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Characterization of a Triglyceride Hydrolase Secreted by Canine Liver Maintained *In Vitro* (39218)

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Studies in our laboratory (1-3) and others (3-6) have shown that postheparin plasma contains at least three distinct long-chain triglyceride hydrolases (TGH). These have the properties described below: (i) Lipoprotein lipase C-I (LPL_{C-I}) is present in postheparin plasma but not in the adipose tissue (1, 2) of normal controls, and is absent from postheparin plasma of type I hyperlipoproteinemic subjects studied in this laboratory (2). LPL_{C-I} is activated by serum or C-I[1] but not C-II[1] and is inhibited by NaCl and protamine sulfate (3). (ii) Lipoprotein lipase C-II (LPL_{C-II}) is present in the postheparin plasma and adipose tissue of man and rats (8). LPL_{C-II} is activated by serum or C-II but not by C-I, and is inhibited by NaCl and protamine sulfate (3). (iii) A third triglyceride lipase (TGL) differs from LPL_{C-I} and LPL_{C-II} in that it is insensitive to protamine sulfate and 1 *M* NaCl (3-5). It does not require serum cofactors or apolipoprotein polypeptides for activation. TGL has been isolated by heparin-sepharose affinity chromatography (6, 9). Evidence has been presented in rats (4, 5) and dogs (11) that TGL is secreted by the liver.

The studies reported here describe investigations to determine the source of LPL_{C-I}. Canine livers were perfused *in vitro* with a [^{3}H]amino acid mixture in the absence and presence of heparin. The perfusate fluid in the hepatic venous end was purified by procedures utilized to isolate and purify LPL_{C-I} and LPL_{C-II} from the postheparin plasma. A polypeptide similar in electrophoretic behavior to plasma LPL could be obtained. This polypeptide isolated from the postheparin perfusate was activated by C-I but not by C-II, hydrolyzed triglyceride in chylomicrons and very low-density lipoproteins (VLDL), and was inhibited by NaCl and protamine sulfate. These properties are similar to LPL_{C-I} isolated from postheparin plasma and are distinct from the TGL described in the liver perfusate and homogenate of rats (5, 10), dogs (9), and man (12).

Materials and methods. Liver perfusion. Overnight fasted (14-16 hr) dogs weighing 25-30 kg were anesthetized by iv injection of pentobarbital sodium (30 mg/kg). After a midline abdominal incision, the liver was mobilized, and the common bile duct was cannulated to remove the bile, after which the duct was ligated. Fluid used for irrigation and perfusion was Tis-U-Sol (Travenol Lab., Morton Grove, Illinois). All manipulations were performed at 37°. The liver was then removed and a cannula (Bardick 16) was inserted into the portal venous end. The liver was immediately irrigated through this cannula with Tis-U-Sol until the effluent became clear. The liver was weighed with the cannula (preperfusion weight) and then placed immediately in the perfusion chamber filled with Tis-U-Sol. In this system the fluid was aerated by a gas mixture containing 95% oxygen and 5% carbon dioxide. The perfusing solution was passed through a filter to arrest air bubbles and entered the liver through the portal venous cannula. The hepatic venous end was open and the fluid coming out of it was collected in the perfusion chamber from which it was circulated. Flow through the liver reached a steady rate of 600-800 ml/min within 20-30 min. The [^{3}H]amino acid mixture (150 μCi, New England Nuclear Corp., Boston, Massachusetts) was then administered, and the time of administration was designated as 0 min. Thereafter 250 ml of fluid was withdrawn from the chamber and replaced by an equal

[1] C-I (apolipoprotein serine) and C-II (apolipoprotein glutamic acid) are the constituent polypeptides of human plasma lipoprotein C. The nomenclature system of plasma lipoproteins and their polypeptides has been described previously (23).

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amount every 5–10 min up to 70 min. The perfusate samples were essentially free of red blood cells. In one set of three experiments, 6000 u of heparin sodium (Upjohn Company, Kalamazoo, Michigan) was administered as a single bolus 15 min after administration of the labeled amino acid mixture. In another set of three experiments, constant infusion of heparin, 30 u per min, was begun 25 min after injection of the amino acid mixture. Liver function was evaluated throughout the experimental period by measuring albumin synthesis, glutamic oxalacetic and pyruvic transaminases, and potassium. Liver weight increased by 4 to 7% during these experiments.

Preparation of triglyceride hydrolase. A triglyceride hydrolase was isolated from each of the postheparin perfusate samples (approximately 200–300 ml) as described previously (13). Protein (14), radioactivity, lipolytic activity, and immunochemical and electrophoretic analyses were performed on the purified fractions.

Isolation of albumin. Albumin was isolated and purified from each perfusate sample by the method of Schwert (15), and analyzed for radioactivity and protein content.

Electrophoresis. Polyacrylamide-gel electrophoresis was performed in 7% gel containing 8 *M* urea at pH 8.9. Protein bands were visualized by staining with Coomassie brilliant blue (16).

Assays of lipolytic activity. [^{14}C]triolein (Amersham Searle Co., Arlington Heights, Illinois), and [^{14}C]mono-olein (Dhom Products, North Hollywood, California) were diluted with nonradioactive mono- and triolein (Analabs, North Haven, Connecticut). Lipolytic activity was assayed against triolein as previously described (1). Monoolein assays were performed by the method of Nilsson-Ehle and Belfrage (17).

Triglyceride from chylomicron and VLDL was also used to measure lipolytic activity. In these instances, 60 mg of albumin (bovine albumin, Fraction V, Armour Laboratories, Kankakee, Michigan) and 25 μmole of ammonium sulfate were added, as well as 2 mg of triglyceride from chylomicrons or VLDL. Sufficient amount of enzyme (0.2 ml) was added to release 2 μmole of FFA when assayed against [^{14}C]triolein activated by 0.05 ml of serum. The mixtures were incubated for 30 min at pH 8.5 and 37°. Fatty acids were extracted and determined as described previously (18).

Isolation of chylomicrons, VLDL, and apolipoprotein polypeptides. Chylomicrons ($S_f > 400$) were isolated from the chyle of a subject who had an extensive fistula of the abdominal thoracic duct after pancreatectomy. Chylomicrons were isolated by preparative ultracentrifugation, as described by Kostner and Holasek (19). VLDL (S_f 20–400) was isolated by ultracentrifugation from the plasma of a type IV hyperlipoproteinemic subject who had been on a high-carbohydrate diet for 2 weeks (20). The lipoprotein fractions were analyzed for triglyceride (21) and phospholipid (22), and by analytical ultracentrifugation to determine the S_f rate of VLDL.

Isolation and characterization of apolipoprotein C (ApoC) polypeptide have been described previously (23).

Inhibitor studies. Assays were performed using [^{14}C]triolein substrate to which sodium chloride (final conc., 1 *M*) or protamine sulfate (final concentration, 3.0 mg/ml enzyme) was added. Purified TGH from the postheparin perfusate was the enzyme source and C-I or serum was used as an activator. In some instances protamine sulfate (3 mg/ml enzyme solution) and 1 *M* NaCl were incubated with the enzyme at 27° for 10 min prior to the 60-min incubation with triolein substrate.

Immunochemical methods. Purified samples of pre- and postheparin perfusate samples were tested by double diffusion (24) analysis against anti-LPL. Purified LPL, 0.5 mg in 2 ml of 0.05 *M* NH_4OH–NH_4Cl buffer, pH 8.5 (from postheparin plasma of normolipidemic human subjects), was mixed with an equal volume of complete Freund's adjuvant, and injected ip into rabbits. Antibodies were detected within 12–21 days. Since these preparations of LPL contained small amounts of ApoC polypeptides (13), it was necessary to remove these antibodies by precipitation with human VLDL in predetermined amounts. The resultant anti-LPL sera exhibited no precipitin lines against ApoC polypeptides or other serum proteins. Preparation of antisera to canine lipoproteins and serum fractions have been

described previously (25).

Results and discussion. The functional status of the liver maintained *in vitro* was satisfactory throughout the experimental period as evidenced by the synthesis of albumin (Fig. 1) and normal values for transaminases and potassium. The data presented in Fig. 1 demonstrate the prompt incorporation of tritiated amino acids into albumin and the triglyceride hydrolase.

The radioactive TGH isolated from the postheparin liver perfusate appeared as a single band on polyacrylamide-gel electrophoresis (Fig. 2) and failed to react with any of the antisera to canine serum fractions (albumin, globulins, high-density and low-density lipoproteins) in an Ouchterlony double immunodiffusion system. However, postheparin liver perfusate TGH demonstrated a precipitin arc when tested against antiserum to human postheparin plasma lipoprotein lipase. This radioactive TGH isolated from perfusate samples obtained after heparin injection exhibited lipolytic activity against [^{14}C]triolein activated by serum or C-I but not C-II. This TGH isolated from timed samples of postheparin liver perfusate had specific activity of 200–250 u/mg of protein (one unit releases 1 μmole FFA/hr, using 0.05 ml of serum as an activator). Postheparin liver perfusate TGH hydrolyzed triolein (212.0 μmole FFA/mg of protein) and mono-olein (20.0 μmole FFA/mg of protein). Lipolytic response of the TGH obtained from postheparin liver perfusate

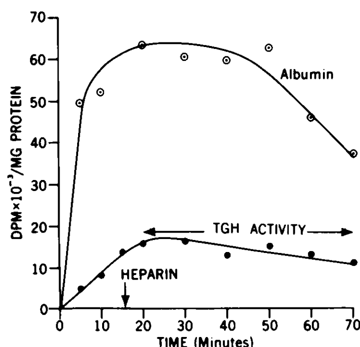

FIG. 1. Incorporation of [^{3}H]amino acids into albumin and TGH isolated from postheparin liver perfusate. [^{3}H]amino acid mixture was administered at 0 time. At the point indicated by arrow, heparin (6000 u) was administered as a single bolus to dog liver perfused *in vitro*.

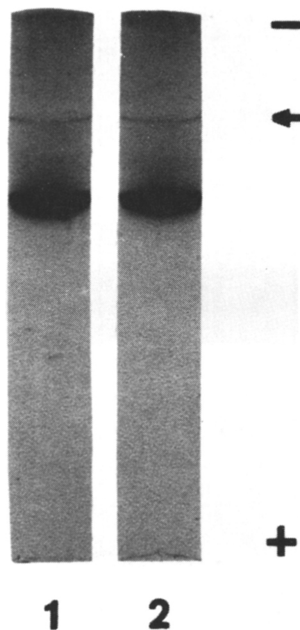

FIG 2. Polyacrylamide disk gel electrophoretic pattern of postheparin perfusate TGH (pattern 1) and a polypeptide (pattern 2) isolated from perfusate samples prior to administration of heparin.

was similar whether heparin was given as a single bolus or as a constant infusion. Triglyceride from chylomicrons and VLDL was hydrolyzed by this liver TGH (Table I). Liver TGH was inhibited by 1 *M* NaCl and protamine sulfate (17 and 21% of control activity, respectively). Values were similar when the enzyme solution was preincubated with the inhibitor (13 and 16% of control activity, respectively). These data indicate that the TGH isolated from postheparin liver perfusate has the characteristics that have been described for LPL_{C-I} in postheparin plasma of normal subjects and laboratory animals: (i) activation by C-I or serum, (ii) ability to hydrolyze triglyceride in chylomicrons and VLDL, and (iii) inhibition by sodium chloride and protamine sulfate. These properties differentiate this TGH (C-I activated) from the triglyceride hydrolase described in rat liver perfusate (5) and homogenates (10), as well as in dog liver perfusate (9), in that the latter does not require serum cofactors, does not hydrolyze lipoprotein triglyceride to any significant extent (5, 10), and is relatively insensitive to NaCl

and protamine sulfate (in fact, 1-3 M NaCl solutions activated this lipase; ref. 5).

It is noteworthy that a polypeptide similar in electrophoretic (Fig. 2) behavior to postheparin liver perfusate TGH could be isolated from the preheparin perfusate samples by the procedure used to obtain enzymatically active TGH from postheparin liver

TABLE I. HYDROLYSIS OF CHYLOMICRON (S_f > 400) AND VLDL (S_f 20–400) TRIGLYCERIDE BY THE TRIGLYCERIDE HYDROLASE FROM CANINE POSTHEPARIN PLASMA AND POSTHEPARIN LIVER PERFUSATE.

	μmole FFA released/ml/hr		
TGH source[a]	[^{14}C]triolein	CM-TG	VLDL-TG
Postheparin plasma	22.6	29.50	21.50
Postheparin liver perfusate (min after heparin infusion)			
10	2.8	2.2	1.3
20	3.2	6.5	2.1
30	3.0	6.3	1.3
40	2.9	8.0	1.5
50	3.2	7.3	1.4

[a] TGH from post-heparin plasma as well as with perfusate samples was isolated and purified by the methods described previously (13).

[b] Abbreviations: CM-TG, chylomicron triglyceride; VLDL-TG, Very low-density lipoprotein triglyceride. Values for FFA release are presented as micromoles per milliliter of enzyme solution. Each milliliter of assay mixture contained 2 mg of triglyceride from either chylomicrons or VLDL, 60 mg of albumin, and 0.2 ml of enzyme solution. In each case, the protein concentration in the enzyme solution was approximately 0.02 mg/ml.

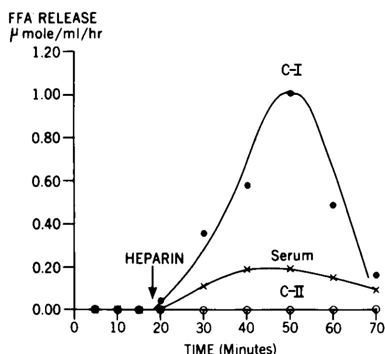

FIG. 3. Lipolytic activity of TGH isolated from postheparin liver perfusate. Each milliliter of assay mixture contained 10 μmole of [^{14}C]triolein, 40 μg of C-I or C-II, or 0.05 ml of serum, 60 μg of phosphatidylcholine, and 0.2 ml of enzyme solution. Results are expressed as μmole of [^{14}C]oleic acid released per milliliter assay mixture per hour.

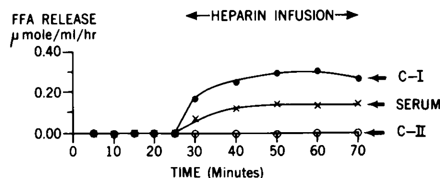

FIG. 4. Lipolytic activity of TGH isolated from postheparin liver perfusate during a constant infusion of heparin (30 units/min). Assays were performed as described in legend for Fig. 3.

perfusate. This polypeptide incorporated [^{3}H]amino acids (Fig. 1), but failed to exhibit lipolytic activity either against triolein activated by serum, C-I, or C-II (Figs. 3, 4), or against lipoprotein triglyceride. Further investigation is required to determine the relationship of this polypeptide to the enzymatically active TGH.

Summary. These studies demonstrate that canine liver synthesizes a TGH activated by C-I but not by C-II. This TGH has properties similar to lipoprotein lipase C-I isolated from postheparin plasma of human subjects, but differs from a previously reported liver triglyceride-lipase or protamine insensitive-lipase in its sensitivity to NaCl and protamine sulfate, as well as in its requirement for a serum cofactor, C-I. These data suggest the possibility that, in dogs, liver is a source of plasma LPL_{C-I}.

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