EVIDENCE FOR THE HEPATIC ORIGIN OF A CANINE POST-HEPARIN PLASMA TRIGLYCERIDE LIPASE

Heiner GRETEN*
Klinisches Institut für Herzinfarktforschung
an der Medizinischen Universitätsklinik Heidelberg, W. Germany

and

Allan D. SNIDERMAN, James G. CHANDLER, Daniel STEINBERG and W. Virgil BROWN

Department of Medicine, School of Medicine,
University of California, San Diego,
La Jolla, California 92037, USA

Received 25 March 1974

1. Introduction

The release of the lipoprotein 'clearing factor' by intravenous heparin was first demonstrated in dogs by Hahn [1]. Although equated for many years with lipoprotein lipase, the post-heparin lipolytic activity (PHLA) of plasma has now been shown to have characteristics that are best explained by the release of several enzymes [2]. Studies of the effects of inhibitors on rat PHLA after varying degrees of partial hepatectomy have strongly suggested the presence of two triglyceride lipases (TGL) [3]. Recently a TGL purified from human plasma was shown to differ from adipose tissue lipoprotein lipase in that (a) there was no requirement for an apolipoprotein cofactor and (b) high NaCl concentrations (0.1 M) produced activation rather than inhibition [4]. The properties of this TGL were similar to those of a lipase activity released from dog and rat liver on perfusion with heparin-containing solutions [5,6]. These purification techniques have now been applied in the study of dog PHLA obtained before and after total hepatectomy. The properties of the purified preparations are compared to those of lipoprotein lipase obtained from dog adipose tissue and of the TGL activity in dog liver homogenates.

2. Materials and methods

Dogs weighing 20–30 kg were fasted for 24 hr, anesthetized with pentobarbital and a siliconized cannula was implanted into the external jugular vein. Plasma samples were collected before and 10 min after injection of heparin (10 U/kg body weight). After returning to their standard diet (Purina Dog Chow) for 3–7 days, the dogs were again anesthetized and a hepatectomy performed. The liver was functionally removed without occlusion or interruption of the inferior vena cava by use of an Eck fistula. The hepatic artery and portal vein were ligated and the hepatic veins were divided individually. Twenty-four hours after hepatectomy, plasma was again collected before and 10 min after intravenous heparin injection. Acetone–ether powders of dog liver and perirenal adipose tissue were prepared as enzyme source for TGL activity as previously described [7]. These powders were solubilized (50 μg/ml) in 0.2 M NaCl containing 0.005 M Na-barbital, pH 7.4.

* Reprint requests should be addressed to Dr. H. Greten.

Dog post-heparin plasma TGL and liver TGL were partially purified by ultracentrifugation (D = 1.21) and affinity chromatography of the infranatant fraction on columns (2.5 X 30 cm) of Sepharose containing covalently-linked heparin by a modification of the method previously described [4]. The infranatant fractions obtained from the ultracentrifugation step were dialyzed versus 0.2 M NaCl, 0.05 M barbital, pH 7.4, and diluted 1:1 with this buffer prior to application to the column. The sample, as obtained from 10 ml of original dog plasma, contained 600–700 mg of protein in the extract of hepatic acetone–ether powders. After the samples were applied, the columns were eluted with 400 ml of the starting buffer and a linear NaCl gradient was then applied from 0.4 to 1.2 M NaCl with 0.05 M sodium veronal, pH 7.4, in all buffers, All proteins were determined by the method of Lowry et al.

TGL assays were carried out by the methods previously reported [4] using ^{14}C-labeled triolein emulsified in gum arabic. Each vial contained in a final volume of 0.5 ml: 0.76 μmoles of triolein (0.008 μC/μmole); 100 μmoles Tris–HCl buffer, pH 8.4, and 5 mg of bovine albumin as fatty acid acceptor; 20 μl of post-heparin plasma, liver or adipose tissue extract or up to 200 μl of column effluents as enzyme source. The concentrations of NaCl in the final assay mixture are indicated (0–1.0 M) in the figures. Total lipids were extracted and ^{14}C-labeled free fatty acids were isolated by the method of Kelley [8]. Normal dog plasma (20 μl) was added to all assays of tissue extracts or column effluents to provide activator apolipoproteins.

3. Results

The effects of increasing concentrations of NaCl on TGL activity in post-heparin plasma and in acetone-ether powder extracts of liver and adipose tissue obtained from the same dog are presented in fig. 1. TGL from the liver extract showed increasing activity with increasing salt concentration over the entire range studied and was not inhibited even by NaCl concentrations up to 1 M. That from the adipose tissue was optimally active at 0.05–0.1 M NaCl and was progressively inhibited at higher concentrations. At high salt concentrations (0.75–1.0 M) the adipose tissue

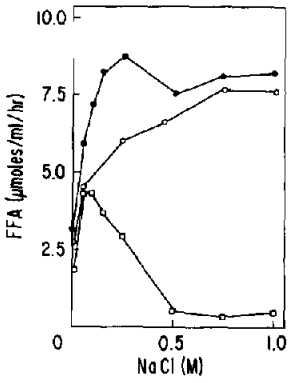

Fig. 1. Effect of sodium chloride concentrations in assay medium on triglyceride lipase activity. The enzyme source was an adipose tissue (□——□) or liver (○——○) extract and post-heparin plasma (●——●) from a normal fasting (24 hr) dog.

TGL was almost totally inhibited. Post-heparin plasma TGL activity from this animal was most active in 0.15 M NaCl but was not suppressed in 0.75 or 1.5 M NaCl. The pattern of response to changing NaCl concentrations is clearly very different for the TGL activity from the two tissues while that of post-heparin plasma TGL, although not identical with either, is most similar to that of the liver activity. In fig. 2, the effects of NaCl concentration on post-heparin plasma TGL activity is shown in three dogs before and after hepatectomy. In two cases the TGL activity before hepatectomy increased with NaCl concentration initially and then decreased to reach a plateau at 0.5 M NaCl with no further suppression at 1 M. In the third dog the TGL activity was even higher at 1.0 M NaCl. The TGL activity in the post-heparin plasma from the three intact animals was similar in that a peak or shoulder was noted in the activity curve between 0.1 and 0.25 M NaCl and activity was retained at 1.0 M NaCl. In contrast, 20 hr after hepatectomy post-heparin plasma TGL obtained from all three dogs was almost totally inhibited at high NaCl levels, a pattern similar to that of the adipose tissue in its response to changing ionic strength.

Post-heparin plasma TGL from each of the three animals was then partially purified by ultracentrifugation and chromatography on heparin–Sepharose. A representative elution pattern from one of these experiments is shown in fig. 3. In each case, a single

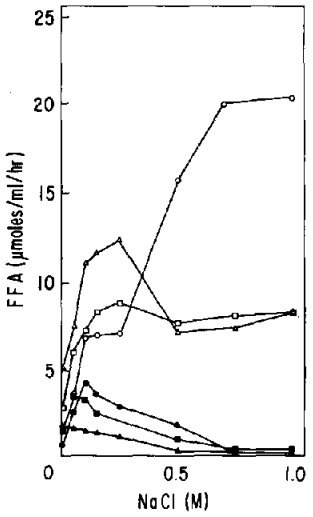

Fig. 2. Effect of sodium chloride concentrations in assay mediums on triglyceride lipase activity of post-heparin plasma from three dogs obtained before (△——△, □——□, ○——○) and after (▲——▲, ■——■, ●——●) hepatectomy.

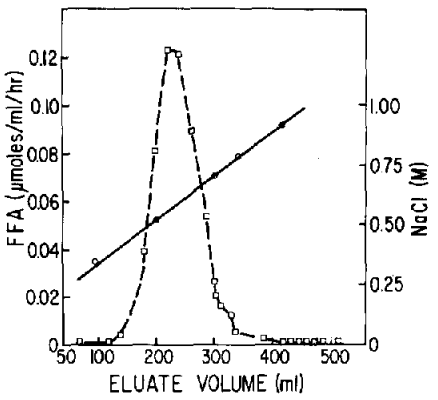

Fig. 4. Heparin-Sepharose chromatography of solubilized acetone-ether powder of dog liver. The chromatography was performed with identical techniques and the triglyceride lipase activity (□——□) determined as in fig. 3. The protein concentration in the eluate containing enzyme activity was 10 to 25 μg/ml.

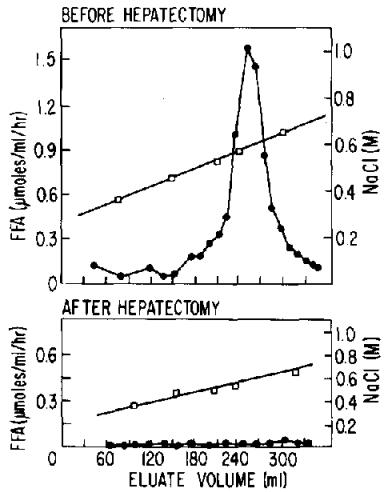

Fig. 3. Heparin-Sepharose chromatography of the post-heparin plasma of a dog before (upper panel) and after (lower panel) hepatectomy. The triglyceride lipase activity (●——●) was assayed in the fractions indicated by addition of 200 μl aliquots to the standard assay system. The increasing NaCl concentration (□——□) was produced by a 400 ml gradient-forming device. The protein concentration in the eluate containing enzyme activity was 2–10 μg/ml.

peak of triglyceride lipase activity was eluted between 0.5 and 0.75 M NaCl. Identical volumes of post-heparin plasma obtained after hepatectomy from each of the dogs was subjected to the same purification procedure using identical conditions. No detectable TGL activity was eluted from the heparin–Sepharose column (in three separate experiments) with the post-heparin plasma samples obtained after hepatectomy. Extracts of the acetone–ether powder prepared from the livers of these dogs were also chromatographed on heparin–Sepharose columns. A single peak of TGL activity was eluted emerging at NaCl concentrations identical to those that eluted the enzyme from post-heparin plasma.

Mixing experiments were performed in which plasma from either intact or hepatectomized dogs was added to the post-heparin plasma obtained from each type of animal. When these preparations were assayed at 0.1 and 1.0 M NaCl concentrations, results identical to those indicated in fig. 2 were obtained. Thus, the alterations in character of the TGL activity in post-heparin plasma after hepatectomy are not explained by the addition of an inhibitor or by the loss of an activator substance consequent to the hepatectomy.

4. Discussion

The co-chromatography on heparin–Sepharose affinity columns of a TGL obtained from post-heparin plasma and a TGL from extracts of liver is further evidence that this organ is one source of TGL in PHLA. In fact, the liver may be the only source of this form of TGL as indicated by its virtual absence from PHLA of hepatectomized animals (figs. 2 and 3). When assayed at lower NaCl concentrations (0.1 M) PHLA in hepatectomized animals was about one-third that seen in the intact animal. However, affinity column chromatography showed no detectable activity corresponding to that isolated from post-heparin plasma of intact animals. Although less total activity was loaded onto the column, the sensitivity of the method is such that as little as 5–10% of this activity would have been detected if it had had the properties of the enzyme found in the animal with a functional liver. The PHLA remaining after hepatectomy was, in its response to changing ionic strengths, identical to adipose tissue lipoprotein lipase and other peripheral tissue lipoprotein lipases [6].

It should be noted that with the procedure used in the present studies, designed for isolation of the hepatic enzyme other forms of post-heparin TGL may not be isolated. Techniques recently developed in the study of swine have allowed purification of a second enzyme from PHLA which has the characteristics of lipoprotein lipase purified from adipose tissue [9]. The relative quantities of the two enzymes in PHLA probably varies considerably from animal to animal, as indicated by the variations in the relative TGL activity noted in the dogs prior to hepatectomy when assayed at 0.1 M (optimum for lipoprotein lipase) or 1.0 M NaCl. In other studies, using non-fasting dogs, the relative activity at the lower salt concentrations has been 2- or 3-fold greater than that obtained when assayed at high ionic strength. This is consistent with the known effects of fasting on lipoprotein lipase levels in adipose tissue [10]. Fasting actually increases lipoprotein lipase in the heart and certain other tissues [11]. Thus, it may be that adipose tissue is quantitavely a more important contributor to PHLA than other peripheral tissues. The physiological role of the liver enzyme characterized in the present studies remains unknown. Studies of the relative amounts of this enzyme and of the 'extra-hepatic enzymes' released by heparin may be helpful in elucidating disorders of lipoprotein metabolism.

Acknowledgements

The authors wish to express their appreciation to Ms. Brigitte Walter, Ms. Virginia Tejada and Ms. Ellen Stanbrough for their excellent technical assistance and to Dr. Marietta Baginsky for her useful suggestions and discussion. This work was supported by a grant of the Deutsche Forschungsgemeinschaft awarded to Dr. Greten and by USPHS grant HL 14197 (Special Center of Research on Atherosclerosis), USPHS grant HL-13304 and USPHS 7 FO5 TW 1774.

References

[1] Hahn, P. F. (1943) Science 98, 19–20.
[2] Greten, H. (1972) Klin. Wschr. 50, 39–41.
[3] Krauss, R. M., Windmueller, H. G., Levy, R. I. and Fredrickson, D. S. (1973) J. Lipid Res. 14, 286–295.
[4] Greten, H., Walter, B. and Brown, W. V. (1972) FEBS Letters 27, 306–310.
[5] Hamilton, Jr., R. L. in: Post-Heparin Plasma Lipase from the Hepatic Circulation, University Microfilms, Ann Arbor, Michigan.
[6] LaRosa, J. C., Levy, R. I., Windmueller, H. G. and Fredrickson, D. S. (1972) J. Lipid Res. 13, 356–363.
[7] Korn, E. D. and Quigley, T. W. (1957) J. Biol. Chem. 226, 833–839.
[8] Kelley, T. F. (1968) J. Lipid Res. 9, 799–800.
[9] Ehnholm, C., Bensadoun, A. and Brown, W. V., submitted for publication.
[10] Garfinkel, A. S. and Schotz, M. C. (1973) Biochim. Biophys. Acta 306, 128–133.
[11] Hollenberg, C. H. (1960) J. Clin. Invest. 39, 1282–1287.