Activation of Lipoprotein Lipase

EFFECTS OF RAT SERUM LIPOPROTEIN FRACTIONS AND HEPARIN

By Thomas F. Whayne, Jr., M.D., and James M. Felts, Ph.D.

ABSTRACT

Guinea pig serum is deficient in high density lipoproteins (HDL); postheparin lipoprotein lipase (LPL) from it hydrolyzes a triglyceride (TG) emulsion very slowly. The rate is markedly increased by the addition of rat serum and even further by rat serum plus heparin. We have studied further the activation of LPL in this system. Rat serum lipoprotein fractions were isolated by ultracentrifugation and added to guinea pig postheparin serum in the presence or absence of heparin. When added in proportion to their original serum concentrations, HDL caused the greatest increase in TG hydrolysis. When each fraction was added at equal protein concentrations, purified low density lipoproteins had almost no effect; both very low density lipoproteins and HDL were very effective in increasing the rate of hydrolysis. Rat serum or rat HDL, added to the assay system in increasing amounts, appeared to increase the effective substrate concentration. In the absence of heparin, increasing the concentration of HDL increased the reaction rate which approached a limiting velocity (Vmax) and produced a hyperbolic curve which conformed to Michaelis-Menten kinetics. In the presence of heparin, increasing the concentration of HDL produced an S-shaped curve and increased the Vmax. These data conformed to the sigmoidal kinetics described by the Hill equation. Our results suggest that (1) heparin may function as a specific ligand which acts as an allosteric modifier of LPL and alters the kinetics of interaction of LPL with the effective substrate and (2) the rate of hydrolysis of the effective TG substrate is regulated by the concentration of this substrate.

ADDITIONAL KEY WORDS triglycerides enzyme activation lipolysis ligand enzyme kinetics guinea pig
heparin serum from dogs (3). In contrast to these findings, Naito and Felts observed that in vitro addition of heparin to postheparin serum from rats resulted in a marked increase in LPL activity (4).

The interaction of LPL in postheparin serum with a TG emulsion in the presence and absence of heparin was the subject of a previous investigation in which six species were studied (5). In five of the species, increasing concentrations of heparin appeared which could account for these observations. However, increasing concentrations of heparin obtained from postheparin serum of guinea serum with a TG emulsion in the presence and to inhibit the serum enzyme. In the rat, we have studied the effects of rat serum and of LPL activation mechanisms because its activity is equivalent to 1 jounole of FFA released per ml of guinea pig postheparin serum per hour. Titrations were usually made on aliquots removed at 0 and 60 minutes from the assay system. The LPL activity of guinea pig postheparin serum in the presence of rat serum (0.4 ml) in the assay system was inhibited 92% by NaCl, 1.0M, and 85% by protamine, 0.3 mg/ml. To inhibit bacterial growth, polymyxin B sulfate (Aerosporin, Burroughs Wellcome & Co., Montreal, Canada) was added to give a serum concentration of 25 U/ml.

Abbreviations

LPL = lipoprotein lipase
g

TG = triglycerides

FFA = free fatty acids

HDL = high-density lipoproteins

LDL = low-density lipoproteins

VLD = very low-density lipoproteins

Methods

Animal Treatment.—Male guinea pigs of the English variety, weighing 600 to 900 g, were maintained on Rockland guinea pig laboratory diet (Teklab Inc., Winfield, Iowa) and fed ad libitum. Male Long-Evans rats, weighing 355 to 375 g, were maintained on Rockland rat laboratory diet and fasted 16 hours before use. Blood was obtained from both rats and guinea pigs by performing midline laparotomies under ether anesthesia followed by collection of blood in glass syringes from the abdominal aorta. The postheparin guinea pig blood was obtained by injecting heparin (20 U/kg) into the inferior vena cava and collecting blood (15 to 20 ml) from the abdominal aorta over the 2- to 3-minute period following injection. Pooled blood samples were then allowed to clot at room temperature; sera were separated by centrifugation at 1,000 g for 10 minutes and kept at 0°C.

LPL Assay System.—Lipase activity in guinea pig postheparin serum was assayed in triplicate by a modification of Robinson's method (6). The assay system consisted of the following: 1.50 ml of TG substrate (one part 10% Intralipid, A. B. Vitrum, Stockholm, Sweden, one part 0.15M NaCl and 8 parts of guinea pig preheparin serum incubated for 15 minutes at 37°C); 0.75 ml of 1.35M Tris buffer, pH 8.4; 2.25 ml of a 15% (w/v) solution of bovine albumin (Pentex Inc., Kankakee, Ill.), pH 9.4; 1.0 ml of 0.025M NH₄OH adjusted to pH 8.6 with HCl; 1.0 ml of guinea pig postheparin serum; and one or more of the following: 0 to 0.9 ml of 0.15M NaCl, 0 to 0.8 ml of rat serum or rat lipoprotein fraction, and 0 to 0.1 ml of a heparin solution. Incubations were carried out at 37°C for 1 hour. Released FFA were extracted by the method of Dole, as modified by Trout et al. (7). The FFA were then titrated by a modification of the method of Salamann and Robinson (8). One unit of LPL activity is equivalent to 1 μmole of FFA released per ml of guinea pig postheparin serum per hour.

Titrations were usually made on aliquots removed at 0 and 60 minutes from the assay system. The rate of release of FFA was linear over a 60-minute interval as tested with the addition of rat serum or rat HDL to the guinea pig LPL assay system. The LPL activity of guinea pig postheparin serum in the presence of rat serum (0.4 ml) in the assay system was inhibited 92% by NaCl, 1.0M, and 85% by protamine, 0.3 mg/ml.

Lipoprotein Fractionation Procedure.—Rat serum was obtained and one part of a solution of disodium ethylenediamine tetracetate (EDTA), 0.25M, and phosphate buffer, 0.5M, pH 7.5, was added to 49 parts of serum to give a concentration of 0.005M EDTA, 0.005M, and phosphate buffer, 0.005M. To inhibit bacterial growth, polymyxin B sulfate (Aerosporin, Burroughs Wellcome & Co., Montreal, Canada) was added to give a serum concentration of 25 U/ml. Rat serum lipoprotein fractions were prepared in a 40.3 rotor on a model L-65B Ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.). Densities were adjusted with a NaCl-KBr solution (9). We altered this solution to contain EDTA, 0.005M, and phosphate buffer, 0.005M, pH 7.5.
Very low-density lipoproteins (VLD) were obtained from serum by flotation at a solvent density (d) of 1.006 for 18 hours at 40,000 rpm (1.13 x 10^15 g-min). LDL were obtained by flotation at a density of 1.033 for 24 hours at 40,000 rpm (1.51 x 10^15 g-min). HDL were obtained by flotation at a density of 1.21 for 24 hours at 40,000 rpm (1.51 x 10^15 g-min). Following flotation, each lipoprotein fraction was separated from the infranatant solution with a tube slicer (Beckman Instruments, Inc.) and the contents of the tube above the blade were removed. The VLD, LDL, and HDL fractions were then washed by flotation at densities of 1.006, 1.063, and 1.21, respectively. The VLD, LDL, HDL, rat serum and d > 1.21 infranatant fraction were then dialyzed for 24 hours against NaCl, 0.15M, containing EDTA, 0.005M, phosphate buffer, 0.01M, pH 7.5, and polymyxin B sulfate, 25 U/ml, following which the serum and its fractions were stored at 4°C in glass test tubes for up to 2 weeks before use. Lipoprotein fractions stored under these conditions showed no alteration in electrophoretic characteristics. When concentration of the VLD or LDL was desired, its fractions were stored at 4°C in glass test tubes. Samples of serum or lipoproteins were prepared for spotting as follows. The agarose solution, 100 μl, at 50°C was added to small test tubes. Samples of serum or lipoproteins were then added to the test tubes in amounts varying from 10 to 100 μl depending on the estimated lipoprotein concentration. After mixing, 5 μl of the agarose solution-sample mixture was pipetted with a micropipet into a sample well. Electrophoresis was performed with a Turner Model 310 power supply and electrophoresis cell (C. K. Turner Associates) for 3 to 4 hours at 50 v and 1.5 mamp/cm of gel width. After electrophoresis, the Cronar film with gel was removed from the mold base, fixed and dried (11). Following this, the dried gel was placed for 18 hours in a solution containing 0.6 g Oil Red O (Allied Chemical, Industrial Chemicals Div., Morristown, N. J.) and 2.4 g Fat Red 7B (Ciba Co., Ltd., Don Mills, Ontario) in 1500 ml of 60% ethanol (C. Naito, unpublished observation) at a temperature of 37°C. Use of this electrophoresis technique established that the mobility of our isolated lipoprotein fractions was not altered by our method of preparation and afforded a check on their purity. In whole rat serum, four lipoprotein fractions were identified according to their purity. In isolated lipoprotein fractions by ultracentrifugation did not alter their mobility appreciably.

**Results**

**Effect of Rat Serum and Heparin on LPL Activity of Guinea Pig Postheparin Serum.**—The addition of rat serum in the absence of heparin produced a stimulation in the LPL activity of postheparin guinea pig serum and maximal rates were approached when 0.4 to 0.8 ml rat serum were added to 1.0 ml guinea pig postheparin serum in the assay system (Fig. 1A, B, C). In the presence of heparin, the shape of the curve was markedly altered. An S-shaped curve was seen which did not reach a plateau at the highest concentration of rat serum added and reached a value of 12 to 28% greater than the activity in the absence of heparin.

**Effect of Rat Serum Lipoprotein Fractions (Added in Proportion to their Original Serum Concentration) on LPL Activity of Guinea Pig Postheparin Serum.**—Dialyzed rat serum (0.4 ml) or lipoprotein fractions, which were isolated by density centrifugation and dialyzed, were added to the LPL assay system supported by the mold base. Serum lipoprotein samples were prepared for spotting as follows. The agarose solution, 100 μl, at 50°C was added to small test tubes. Samples of serum or lipoproteins were then added to the test tubes in amounts varying from 10 to 100 μl depending on the estimated lipoprotein concentration. After mixing, 5 μl of the agarose solution-sample mixture was pipetted with a micropipet into a sample well. Electrophoresis was performed with a Turner Model 310 power supply and electrophoresis cell (C. K. Turner Associates) for 3 to 4 hours at 50 v and 1.5 mamp/cm of gel width. After electrophoresis, the Cronar film with gel was removed from the mold base, fixed and dried (11). Following this, the dried gel was placed for 18 hours in a solution containing 0.6 g Oil Red O (Allied Chemical, Industrial Chemicals Div., Morristown, N. J.) and 2.4 g Fat Red 7B (Ciba Co., Ltd., Don Mills, Ontario) in 1500 ml of 60% ethanol (C. Naito, unpublished observation) at a temperature of 37°C. Use of this electrophoresis technique established that the mobility of our isolated lipoprotein fractions was not altered by our method of preparation and afforded a check on their purity. In whole rat serum, four lipoprotein fractions were identified according to their purity. In isolated lipoprotein fractions by ultracentrifugation did not alter their mobility appreciably.
of guinea pig postheparin serum. The lipoprotein fractions were added in an amount equivalent to that contained in 0.4 ml of pooled rat serum. The stimulation of the LPL activity of guinea pig postheparin serum by these additions is shown in Table 1. HDL were by far the most effective lipoprotein fraction in rat serum for the activation of the LPL of guinea pig postheparin serum.

TABLE 1
Effect of Isolated Rat Serum Fractions on LPL Activity of Guinea Pig Postheparin Serum

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Addition to assay system</th>
<th>Protein added (mg)</th>
<th>Increase in LPL activity due to addition</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole serum</td>
<td>24</td>
<td>17</td>
<td>1300</td>
</tr>
<tr>
<td>2</td>
<td>VLD</td>
<td>0.017</td>
<td>3.2</td>
<td>240</td>
</tr>
<tr>
<td>1</td>
<td>LDL</td>
<td>0.014</td>
<td>0.35</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>HDL</td>
<td>0.010</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>d &gt; 1.21 fraction</td>
<td>0.270</td>
<td>10</td>
<td>1400</td>
</tr>
<tr>
<td>2</td>
<td>0.190</td>
<td>12</td>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>d &gt; 1.21 fraction</td>
<td>0.00</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Units of activity = micromoles FFA per ml guinea pig postheparin serum per hour. Fractions of rat serum were isolated by ultracentrifugation (see text) and reconstituted to the original serum volume from which they were obtained. Each was added to the assay system in 0.4 ml. Baseline LPL activity of guinea pig postheparin serum was 1.3 units in experiment 1 and 0.42 units in experiment 2. LPL = lipoprotein lipase; VLD = very low density lipoproteins; LDL = low-density lipoproteins; HDL = high-density lipoproteins.
pig postheparin serum. The rat serum (d > 1.21) infranatant fraction did not stimulate the LPL activity of guinea pig postheparin serum. Examination of each lipoprotein fraction by gel electrophoresis showed the following: VLD were a single band with no detectable contamination; LDL were a single band with very slight contamination with both VLD and HDL-A; HDL consisted of two α bands (HDL-A and HDL-B) with no detectable contamination.

Effect of Rat Serum Lipoprotein Fractions (Added in Equivalent Amounts of Protein) on LPL Activity of Guinea Pig Postheparin Serum.—Rat serum lipoprotein fractions were isolated by density centrifugation, dialyzed, concentrated and then added to the LPL assay system of guinea pig postheparin serum in a volume so that an equal amount of protein from each fraction was added. Under these conditions, it was found that the VLD fraction was almost as effective as HDL on a milligram protein basis in stimulating activity (Table 2). The LDL were also found to have considerable activity when isolated by our usual technique (see Methods); but as noted above, when each of these fractions was examined by gel electrophoresis, it was found that the LDL was slightly contaminated by small amounts of VLD and HDL-A. Isolation of an electrophoretically pure LDL fraction was achieved by the following technique. Serum was adjusted to a density of 1.010 and then centrifuged for 18 hours at 40,000 rpm, as previously described, to isolate the VLD. The d > 1.010 infranatant fraction was then adjusted to a density of 1.045 and centrifuged for 24 hours at 40,000 rpm. The densities used here for the LDL purification are similar to those used by Windmueller and Levy (13) and by Koga et al. (14). The top fraction containing LDL was saved after cutting the tube with a tube slicer. It was readjusted to a density of 1.020 and then centrifuged as above to assure complete removal of the VLD. The infranatant fraction containing LDL was saved. The original d > 1.045 infranatant fraction was adjusted to a density of 1.21 and centrifuged to float the HDL. The VLD, LDL and HDL fractions were then washed by flotation at densities of 1.020, 1.055, and 1.21, respectively. The isolated lipoprotein fractions were then dialyzed. Examination of each fraction by gel electrophoresis showed that VLD and LDL were without contamination; however, more sensitive techniques, such as immunoelectrophoresis, may have detected contamination not detected by our electrophoresis system. The HDL had a barely detectable LDL band. These rat serum lipoprotein fractions were then added to the LPL assay system of guinea pig postheparin serum in a volume so that an equal amount of protein from each fraction was added and the activation response of the LPL of guinea pig postheparin serum was tested (Table 3). Again, VLD were almost as effective in LPL activation as HDL but the purified LDL had almost no effect.

**Table 2**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Additions to assay systems</th>
<th>Protein added (mg)</th>
<th>Increase in LPL activity due to addition (units/mL)</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VLD 0.16</td>
<td>4.5</td>
<td>820</td>
<td>820</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>11</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>6.9</td>
<td>1100</td>
<td>1100</td>
</tr>
<tr>
<td>1</td>
<td>LDL 0.16</td>
<td>1.3</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>4.4</td>
<td>340</td>
<td>340</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>5.1</td>
<td>850</td>
<td>850</td>
</tr>
<tr>
<td>1</td>
<td>HDL 0.16</td>
<td>5.8</td>
<td>1100</td>
<td>1100</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>12</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>9.5</td>
<td>1600</td>
<td>1600</td>
</tr>
</tbody>
</table>

*Units of activity = micromoles FFA per ml guinea pig postheparin serum per hour. Fractions of rat serum were isolated by ultracentrifugation (see text). Equivalent amounts of protein from each fraction were added to LPL assay system of the guinea pig postheparin serum for each experiment. Baseline LPL activity of guinea pig postheparin serum was 0.94 units for experiment 1, 1.3 units for experiment 2, and 0.90 units for experiment 3. Abbreviations as in Table 1.

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TABLE 3
Effect of Purified Rat Serum Fractions on LPL Activity of Guinea Pig Postheparin Serum

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Addition to assay system</th>
<th>Protein added (mg)</th>
<th>Increase in LPL activity due to addition</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VLD</td>
<td>0.15</td>
<td>2.4</td>
<td>310</td>
</tr>
<tr>
<td>2</td>
<td>VLD</td>
<td>0.11</td>
<td>6.6</td>
<td>1000</td>
</tr>
<tr>
<td>1</td>
<td>LDL</td>
<td>0.15</td>
<td>0.14</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>LDL</td>
<td>0.11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>HDL</td>
<td>0.15</td>
<td>2</td>
<td>350</td>
</tr>
<tr>
<td>2</td>
<td>HDL</td>
<td>0.11</td>
<td>11.1</td>
<td>1800</td>
</tr>
</tbody>
</table>

*Units of activity = micromoles FFA per ml guinea pig postheparin serum per hour. Fractions of rat serum were isolated by ultracentrifugation (see text). VLD and LDL were electrophoretically pure; there was very slight β-band contamination on electrophoresis of HDL. Equivalent amounts of protein from each fraction were added to the LPL assay system of the guinea pig postheparin serum for each experiment. Base-line LPL activity of guinea pig postheparin serum was 0.77 units for experiment 1 and 0.63 units for experiment 2.

Abbreviations as for Table 1.

different parts of the rat HDL spectrum, d = 1.115 to 1.045 (HDL-A) and d = 1.21 to 1.115 (HDL-B) on the LPL of guinea pig postheparin serum were studied. Centrifugation of the unfractionated HDL at these densities effectively separated the HDL-A from the HDL-B. Electrophoresis of these fractions showed that HDL-B (slower mobility) was a single band while HDL-A

TABLE 4
Effect of Isolated Rat HDL Subfractions on LPL Activity of Guinea Pig Postheparin Serum

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Addition to assay system</th>
<th>HDL protein contained in addition (mg)</th>
<th>Increase in LPL activity due to addition</th>
<th>% increase</th>
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<tr>
<td>1</td>
<td>HDL</td>
<td>0.18</td>
<td>6.9</td>
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<td>HDL</td>
<td>0.28</td>
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<td>HDL</td>
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<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>1</td>
<td>Serum</td>
<td>0.18</td>
<td>7.7</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>0.28</td>
<td>3.2</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>0.18</td>
<td>6.3</td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>HDL plus d &gt; 1.210</td>
<td>0.18</td>
<td>6.6</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>HDL plus d &gt; 1.210</td>
<td>0.28</td>
<td>4.4</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>HDL plus d &gt; 1.210</td>
<td>0.18</td>
<td>5.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

*Units of activity = micromoles FFA per ml guinea pig postheparin serum per hour. HDL subfractions of rat serum were isolated by ultracentrifugation (see text). Equivalent amounts of protein from HDL and the HDL subfractions were added to the LPL assay system of guinea pig postheparin serum for each experiment. Base-line LPL activity of guinea pig postheparin serum was 0.77 units in experiment 1, and 0.63 units in experiment 2.

Abbreviations as in Table 1.
ACTIVATION OF LIPOPROTEIN LIPASE

(fastest α mobility) had a very slight contamination with LDL. HDL-A was by far the largest component of rat HDL, constituting 88% of the total protein. The activation effect of each subfraction and the unfractionated HDL (added in equal amounts of protein) on the LPL of guinea pig postheparin serum was tested. The LPL activation effect of HDL-A was much greater than the effect of HDL-B and even exceeded the effect of unfractionated HDL. (Table 4).

Effect of Rat Serum, HDL, d>1.21 Infranatant Fraction and Heparin on LPL Activity of Guinea Pig Postheparin Serum.—The effects of heparin on LPL activation of guinea pig postheparin serum in the presence of the following additions to the assay system were compared: (1) 0.4 ml of rat HDL solution containing rat HDL in concentration equivalent to the HDL in 0.4 ml of pooled rat serum; (2) 0.4 ml of pooled rat serum; and (3) 0.4 ml of rat d>1.21 infranatant fraction containing added rat HDL both in amounts equivalent to that contained in 0.4 ml of pooled rat serum. The effect of each addition on LPL activation in the presence and absence of heparin at an in vitro concentration of 1.0 U/ml was studied (Table 5). Rat serum and the rat d>1.21 infranatant fraction containing rat HDL caused increased LPL activation in the presence of heparin whereas LPL activation by rat HDL alone was barely altered in the presence of heparin.

Effect of Increasing Concentrations of Rat HDL and Heparin on LPL Activity of Guinea Pig Postheparin Serum.—Electrophoretically pure rat HDL were diluted to a protein concentration of 0.50 mg/ml. The HDL were then added in increasing amounts (0, 0.1, 0.2, 0.4, and 0.8 ml) to the guinea pig LPL assay system, with and without in vitro heparin in a concentration of 1.0 U/ml in the assay system. LPL activity was increased by rat HDL and followed a hyperbolic curve (Fig. 2A, B, C). In the presence of heparin, increasing amounts of HDL produced an S-shaped curve of LPL activity and an increase in the maximum rate of hydrolysis.

Discussion

The individual functions of heparin and lipoprotein as co-factors in the activation of the LPL enzyme system have not been fully elucidated. From studies of the tissue enzyme, Korn postulated that heparin functions as a prosthetic group for LPL (1). A role for
heparin in the formation of an enzyme-substrate complex has also been suggested (15). Both whole human serum and its HDL fraction have been shown to activate an artificial TG emulsion (2, 10) and it is possible that the altered surface of the TG particles produces an effective substrate which is then able to attach to the enzyme. We have explored further the roles of serum fractions and of heparin in LPL activation.

Isolated lipoprotein fractions from rat serum were added to our LPL assay system of guinea pig postheparin serum in an amount equivalent to that contained in 0.4 ml of pooled rat serum. VLD increased LPL activity by 200 and 240%, LDL by 0 and 26%, and HDL stimulated activity 1400 and 2800% (Table 1). Bier and Havel have reported a similar effect with human VLD, LDL, and HDL added to LPL from fresh cow's milk (17). Fielding has recently described the activation of highly purified (16,000-fold) LPL in human postheparin plasma by human lipoprotein fractions (18). He found that significantly higher rates of hydrolysis were obtained with HDL-activated TG emulsion than with VLD- or LDL-activated TG emulsions. The effect of LDL was less than the effect of VLD. Fielding has also found that in his assay system, mixture of VLD or LDL with HDL increased LPL activity by an average of 85%. At the concentration of HDL selected for these studies, heparin essentially had no effect on LPL activation by HDL. However, distinct increases in LPL activation occurred in the presence of heparin when HDL were combined with the d > 1.21 infranatant fraction. A possible explanation for these observations is that a third LPL activation factor beside heparin and HDL may be present in rat serum, which remains in the d > 1.21 infranatant fraction.

When two electrophoretic components of HDL were subfractionated by ultracentrifugation and the LPL activation by the subfractions tested at equivalent protein concentrations, HDL-A had the greater stimulatory effect while HDL-B had a much smaller effect. The effect of HDL-A was even greater than that of unfractionated HDL when added to the LPL assay system for guinea pig postheparin serum. These results suggest that the activation of LPL is effected by common apoproteins of VLD and HDL, most likely the protein components of apo HDL which have been shown by disc gel electrophoresis to be present in both rat HDL and rat VLD (14). Rat VLD has been shown to contain three distinct antigenic components, one of which reacts with anti-HDL antibodies (14, 20), another with anti-LDL antibodies, and a third which is unidentified (14). There are at least two possibilities for the close proximity in effectiveness of the rat VLD fraction to the effectiveness of the rat HDL fraction in stimulating the LPL activity of guinea pig postheparin serum. The percent of total apo HDL or a subfraction present in VLD may be sufficiently high to account for the effect; measurements of this percent do not appear to be available for the rat. Alternatively, there may be an unidentified VLD apoprotein with an LPL activating effect.

Comparison of the effects of in vitro heparin (1.0 U/ml) on the LPL activation of guinea pig postheparin serum by rat serum, by HDL and by HDL in combination with the d > 1.21 infranatant fraction was made. Heparin increased the LPL activation by whole rat serum by an average of 85%. At the concentration of HDL selected for these studies, heparin essentially had no effect on LPL activation by HDL. However, distinct increases in LPL activation occurred in the presence of heparin when HDL were combined with the d > 1.21 infranatant fraction. A possible explanation for these observations is that a third LPL activation factor beside heparin and HDL may be present in rat serum, which remains in the d > 1.21 infranatant fraction.

When two electrophoretic components of HDL were subfractionated by ultracentrifugation and the LPL activation by the subfractions tested at equivalent protein concentrations, HDL-A had the greater stimulatory effect while HDL-B had a much smaller effect. The effect of HDL-A was even greater than that of unfractionated HDL when added to the LPL assay system for guinea pig postheparin serum. These results suggest that the activation of LPL is effected by common apoproteins of VLD and HDL, most likely the protein components of apo HDL which have been shown by disc gel electrophoresis to be present in both rat HDL and rat VLD (14). Rat VLD has been shown to contain three distinct antigenic components, one of which reacts with anti-HDL antibodies (14, 20), another with anti-LDL antibodies, and a third which is unidentified (14). There are at least two possibilities for the close proximity in effectiveness of the rat VLD fraction to the effectiveness of the rat HDL fraction in stimulating the LPL activity of guinea pig postheparin serum. The percent of total apo HDL or a subfraction present in VLD may be sufficiently high to account for the effect; measurements of this percent do not appear to be available for the rat. Alternatively, there may be an unidentified VLD apoprotein with an LPL activating effect.

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in equal amounts of protein. This indicates that HDL-A contains the greatest amount of LPL activation factor present in unfractionated HDL.

When either rat serum or isolated HDL were added to the LPL assay system of guinea pig postheparin serum in increasing amounts, activity was seen to approach a limiting velocity and followed a hyperbolic curve typical of Michaelis-Menten kinetics. This suggests that increasing concentrations of either whole serum or HDL increased the
effective substrate concentration by converting the inactive TG emulsion to an activated TG emulsion. With heparin present, both rat HDL and rat serum produced similar S-shaped curves and both produced increases in the maximum rate of hydrolysis ($V_{max}$). The appearance of the S-shaped curves in the presence of heparin suggests that heparin may function as a specific ligand which acts as an allosteric modifier of LPL and which alters the kinetics of interaction of LPL with the effective substrate (21, 22). This would suggest that over a certain critical range, the rate of hydrolysis of the effective TG substrate is finely regulated by the concentration of the effective TG substrate itself.

The extent to which our experimental curves conform to mathematical descriptions of enzyme kinetics was tested for the experiments with HDL additions (23). When the data for the hyperbolic curves were plotted according to Lineweaver-Burk, a perfect straight line relationship was obtained (Fig. 3). When the data for the S-shaped curves were plotted according to the linear form of the Hill equation, a perfect straight line relationship was obtained (Fig. 4). The slope, $n$, is approximately two for each of the Hill plots. The fact that the data conform so precisely to these mathematical formulations strongly suggests that heparin produces a conformational change in LPL which alters its kinetic properties.

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**References**


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ACTIVATION OF LIPOPROTEIN LIPASE

Activation of Lipoprotein Lipase: EFFECTS OF RAT SERUM LIPOPROTEIN FRACTIONS AND HEPARIN
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