With ethionine	0.219 ± 0.061 (3)[a]
5 °C adapted mice	
D. Control	0.129 ± 0.032 (6)
E. With CCl4	0.147 ± 0.030 (8)
F. With ethionine	0.157 ± 0.027 (4)

Values represent the mean ± SD. In parentheses animals number.
a: indicates difference statistically significant with its control (p < 0.01).

Ethionine administration also induced a significant enhancement of the liver lipoprotein lipase activity in 27 °C adapted mice (Table I). As it is usual, these animals presented a markedly increase in the hepatic lipid content (28.40 ± 8.58 g%) distributed in large cytoplasmic fat droplets in the centrilobular and midzonal area. Necrotic processes were present near of central vein.

Mice acclimatized at 5 °C did not develop fatty liver neither by CCl_4 nor ethionine administration. Liver histology showed absence of fatty infiltration, although mild necrosis was noted in the centrilobular zone. The liver lipid concentration was reduced in both CCl_4-fed or ethionine-treated animals maintained at 5 °C (6.77 ± 1.88 and 5.53 ± 0.89, respectively). In both groups, the hepatic lipoprotein lipase activity did not differ of 5 °C controls values. Furthermore, this controls values were not significantly differents of those found in 27 °C control mice (Table I).

In this experiment we observed that the increased activity of liver-LPL induced by CCl_4, presented early onset (Table II). Two hours after treatment, the enzymatic activity was enhanced two fold in comparison to control one. This enhancement remained during the following 48 hr.

TABLE II

Effect of the carbon tetrachloride on the hepatic lipoprotein lipase activity of mice kept at laboratory temperature (27 ± 2 °C)

Time after treatment hours	Lipoprotein lipase activity μEq/h/mg protein
0	0.104 ± 0.076 (7)
2	0.240 ± 0.050 (3)[a]
4	0.210 ± 0.040 (3)[a]
12	0.230 ± 0.010 (3)[a]
24	0.240 ± 0.040 (3)[a]
48	0.259 ± 0.062 (13)[a]

Each value represents the mean ± SD. In parentheses number of animals.
a: indicates difference statistically significant with the control (p < 0.01).

DISCUSSION

The existence of LPL activity in the liver is well documented. The enzyme synthesis occurs endogenously in the hepatocyte (Jensen et al., 1980) and its activity is larger in the prenatal phase (Reina et al., 1987). It is possible that in the adult, the enzyme may be in a less active form (Ailhaud, 1990); however, its occurrence in the adult liver has been repeatedly observed in hyperlipemic condition or in pregnant rat submitted to starvation (Vilaró et al., 1990).

The results obtained in this work have indicated that the hepatic-LPL activity was significantly increased in animals maintained to 27 °C under fatty liver condition (Table I, B and C groups). This fact is in agreement with previous finding obtained in others tissues. It has been reported in newborn rats that the increase of the triglyceride-adipocyte is in concordance with the enhancement of adipocyte tissue lipoprotein lipase (Ramirez et al., 1983). The other hepatic triacilglycerol lipase (LH) is unaffected in either adult or neonate rat liver under hyperlipemic conditions (Burgaya et al., 1988).

We have determined that the increment of LPL activity had an early onset (Table II), which is in agreement with the rapid hepatic TG-accumulation observed in CCl_4-induced fatty liver. It has been reported that the hepatic-TG duplicate their concentration, three hours after ingestion of hepatotoxic (Lombardi, 1965).

The fact that hepatic-LPL activity was increased in fatty liver induced by CCl4 or ethi-

onine, it could be indicative that this modification is not specific for a particular fatty liver inducing agent. It may correspond, instead, to a general mechanism by which the high LPL activity could favor the uptake of fatty acids by the liver and their following accumulation as triglycerides. It has been suggested that the LPL activity confers to the liver the capacity of TG-importer (López-Tejero et al., 1988). In others cells, such as macrophages, the predominant mechanism by which the triglycerides are intracellulary accumulated is through the action of lipoprotein lipase (Bates et al., 1984).

In cold-acclimatization, our results had indicated that the LPL activity in the liver was not increased when animals were submitted to hepatoesteatosis inducers (Table I, E and F groups), e.i. mice did not develop fatty liver. In addition, control animals at 5 °C presented the same enzyme activity that the 27 °C-control (Table I, A and D groups). This fact is in agreement with others authors that had reported no effect of cold exposure on the hepatic-lipoprotein lipase activity in normal rats (Radomski & Orme, 1971). It is possible that in cold-acclimatization the triglycerides of the blood lipoproteins could be preferentially taken by brown adipose tissue. Chronic exposure to cold induces a constant rise in energetic metabolism.

Based in these results it could be concluded that in the liver of cold-acclimatized mice the enhancement of LPL-activity did not take place by the action of CCl_4 or ethionine, as it was observed when animals were kept at room temperature. These agents were unable to induce fatty liver in cold-adapted animals.

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