MONOACYLGLYCEROL HYDROLASE IN HUMAN PLATELETS

Effect of platelets on the hydrolysis of chylomicron acylglycerols by lipoprotein lipase in vitro

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1. Introduction

Monoacylglycerols accumulate during the hydrolysis of chylomicron acylglycerols by lipoprotein lipase (LPL) in vitro [1]. However, no accumulation of monoacylglycerols has been observed in vivo. Several enzymes possessing monoacylglycerol hydrolase activity may be involved in the hydrolysis of the monoacylglycerols formed during the LPL-reaction. These include the monoacylglycerol hydrolase of adipose tissue [2] as well as postheparin plasma lipases (hepatic lipase) [3]. Monoacylglycerol hydrolase has been recently shown to be present in human blood platelets [4]. The enzyme could not be released from the cells by heparin and it is therefore distinct from the postheparin plasma lipases [4]. The other blood cells were devoid of this enzymatic activity [4].

Experiments were designed to study if the monoacylglycerol hydrolase of platelets acts on the monoacylglycerol generated in the LPL-catalyzed hydrolysis of chylomicron acylglycerols. Chylomicrons were reacted in vitro with LPL in the presence and absence of platelets under conditions where accumulation of monoacylglycerol could be expected [1].

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2. Materials and methods

2.1. *Isolation of platelets*

Platelets were isolated as described [5]. The isolated cells were suspended in phosphate buffered saline (PBS).

2.2 *Preparation of rat lymph chylomicrons*

Rats were prepared with indwelling catheters in the abdomen and in the main lymphatic trunk draining the intestine [6]. They were given through a gastric cannula a 2 ml bolus of Intralipid® (Kabi Vitrum, Sweden) containing 750 μCi tri[9,10(n)-^{3}H]oleoyl glycerol (spec. act. 540 Ci/mol Radiochemical Centre, Amersham) followed by a constant infusion of Intralipid® at 0.75 ml/h. Intestinal lymph was collected for 5 h into tubes held on ice. Chylomicrons were separated by layering 4 ml lymph under 7 ml 0.9% (w/v) NaCl and by centrifuging for 30 min at 20 000 × g_{av} in a Sorvall OTD-2 centrifuge using a Beckman Ti-50 angle-head rotor at +2°C. The upper 4 ml of the tube was collected by slicing. Penicillin (100 IU/ml) and streptomycin (50 μg/ml) were added to prevent microbial growth. Chylomicrons were stored at +4°C and used within one week from the preparation.

2.3. *Source of lipoprotein lipase*

Lipoprotein lipase (LPL) was purified from bovine milk as in [7]. The specific activity of the purified LPL was 29 mmol free fatty acid . mg protein^{-1} . h^{-1} when assayed using gum arabic stabilized tri[1-^{14}C]-oleoylglycerol as a substrate [7].

2.4. *Analytical methods*

The lipids were extracted according to [8] and separated by thin-layer chromatography on silica gel coated plates (DC-Plastikfolien, Kieselgel 60, F_{254}, Merck, Darmstadt) developed with hexane/diethyl-ether/acetic acid (70/20/2, v/v/v). Lipid radioactivity was measured with a liquid scintillation spectrometer using a toluol based Bray's solution [9]. From the distribution of lipid radioactivity, the molar percentages of the different acylglycerols and the amount of free fatty acids (FFA) were calculated.

3. Results

3.1. *Hydrolysis of chylomicron acylglycerols by LPL*

Chylomicron acylglycerols were hydrolyzed in vitro by homogeneous LPL. The assay conditions were chosen to obtain monoacylglycerol accumulation [1]. The initial molar ratio of chylomicron triacylglycerols to albumin was 1.7. Assuming 7 fatty acid binding sites/albumin molecule [1], this corresponds to a ratio of 1.4 of binding sites to the total amount of fatty acids (BS/FA). The final albumin concentration was 4.9% (w/v), and the amount of enzyme 0.58 μg LPL/μmol chylomicron triacylglycerol.

During the first 10 min incubation an accumulation in the monoacylglycerol fraction up to 32 mol% of the initial acylglycerols was observed (fig.1C). Concomitantly, the molar percentage of triacylglycerols decreased from 92 to 19% (fig.1A). At the same time the molar percentage of FFA increased from 3 to 68% of the total fatty acids (fig.1B). During the next 50 min incubation the molar percentage of triacylglycerols further declined to 2% (fig.1A) and the molar percentage of FFA increased to 94% (fig.1B). Simultaneously, the molar percentage of monoacylglycerols decreased to 9% (fig.1C). The molar percentage of diacylglycerols was <6% during the whole incubation period (fig.1D).

3.2. *Hydrolysis of chylomicron acylglycerols by LPL in the presence of platelets*

The conditions for the hydrolysis of chylomicron acylglycerols by LPL were otherwise similar to those above, but the incubation mixture also contained isolated human platelets in a final concentration of 6×10^8 platelets/ml. No significant accumulation of

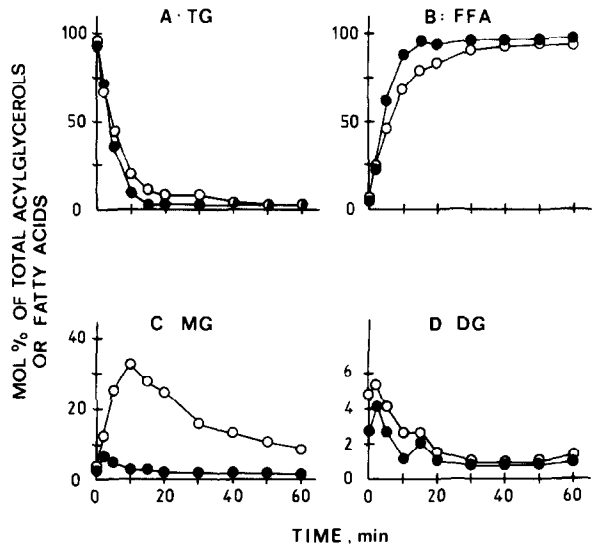

Fig.1. Effect of platelets on the hydrolysis of chylomicron acylglycerols by LPL at different time intervals. The incubation mixtures contained in 1 ml 0.18 M NaCl, 80 mM Tris–HCl buffer (pH 8.6) the following: 0.72 μmol BSA; 1.2 μmol chylomicron triacylglycerol; 0.69 μg LPL. (○---○) Values obtained in the absence of platelets. (●---●) Values from an experiment containing 6×10^8 platelets/ml reaction mixture described above. Incubation was at 28°C. At the indicated times aliquots of 0.45 ml were withdrawn and analyzed for lipid radioactivity as in section 2.

monoacylglycerols occurred. The molar percentage of monoacylglycerols was ≤6% of the initial acylglycerols (fig.1C). During the first 2 min of incubation, the molar percentage of monoacylglycerols increased from 1 to 6% and declined to 3% during the period between 2 and 10 min (fig.1C). During the first 10 min incubation the molar percentage of triacylglycerols decreased from 96 to 9% (fig.1A). The molar percentage of fatty acids released increased simultaneously from 2 to 89% of the total fatty acids present (fig.1B). The hydrolysis by LPL of triacylglycerols to FFA was thus slightly accelerated by platelets.

During the next 50 min the molar percentage of monoacylglycerols decreased to 1% (fig.1C) and the molar percentage of triacylglycerols decreased in 2% (fig.1A). The molar percentage of FFA increased at the same time to 97% of the total fatty acids (fig.1B). The molar percentage of diacylglycerols was ≤4% of the initial acylglycerols (fig.1D).

3.3. *Effect of platelet concentration of the hydrolysis of chylomicron acylglycerols*

To further study the effect of platelets on the hydrolysis of chylomicron acylglycerols by LPL and to estimate the concentration of platelets needed, the amount of platelets in the incubation mixture was varied. The hydrolysis was conducted both at a large and small excess of fatty acid binding sites available per the total number of fatty acids present, the BS/FA ratios being 6.1 and 1.4 [1]. The final albumin concentration was 4% (w/v) in both cases. The mixtures were incubated at 28°C for 10 min, whereafter the reaction was stopped and the distribution of lipid radioactivity was determined.

Using ratio BS/FA of 6.1 the molar percentage of monoacylglycerols in the absence of platelets was 33% of the initial acylglycerols (fig.2C). The corresponding value was 5% in the presence of 3×10^8 platelets/ml incubation mixture (fig.2C). When the ratio BS/FA was 1.4, the molar percentages of monoacylglycerol were 21% and 3%, in the absence and presence of platelets, respectively (fig.2C). Thus, both at a large and at a small excess of albumin fatty acid binding sites, the amounts of monoacylglycerol formed in the presence of 3×10^8 platelets/ml were ~14% of the amount of monoacylglycerols formed in the absence of platelets.

When the ratio BS/FA was 6.1, the molar percentage of fatty acids released from the total amount of fatty acids present was 63% in the absence of platelets and 83% in the presence of 3×10^8 platelets/ml (fig.2B). At the BS/FA ratio of 1.4, the respective values were 71% in the absence and 84% in the presence of 3×10^8 platelets/ml (fig.2B).

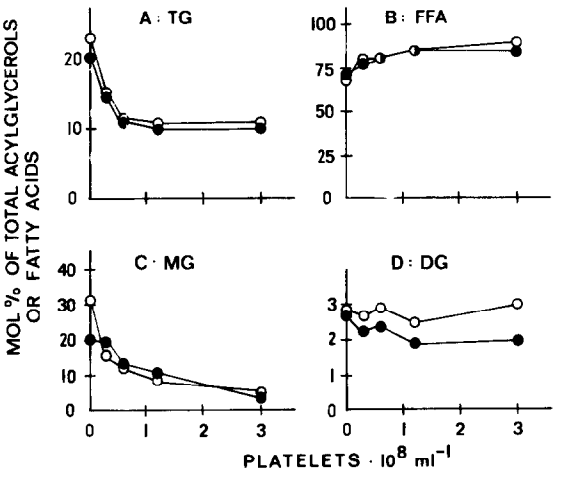

Fig.2. Effect of different amounts of platelets on the hydrolysis of chylomicron acylglycerols by LPL. (o---o) at initial triacylglycerol/albumin molar ratio of 0.38 and initial LPL/triacylglycerol ratio of 0.57 (μg/μmol). The incubation mixtures contained in 1 ml 0.18 M NaCl, 80 mM Tris–HCl buffer (pH 8.6) the following: 0.59 μmol BSA; 0.23 μmol chylomicron triacylglycerol; 0.13 μg LPL; the indicated amounts of platelets. (•---•) At initial triacylglycerol/albumin molar ratio of 1.64 and initial LPL/triacylglycerol ratio of 0.52 (μg/μmol). The incubation mixtures contained in 1 ml of 0.18 M NaCl, 80 mM Tris–HCl buffer (pH 8.6) the following: 0.59 μmol BSA; 0.97 μmol chylomicron triacylglycerol; 0.50 μg LPL; the indicated amounts of platelets. Incubation was at 28°C for 10 min.

4. Discussion

Under specific conditions monoacylglycerols are known to accumulate in the LPL-catalyzed in vitro hydrolysis of artificial triacylglycerol emulsions [10] as well as of chylomicron acylglycerols [1]. One reason for this accumulation is thought to be the positional specificity of lipoprotein lipase. LPL is able to hydrolyze 1(3)-monoacylglycerol, but probably not its 2-isomer [10,11]. Isomerization of the 2-isomer to 1(3)-monoacylglycerol has been suggested to be necessary for the complete hydrolysis of acylglycerol by LPL to FFA and glycerol. Some of the monoacylglycerol accumulation may also be due to its absorption to the FFA carrier molecule, albumin, out of the reach of LPL [1].

Our purpose was to study the effect of platelets on the hydrolysis of the monoacylglycerols formed during the LPL-catalyzed hydrolysis of chylomicron acylglycerols. We hydrolyzed in vitro radioactivity labelled chylomicrons by LPL with and without platelets under conditions where monoacylglycerol accumulation has been observed to occur [1]. In the absence of platelets, monoacylglycerol accumulated (fig.1C), as reported earlier [1]. No accumulation took place in the presence of platelets (fig.1C).

When the number of platelets in the incubation mixture was varied, an inverse relationship between the monoacylglycerols formed and the number of platelets was observed (fig.2A). When the amount of platelets was 3×10^8/ml, which corresponds to the

normal platelet concentration in the blood, the amount of monoacylglycerols formed during the reaction was 14% of the monoacylglycerol formed in the absence of platelets under otherwise similar conditions. The same result was found both at a large and at a small excess of fatty acid binding sites on albumin per total number of fatty acids present. The amount of FFA was proportional to the amount of platelets present (fig.2B).

Several theories have been proposed to explain the fate of the monoacylglycerols formed during the hydrolysis of chylomicron acylglycerols by LPL. Some of these monoacylglycerols may enter the adipose tissue to be hydrolyzed there by the adipose tissue monoacylglycerol lipase [2]. Since this enzyme lacks positional specificity, no isomerization would be needed prior to the hydrolysis [12]. In addition, it has been suggested that the monoacylglycerols, if not absorbed by the extrahepatic tissue, would be carried in albumin, high density lipoprotein, and in low density lipoprotein to the liver to be hydrolyzed there by the hepatic lipase [13]. The substrate specificity reported for the hepatic lipase [13] is, however, likely to be an artefact arising during the enzyme isolation [14]. In vivo, the hydrolysis of chylomicron triacylglycerol by LPL occurs in the bloodstream on the endothelial cells of capillaries [15]. Thus, the monoacylglycerols formed during this hydrolysis are immediately exposed to the blood. The presence in the blood platelets of a monoacylglycerol hydrolase which hydrolyzes in vitro the monoacylglycerols formed in the LPL–chylomicron reaction, suggests an additional pathway for monoacylglycerols. The adherence of platelets to chylomicrons, known to occur in hyperlipemic plasma [16], supports this possibility. The in vivo significance of this pathway is still to be established.

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