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SHORT COMMUNICATIONS

Effect of an antimitotic agent (cyclophosphamide) on post-heparin plasma lipoprotein lipase activity in rabbit

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Hyperlipidemia occurs in male New Zealand rabbits after cyclophosphamide treatment. It is characterized by hypertriglyceridemia, hypercholesterolemia, VLDL accumulation and HDL* decrease [1]. Moreover, the lipoprotein lipase post heparin is inhibited after the treatment. The cyclophosphamide action is similar to that observed with ionizing radiations [2, 3]. A modification of the apolipoprotein composition of VLDL appears in irradiated rabbits as in cyclophosphamide treated rabbits [1, 2, 4]. Two new apolipoproteins X_1 and X_2 are visualized, their essential characteristics being the apparent molecular weight of about 10,000 and their isoelectric points of 6.10, 6.42 and 6.72. The present report tries to investigate the post-heparin plasma lipoprotein lipase activities and the effect of new apolipoproteins on these enzymes, in order to explain the mechanisms of hypertriglyceridemia.

Materials and methods

Animals. Male New Zealand white rabbits weighing approximately 2–2.5 kg were used. All the animals were fasted for 15 hr before treatment. Treated rabbits received different cyclophosphamide doses (8.25, 16.5, 32.5, 65 mg/kg), in the ear vein. The maximal dose used represented 1/2 LD_{50} (lethal dose has been determined by Brock [5]). Post-heparin blood was drawn according to a previously described method [1].

Lipoprotein preparations. Lipoprotein fractionation was performed according to a previously reported method [1]. For apolipoprotein purification, the VLDL were previously dialyzed against a standard buffer containing 5 mM Tris, 0.5 mM EDTA, 150 mM NaCl, pH 7.4

Post-heparin lipase preparation. Hepatic triacylglycerol lipase (H-TGL) and lipoprotein lipase (LPL) were fractionated from control and treated rabbit post-heparin plasma. An affinity chromatography was carried out on heparin sepharose CL 6 B (Pharmacia) [6] using 0.005 M sodium barbital buffer, pH 8.4, containing 20% (v/v) glycerol and NaCl of increasing molarity (0.72 M and 1.5 M NaCl for H-TGL and LPL respectively). Column fractions were collected in tubes containing a final concentration of 6.7% fatty acid-free albumin to further stabilize the enzymes.

Apolipoprotein preparation. The lyophilized VLDL were delipidated by extraction with cold ethanol/diethyl ether according to the method of Brown *et al.* [7]. The apolipoproteins X_1 and X_2 (25% of total soluble apolipoproteins) which appeared in treated rabbits with respective pI values 6.72, 6.42 and 6.10 were fractionated by preparative electric focusing according to the procedure of Marcel *et al.* [8]. The protein bands on the gel were visualized using a prewetted-paper print technique [8]. The regions of the gel corresponding to the individual stained bands were eluted with 1.5–2 ml 0.5% sodium decyl sulfate in 0.01 M Tris–HCl, pH 8.2 [9]. The apolipoproteins X_1 and X_2 fractions were dialysed against a buffer (10 mM, 1 mM EDTA, pH 8) and concentrated by membrane ultrafiltration. These apolipoproteins were pure as tested by analytical isoelectric focusing [10, 11].

Post-heparin lipolytic activity determination. LPL and H-TGL activities after purification were measured according to Nilsson-Ehle and Schotz [12] using a sonicated emulsion of tri[9,10(*n*)-^{3}H] oleoyl glycerol in glycerol stabilized with lecithin.

The effects of purified apolipoproteins X_1 and X_2 on LPL and H-TGL of control rabbit post-heparin plasma were tested by the method of Ekman and Nilsson-Ehle [13]. To test the apolipoprotein action on LPL, 50 μl of VLDL were also added to the preparation; they corresponded approximately to 5 μg/ml of apo CII (final concentration) used as an LPL activator [14]. In the first place, apolipoproteins and enzymes were pre-incubated at 0° for 10 min; then tri[^{3}H]oleoylglycerol was deposited and incubation was performed for 15 min at 37°. The labelled free fatty acids were extracted according to the methods of Belfrage *et al.* [15]. One mUnit of enzyme activity was defined as the amount of enzyme in 1 ml of enzyme preparation which released 1 nmole fatty acid per min at 37°.

Protein was measured using bovine serum albumin as standard by the method of Lowry *et al.* [16].

Assay of lipoprotein lipase activity in adipose tissue. A radioactively-pure substrate was prepared as previously [12, 17]. Perirenal adipose tissue of normal and treated rabbits weighing 50 mg was cut into small pieces and incubated with shaking at 37° for 1 hr in Krebs–Ringer 0.1 M Tris–HCl buffer, pH 8.4, containing 1 g/100 ml bovine serum albumin and 2 IU heparin. The incubation medium contained [^{3}H]triolein substrate mixture and pre-heparin plasma as source of apo CII. The LPL activity was expressed as mU/min/g tissue.

Results and discussion

The data in Fig. 1 and Table 1 show an important LPL activity decrease while the H-TGL activity remained constant in treated rabbits. The LPL activity decline was accentuated with the cyclophosphamide dose increase.

The lipoprotein lipase catalyses the hydrolysis of plasma chylomicrons and VLDL triacylglycerols and thereby facilitates the removal of triacylglycerol fatty acid from the circulation [18]. This LPL activity decline would explain hypertriglyceridemia and hyperlipoproteinemia in treated rabbits. As according to Kekki [19], the greater part of the HDL present in blood results from LPL action. The absence of HDL observed in rabbits treated [1] at 1/2 LD_{50} may be due to LPL deficiency. This LPL diminution, as well as the presence of new apolipoproteins led us to study their action on these two partially purified enzymes.

As regards H-TGL, the apolipoproteins with pI 6.10, 6.42 and 6.72 play an inhibitor role (Fig. 2a). This inhibitory effect was important for small apolipoprotein quantities *in vitro*. H-TGL would be present in treated rabbits but inhibited *in vivo* by these apolipoproteins and thereby they would be implicated in provoked hypertriglyceridemia. The apolipoprotein action on LPL was less clear but the two apolipoproteins, X_1 (pI: 6.72) and X_2, seemed to inhibit LPL activity (Fig. 2b). Their effect on this enzyme *in vitro* was complex, yet the two new synthesized apolipoproteins played an important role for triglyceride hydrolysis and VLDL metabolism, since they highly decreased H-TGL activity and partly diminished LPL activity. However, this inhibition towards LPL was not responsible for the total

* Abbreviations used: HDL, high density lipoprotein(s); H-TGL, hepatic triacylglycerol lipase; LPL, lipoprotein lipase; VLDL, very low density lipoprotein(s).

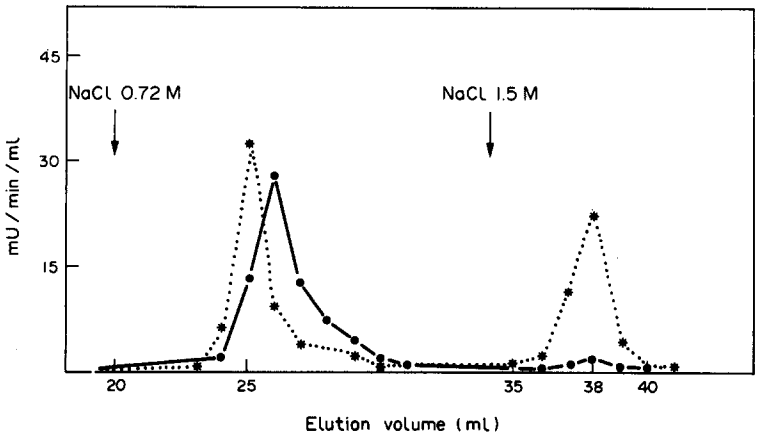

Fig. 1. Activity of hepatic triglyceride lipase and lipoprotein lipase eluted from heparin-sepharose CL 6 B column. Post-heparin plasma was applied on this affinity chromatography column (1 × 15 cm) equilibrated with 5 mM Na barbital buffer, pH 7.4, containing 20% v/v glycerol. H-TGL and LPL were eluted at 0.72 M and 1.5 M NaCl (in sodium barbital buffer), respectively *·····*, control rabbit post-heparin plasma; ●——●, treated rabbit post-heparin plasma (65 mg/kg), 16 hr after injection. The data were obtained from a single preparation but are representative of three or four purification experiments.

Table 1. Activity of post-heparin lipoprotein lipase and triglyceride lipase eluted from heparin-sepharose CL 6 B column; relation with cyclophosphamide dose

Cyclophosphamide (mg/kg)	0	12.5	32.5	65.0
LPL activity (mU/min/ml)	22.3 ± 3.2	16.75 ± 2.99	10.6 ± 6.2	4.89 ± 3.1
	(4)	(3)	(3)	(3)
H-TGL activity (mU/min/ml)	21.1 ± 13.87	25.2 ± 2.82	35.8 ± 9.12	27.46 ± 5.87
	(4)	(3)	(3)	(3)

Results represented cyclophosphamide action 16 hr after treatment. Values are mean ± S.D.; in parentheses, number of experiments.

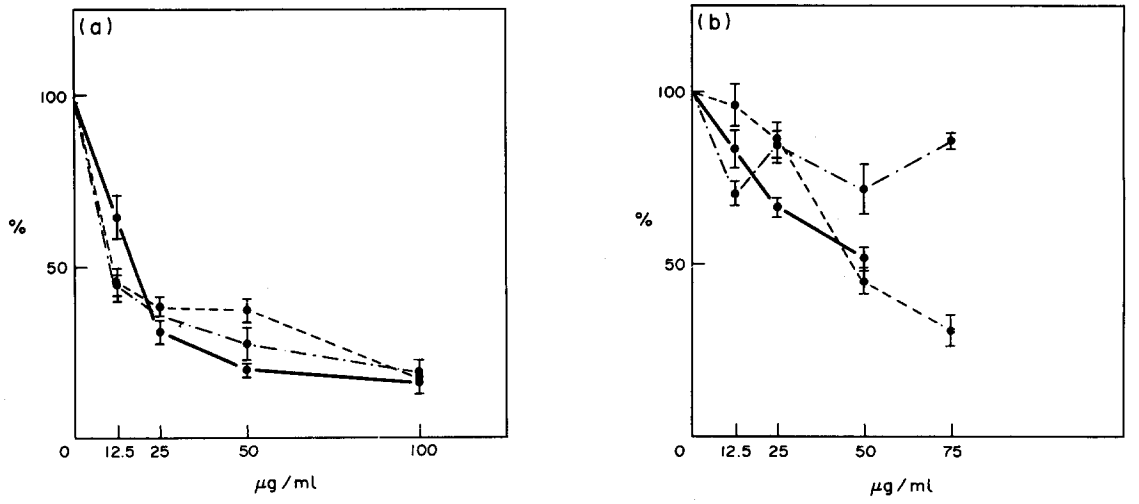

Fig. 2. Effect of new apolipoprotein X_1 and X_2 on the lipase activity. Activity results are expressed in percent as a function of apolipoprotein concentration. ———, apolipoprotein X_2 with pI 6.10; —·—, apolipoprotein X_1 with pI 6.42; – – – –, apolipoprotein X_1 with pI 6.72: (a) effect on hepatic triglyceride lipase; (b) effect on lipoprotein lipase. Values are mean ± S.D. ($N = 4$).

absence of LPL activity found in treated rabbits (65 mg/kg); therefore, we tried to dose this enzyme in adipose tissue.

LPL is thought to be synthesized in various extra-hepatic tissues among which adipose tissue and secreted by cells before being transported to a site at the luminal surface of the capillary endothelial cells where it exerts its activity [20]. LPL activity in perirenal adipose tissue in treated rabbit was similar to that of control rabbits. These experiments permitted us only to estimate a difference between adipose LPL of control and treated rabbits; for real activity values LPL purification could be used. Therefore, it was possible that LPL would be normally synthesized and activated in the adipocyte. A defect of LPL secretion, resulting from treatment, is followed by an accumulation of this enzyme in adipose tissue. LPL would exist in adipocyte under a proenzyme form which would be activated in this cell before being secreted [21–23]. In our experiment, the synthesized LPL would pass from the proenzyme stage to an active enzyme which was never secreted: a change in the adipocyte membrane or in an LPL active structure could be the cause. Further experiments using isolated adipocytes should be carried out to confirm this hypothesis.

In short, the patterns of separation of hepatic triglyceride lipase and lipoprotein lipase by chromatography on heparin-sepharose showed that extra-hepatic lipoprotein lipase was deficient in treated rabbit plasma. The effect of apolipoproteins of molecular weights of about 10,000 on control rabbit post-heparin plasma hepatic triacylglycerol lipase and extra-hepatic lipase was observed. These apolipoproteins inhibited both activities *in vitro*. By their inhibitory effect on these enzymes, these apolipoproteins might play a great part in the development of hypertriglyceridemia after cyclophosphamide injection.

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Involvement of catechol-*O*-methyl transferase in the metabolism of the putative dopamine autoreceptor agonist 3-PPP(3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine)

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3-PPP(3-(3-hydroxyphenol)-*N*-*n*-propylpiperidine) has been suggested to act as an agonist at dopamine autoreceptors in the rat brain [1, 2], raising the possibility that this compound may be a potential anti-psychotic agent in man [3]. The compound has a rather low bioavailability in the rat with the bulk of the orally administered 3-PPP being excreted as 3-PPP-glucuronide in the urine [4]. A recent study by Rollema and Mastebroek [5] has also suggested that 3-PPP is hydroxylated to its catechol analogue 4-hydroxy-3-PPP by microsomal enzymes. The authors could demonstrate the presence of 4-hydroxy-3-PPP after incubation of rat liver microsomes with 3-PPP *in vitro*, but *in vivo* the catechol was found in the brain only after treatment of the animals with tropolone, a catechol-*O*-methyl transferase inhibitor [5], raising the possibility that 4-hydroxy-3-PPP is a substrate for this enzyme. This possibility has been investigated in the present study.

Materials and methods

Catechol-*O*-methyl transferase (EC 2.1.1.6, COMT) was prepared from ox liver by the method of Gulliver and Tipton [6] up to, and including, the gel-filtration step. COMT activity was assayed spectrophotometrically at 37° by the coupled assay of Coward and Wu [7] as modified by Gulliver and Tipton [6], in which the *S*-adenosylhomocysteine formed in the reaction is converted to *S*-inosylhomocyteine by the action of adenosine deaminase (EC 2.5.4.4). The reaction mixture contained, in a total volume