Comparison of the Triacylglycerol Hydrolase Activity of Human Post-Heparin Plasma Lipoprotein Lipase and Hepatic Triacylglycerol Lipase. A Monolayer Study†

Richard L. Jackson,* Elvira Ponce,‡ and Larry R. McLean

Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, and Merrell Dow Research Institute, Cincinnati, Ohio 45215

Rudy A. Demel

Biochemical Laboratory, The State University of Utrecht, Padualaan 8, Utrecht, The Netherlands

Received April 12, 1985

ABSTRACT: Interfacial catalysis of hepatic triacylglycerol lipase (H-TGL) and lipoprotein lipase (LpL) isolated from human post-heparin plasma was investigated with mixed monolayers of trioleoylglycerol (TO) and egg phosphatidylcholine. Rates of enzyme catalysis were dependent on surface pressure, substrate concentration, apoC-II (the activator protein for LpL), and cholesteryl oleate (CO). LpL showed a surface pressure optimum between 22 and 24 mN m^{-1}, whereas H-TGL activity decreased at pressures >20 mN m^{-1}. LpL activity was enhanced >10-fold by apoC-II; 1 M NaCl inhibited enzyme activity. ApoC-II, apoC-III, apoA-I, apoA-II, and 1 M NaCl had no effect on H-TGL activity. The substrate (TO) dependency was different for the two lipases. For LpL, there was a marked enhancement of enzyme activity between 2 and 4 mol % TO, whereas for H-TGL, enzyme activity increased linearly between 1 and 10 mol % TO. LpL activity toward monolayers containing 2 mol % TO was enhanced 2.6-fold by the addition of 5 mol % CO; cholesteryl ester had no effect on H-TGL activity. These findings suggest that the two lipolytic enzymes have different interfacial properties, which may have relevance to the rates of hydrolysis of triacylglycerols at a lipoprotein interface.

Lipoprotein lipase (LpL)[1] and hepatic triacylglycerol lipase (H-TGL) are lipolytic enzymes that play important roles in the clearance of triacylglycerols from the circulation. [For review, see Jackson (1983), Kinnunen et al. (1983), Kinnunen (1983), and Smith & Pownall (1983)]. In the absence of these enzymes, as occurs in the rare disorders of LpL deficiency (Nikkila, 1983) or H-TGL deficiency (Breckenridge et al., 1982), triacylglycerols are elevated to levels >1000 mg/dL of plasma. Although both LpL and H-TGL hydrolyze triacylglycerols, they differ in their properties and specificities for lipoprotein substrates. The criteria for distinguishing between these enzymes are that LpL requires apolipoprotein C-II, a protein constituent of triacylglycerol-rich lipoproteins, for maximal activity and is inhibited by 1 M NaCl, whereas H-TGL is active in 1 M NaCl and does not require any protein activator for maximal activity. The preferred lipoprotein substrates for LpL are chylomicrons and very low density lipoproteins (VLDL). H-TGL has only limited activity toward these triacylglycerol-rich lipoproteins but prefers remnant triacylglycerol-rich lipoproteins produced from the action of LpL on chylomicrons and VLDL, and high-density lipoproteins (HDL), particularly the subfraction corresponding to HDL_2 (Jackson, 1983; Kinnunen et al., 1983).

Although LpL and H-TGL differ with respect to their properties and specificity toward lipoprotein substrates, it is assumed that both enzymes catalyze the hydrolysis of triacylglycerols contained within the outer monolayer of the lipoprotein particle. The lipoprotein monolayer contains mainly phosphatidylcholine, sphingomyelin, unesterified cholesterol, protein, and small amounts of the neutral lipids, triacylglycerols and cholesteryl esters. Because of our interest in understanding interfacial enzyme catalysis (Quinn et al., 1983), we have developed a monolayer system that allows for the study of lipolytic enzymes with long-chain, physiological lipids (Demel et al., 1982, 1984; Demel & Jackson, 1985). Since LpL and H-TGL show markedly different properties with respect to their specificity for lipoprotein substrates, it was of interest to determine whether the differences in enzyme activity could be explained by the properties of the monolayer itself. The results show that the rates of the LpL- and H-TGL-catalyzed hydrolysis of trioleoylglycerol in a monolayer differ markedly with respect to surface pressure, substrate concentration, and the amount of neutral lipids.

Materials and Methods

Materials. Tri[1-^{14}C]oleylglycerol (47.5 mCi/mmol) was obtained from Amersham. Fatty acid free bovine serum albumin (BSA; fraction V), aprotinin, leupeptin, Sepharose CL-4B, phenyl-Sepharose, hydroxyapatite, DEAE-Sephacel, and heparin (169 units/mg) were purchased from Sigma. Heparin–Sepharose CL-4B was prepared as described previously (Matsuoka et al., 1980). Egg yolk phosphatidylcholine

†Supported by U.S. Public Health Service Grants P01 HL-22619 and R01 HL-23019 and by General Clinical Research Center and CLINFO Grant NIH RR 00068. L.R.M. is supported by Molecular and Cellular Biology Training Grant NIH HL-07527.

*Address correspondence to this author at the Merrell Dow Research Institute.

‡Permanent address: Catedra de Bioquimica, Escuela de Medicina J. M., Vargas, Universidad Central de Venzuela, Caracas, Venezuela.

[1] Abbreviations: LpL, lipoprotein lipase; H-TGL, hepatic triacylglycerol lipase; TO, trioleoylglycerol; CO, cholesteryl oleate; apoC-II, apoC-III, apoA-I, and apoA-II, apolipoproteins of human plasma lipoproteins; PC, phosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/86/0425-1166$01.50/0

was isolated and purified by high-performance liquid chromatography as described previously (Geurts van Kessel et al., 1981). ApoC-II and apoC-III$_2$ (2 mol of sialic acid) were isolated from plasma triacylglycerol-rich lipoproteins ($d < 1.02$ g/mL) of subjects with fasting chylomicronemia (type V hyperlipoproteinemia) (Cardin et al., 1984). ApoA-I and apoA-II were isolated from HDL of normal subjects (Cardin et al., 1984). The isolated apoproteins were homogeneous by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (apoA-I and apoA-II) and by isoelectrofocusing (apoC-II and apoC-III$_2$) and had amino acid compositions consistent with their known sequences.

Isolation of Human Post-Heparin Plasma LpL and H-TGL. LpL and H-TGL were isolated from post-heparin plasma by chromatography on heparin–Sepharose. In a typical isolation, post-heparin plasma (2800 mL) was mixed with an equal volume of 10 mM potassium phosphate, pH 6.8, 0.45 M NaCl, 1 mM EDTA, and 400 mL heparin–Sepharose CL-4B. After being stirred for 2 h at 4 °C, the heparin–Sepharose was collected on a sintered glass funnel and the gel washed in 500-mL batches as follows: 4 L of 5 mM potassium phosphate, pH 6.8, 0.3 M NaCl, and 1 mM EDTA; 8 L of 5 mM potassium phosphate, pH 6.8, and 1 mM EDTA; 1 L of 5 mM potassium phosphate, pH 6.8, containing 0.2% Triton N-101; 8 L of 5 mM potassium phosphate, pH 6.8, and 1 mM EDTA; 2 L of 5 mM potassium phosphate, pH 6.8, 0.3 M NaCl, and 1 mM EDTA. The gel was poured into a column (5.0 × 45 cm), and protein was eluted (250 mL/h) with 5 mM potassium phosphate, pH 6.8, containing 2.4 M NaCl and 10% glycerol; aprotinin and leupeptin were added to final concentrations of 50 kallikrein inhibitor units/mL and 10 μg/mL, respectively. The protein solution was diluted with 5 mM potassium phosphate, pH 6.8, 1 mM EDTA, and 10% glycerol, to give a final NaCl concentration of 0.4 M. The sample was then applied to a second column (3.0 × 30 cm) of heparin–Sepharose. Protein was then eluted with a NaCl gradient (0.4–2.5 M). H-TGL was further purified by chromatography on phenyl-Sepharose and DEAE-Sephacel.[2] The purified enzyme had a specific activity of 192 μmol of oleic acid released h^{-1} (mg of protein)$^{-1}$, corresponding to a purification of >25 000-fold; activity was not affected by the addition of 1 M NaCl. LpL was further purified by chromatography on hydroxyapatite.[2] The purified enzyme had a specific activity of 74 μmol of oleic acid released h^{-1} (mg of protein)$^{-1}$, corresponding to a purification of 20 000-fold. LpL activity was enhanced ~10-fold by apoC-II and was inhibited by 1 M NaCl.

Other Methods. Lipase activities were determined with a Triton X-100 emulsified tri[1-^{14}C]oleoylglycerol substrate as described previously (Matsuoka et al., 1980); the LpL assays contained 4 μg/mL apoC-II and 0.15 M NaCl whereas the H-TGL assays contained 1 M NaCl and no apoC-II. Protein was determined by the method of Lowry et al. (1951) with BSA as standard protein.

Monolayer experiments were performed in a 15-mL Teflon trough (5.4 × 5.9 × 0.5 cm) as described previously (Demel et al., 1982, 1984). The subphase contained 15 mL of 10 mM Tris-HCl, pH 8.4, and 0.15 M NaCl and was stirred with a magnetic bar. Surface pressure was determined with a recording Beckman LM 500 electrobalance equipped with a platinum plate 1.96 cm wide. Surface radioactivity was measured with a gas-flow detector (Nuclear Chicago) positioned 0.3 cm above the interface; the gas was helium/1.3% butane. Measurements were performed at 33.0 ± 0.1 °C in a thermostatically controlled box. Lipids were spread from chloroform solutions to the desired surface pressure. After the films stabilized (2–3 min), solutions were injected under the film through a 0.8-cm hole at an extended cover of the Teflon trough. Enzyme activities are expressed as the nanomoles of [^{14}C]oleic acid released per hour per milligram of injected enzyme. Results represent the average of duplicate values and are ±10%.

[2] E. Ponce, L. R. McLean, A. Rechtin, and R. L. Jackson, unpublished results.

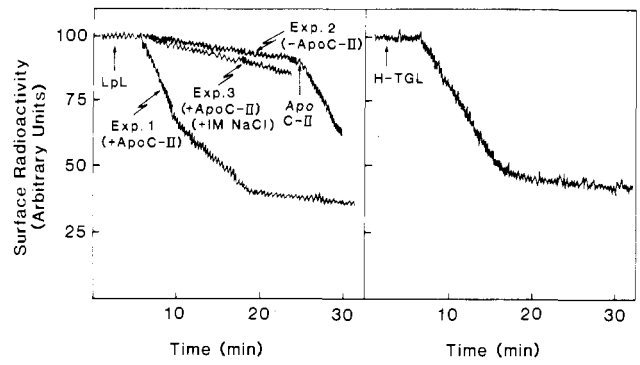

FIGURE 1: LpL- and H-TGL-catalyzed hydrolysis of 4 mol % TO in egg PC. The monolayers contained 8.4 nmol of egg PC and 4 mol % tri[1-^{14}C]oleoylglycerol (10 000 cpm) at a surface pressure of 22 mN m^{-1}. The subphase contained 15 mL of 10 mM Tris-HCl, pH 8.4, and 0.15 M NaCl. Temperature was maintained at 33.0 ± 0.1 °C. After the monolayer stabilized, 0.02 mL of heparin (2.5 mg/mL), 0.05 mL of fatty acid free BSA (5 mg/mL), 0.01 mL of apoC-II (0.1 mg/mL in 6 M guanidine hydrochloride), and LpL (16.75 μg, left panel) or H-TGL (10.5 μg, right panel) were added. Surface pressure and radioactivity were determined as described under Materials and Methods.

RESULTS

Monolayer Lipid Studies. Representative tracings of the LpL and H-TGL-catalyzed hydrolysis of 4 mol % tri[1-^{14}C]oleoylglycerol in a monolayer of egg PC are illustrated in Figure 1. As enzyme catalysis proceeds, [^{14}C]oleic acid is removed from the interface by the action of BSA. The rates of enzyme catalysis are determined by measuring the initial rate of decrease of radioactivity. As shown previously (Demel et al., 1982), the BSA-mediated removal of oleic acid from the interface is not a rate-limiting factor in enzyme catalysis. Although LpL and H-TGL are specific for the glycerol ester bonds in position 1 (3), the kinetics of hydrolysis were different for the two enzymes. For LpL, in the presence of apoC-II (Figure 1, experiment 1), the initial rate of catalysis was 158.4 nmol h^{-1} mg^{-1}; after 30% of the trioleoylglycerol was hydrolyzed, the rate was 67.4 nmol h^{-1} mg^{-1}. Maximum hydrolysis corresponded to the release of two oleic acid groups (62% decrease in radioactivity); monooleoylglycerol remains in the monolayer and is not further hydrolyzed (Demel et al., 1982). In the absence of apoC-II, LpL had an activity of 11.8 nmol h^{-1} mg^{-1} (Figure 1, experiment 2); addition of the activator protein after a 20 min incubation gave a rate of LpL hydrolysis nearly the same as that in experiment 1, indicating that, in the absence of apoC-II, the enzyme is not inactivated at the interface. In the presence of apoC-II and 1 M NaCl (Figure 1, experiment 3), the rate of enzyme hydrolysis was 17.6 nmol h^{-1} mg^{-1}, a value nearly identical with that for the enzyme in the absence of the activator protein.

In contrast to LpL, the rate of H-TGL catalysis (99.9 nmol h^{-1} mg^{-1}) was linear to >50% hydrolysis (Figure 1); after 60 min the final extent of hydrolysis was 65%. The rate of H-TGL hydrolysis was the same in the presence or absence of

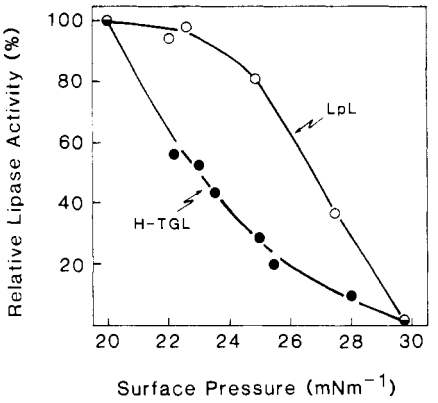

FIGURE 2: Effect of surface pressure on the LpL- and H-TGL-catalyzed hydrolysis of 3 mol % TO in egg PC. Relative activities of LpL and H-TGL were determined at the indicated surface pressures with the conditions described in Figure 1.

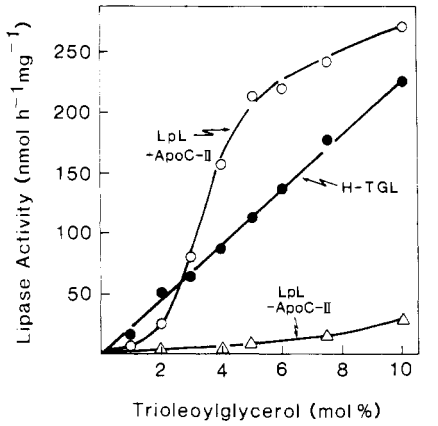

FIGURE 3: Dependence of LpL and H-TGL activity on TO concentration. The monolayers contained egg PC and the indicated concentration of TO at a surface pressure of 20 mN m^{-1}. LpL activity was determined in the presence of 1 μg of apoC-II (○) or in the absence of apoC-II (△). H-TGL activity was determined in the absence of apoC-II (●). Other experimental conditions are the same as those described in Figure 1.

1 M NaCl. Enzyme activity was not stimulated or inhibited by the addition of up to 1 μg of apoC-II, apoC-III, apoA-I, or apoA-II (data not shown). Addition of >1 μg apoprotein caused a decrease in H-TGL activity that was not specific for any one apoprotein as previously shown for LpL (Jackson et al., 1980). As shown below, H-TGL activity decreases at surface pressures >20 mN m^{-1}; with the addition of an excess of any apoprotein, there is an increase in surface pressure that is concomitant with a decrease in enzyme activity.

The effect of lipid packing on LpL and H-TGL activity is shown in Figure 2. In these experiments, the monolayer contained 3 mol % TO in egg PC at the indicated surface pressure. With these conditions, TO is completely miscible with PC in the monolayer (Demel et al., 1982). For H-TGL, enzyme activity decreased from 65.0 nmol h^{-1} mg^{-1} at 20 mN m^{-1} to <20% of this value at surface pressures >25 mN m^{-1}; the decrease in enzyme activity was independent of the amount of H-TGL added to the subphase. For LpL, there was an apparent surface pressure optimum between 20 and 24 mN m^{-1}, and then enzyme activity decreased at higher surface pressures. For both H-TGL and LpL, activities were undetectable at pressures >30 mN m^{-1} even with 10-fold higher enzyme concentrations.

The dependency of enzyme activity on interfacial TO concentration is shown in Figure 3; both enzyme activities were determined at 20 mN m^{-1}. For H-TGL, enzyme activity

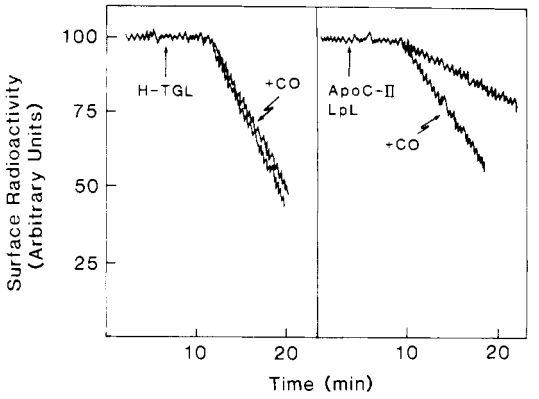

FIGURE 4: Effect of cholesteryl oleate (CO) on the LpL- and H-TGL-catalyzed hydrolysis of TO. The monolayers contained 2 mol % TO with or without 5 mol % CO (as indicated) in egg PC at a surface pressure of 20 mN m^{-1}. The experimental conditions are the same as those described in Figure 1.

increased linearly from 1 to 10 mol % TO. In contrast, LpL activities were nonlinear with increasing TO concentrations. At a substrate concentration of 2 mol % TO, LpL activity was markedly enhanced; above 5–6 mol % TO, LpL activity increased less rapidly with increasing TO concentrations.

Effect of Cholesteryl Oleate on the Enzyme-Catalyzed Hydrolysis of Trioleoylglycerol. In a recent report (Demel & Jackson, 1985), we showed with bovine milk LpL that 5 mol % CO in a monolayer containing 2.5 mol % TO in egg PC enhanced the LpL-catalyzed hydrolysis of TO; at TO concentrations >5 mol %, CO inhibited catalysis presumably by competing with the triacylglycerol for the interface. The results shown in Figure 4 indicate that the activity of post-heparin plasma LpL is also enhanced by 5 mol % CO in a monolyer of 2 mol % TO. In the presence of apoC-II and at a surface pressure of 20 mN m^{-1}, LpL activity was 26 nmol h^{-1} mg^{-1} in a monolayer containing 2 mol % TO. With the same assay conditions in a monolayer containing 2 mol % TO and 5 mol % CO, enzyme activity was enhanced 2.6-fold (65 nmol h^{-1} mg^{-1}). In the absence of apoC-II, enzyme activities were low. However, CO enhanced LpL activity approximately 2-fold (from 1.7 to 3.0 nmol h^{-1} mg^{-1}). In contrast, the addition of 5 mol % CO to a monolayer of 2 mol % TO in egg PC did not significantly alter H-TGL activity (Figure 4); the rates were 65 and 56 nmol h^{-1} mg^{-1}, in the presence and absence of CO, respectively.

Discussion

The monolayer system described in this study has allowed for a detailed comparison of the triacylglycerol-hydrolyzing activities of LpL and H-TGL at a lipid interface. The primary advantage of this system is that controlled changes in the lipid composition and properties of the monolayer are possible, and as a result, the conditions that might arise during the catabolism of large, triacylglycerol-rich lipoproteins may be simulated. In the monolayer system, the rates of trioleoylglycerol hydrolysis by LpL and H-TGL were shown to depend on surface pressure and on apoC-II, TO, and cholesteryl oleate concentrations. The effect of each of these factors on TO hydrolysis differed for the two enzymes, suggesting that LpL and H-TGL have different interfacial properties.

The rate of hydrolysis of TO in a PC monolayer catalyzed by human post-heparin plasma LpL is enhanced >10-fold by apoC-II (1 μg/15 mL). In contrast, H-TGL activity is unaffected by the same concentration of apoC-II, apoC-III, apoA-I, or apoA-II; in the monolayer system, apoA-II did not activate H-TGL as reported by Jahn et al. (1981, 1983) with

phospholipid-emulsified trioleoylglycerol as substrate. At higher concentrations of added apolipoproteins, the activity of H-TGL is decreased toward lipoproteins (Kuusi, 1979; Shinomiya et al., 1982) and lipid emulsions (Kinnunen & Ehnholm, 1976; Kubo et al., 1982). Inhibition of H-TGL in activity could be explained by competition of the apolipoproteins and H-TGL for the lipid interface, as suggested by Kubo et al. (1982), or alternatively we suggest that apoproteins increase surface packing of the lipid.

It is well established that the physiological substrates for LpL are large triacylglycerol-rich lipoproteins; LpL represents a rate-limiting enzyme in triacylglycerol metabolism (Reardon et al., 1982). Although the role of H-TGL in lipoprotein metabolism is less well established, two functions have been suggested: (1) H-TGL has been implicated in the catabolism of remnant lipoproteins, also referred to as intermediate-density lipoproteins of S_f 12–60 (Grosser et al., 1981; Murase & Itakura, 1981; Goldberg et al., 1982; Yamada et al., 1981; Musliner et al., 1979), and (2) H-TGL has been shown to play a role in the hydrolysis of HDL triacylglycerols, HDL phosphatidylcholine, and HDL phosphatidylethanolamine (Shirai et al., 1981; Landin et al., 1984; Rao et al., 1982; Kuusi et al., 1979, 1980; Jansen et al., 1980; Groot et al., 1981, 1983); the HDL subfraction corresponding to HDL_2 is the preferred substrate for H-TGL.

Although it is not possible to predict kinetic behavior in lipoprotein systems from monolayer results, the model monolayer system may suggest certain kinetic events that explain the preference of LpL and H-TGL for different lipoprotein substrates. As shown in Figure 2, the effect of surface pressure on LpL catalysis differs markedly from that observed for H-TGL. H-TGL activity decreases rapidly with increasing surface pressure, whereas LpL activity remains relatively constant from 20 to 23 mN m^{-1}. The surface pressure of a lipoprotein interface is not known. Differential scanning calorimetric (Suurkuusk et al., 1976) and Raman spectroscopic (Mendelsohn et al., 1976; Gaber & Peticolas, 1977) data are consistent with decreasing interchain interactions in PC bilayers of increasing curvature. On the basis of these data, it seems reasonable to propose that large chylomicrons and VLDL have a higher surface monolayer packing density than smaller particles. Since LpL activity remains high at surface pressures where H-TGL activity is low, the putative higher interfacial packing density of the chylomicron would favor hydrolysis of chylomicron triacylglycerols by LpL rather than H-TGL. For example, at a presumed packing density of a chylomicron interface corresponding to a surface pressure of $\sim$25 mN m^{-1} the activity of LpL would be >3 times higher than that for H-TGL relative to their activities at 20 mN m^{-1} (Figure 2). In contrast to the large triacylglycerol-rich lipoproteins, small particles would likely have a lower surface pressure and decreased amounts of apoC-II. These conditions would favor the action of H-TGL.

The concentration of neutral lipids in the monolayer may also be a factor that regulates lipolysis and explains the differences in lipoprotein substrate specificities of LpL and H-TGL. As shown in Figure 3, at 20 mN m^{-1} surface pressure, LpL is activated by TO concentrations in a range of 2–4 mol %, corresponding to the estimated concentration of TO in the surface of chylomicrons and VLDL (Miller & Small, 1983). These data suggest that the activity of LpL would be low relative to H-TGL when the interfacial concentration of TO in a chylomicron is <3 mol %. In the case of large chylomicrons, a high surface pressure may decrease the concentration of TO in the interface (Demel et al., 1983), favoring H-TGL rather than LpL catalysis. However, at a surface pressure of $\sim$25 mN m^{-1}, H-TGL activity is negligible compared to LpL activity. In addition, even at TO concentrations as low as 2 mol %, LpL activity is increased 2.6-fold in the presence of 5 mol % CO; CO has no effect on H-TGL activity. Cholesteryl esters may be important in vivo since, during the catabolism of chylomicrons, the concentration of cholesteryl esters in the lipoproteins increases due to triacylglycerol hydrolysis and transfer of cholesteryl esters from HDL.

In summary, we have shown that two factors are significant in determining the catalytic preference of LpL and H-TGL for TO in a phospholipid monolayer: (1) surface pressure, which favors LpL catalysis in the range 22–28 mN m^{-1}, and (2) neutral lipid concentrations, which regulate the catalytic rate of LpL within a narrow concentration range. We speculate that these differences in interfacial properties may account for the lipoprotein specificities of these important lipolytic enzymes.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. E. A. Stein and Dr. M. Kashyap for providing the post-heparin plasma used in this study. We especially thank Gwen Kraft for preparing the figures and Robin Wright for preparing the manuscript for publication.

Registry No. LpL, 9004-02-8; TGL, 9001-62-1; TO, 122-32-7; CO, 303-43-5.

REFERENCES

Breckenridge, W. C., Little, J. A., Alaupovic, P., Wang, C. S., Kuksis, A., Kakis, G., Lindgren, F., & Grardiner, G. (1982) *Atherosclerosis (Shannon, Irel.) 45*, 161–179.

Cardin, A. D., Holdsworth, G., & Jackson, R. L. (1984) *Methods Pharmacol. 5*, 141–166.

Demel, R. A., & Jackson, R. L. (1985) *J. Biol. Chem. 260*, 9589–9592.

Demel, R. A., Shirai, K., & Jackson, R. L. (1982) *Biochim. Biophys. Acta 713*, 629–637.

Demel, R. A., Dings, P. J., & Jackson, R. L. (1984) *Biochim. Biophys. Acta 793*, 399–407.

Gaber, B. P., & Peticolas, W. L. (1977) *Biochim. Biophys. Acta 465*, 260–274.

Geurts van Kessel, W. S. M., Tieman, M., & Demel, R. A. (1981) *Lipids 16*, 58–63.

Goldberg, I. J., Le, N.-A., Paterniti, J. R., Ginsberg, H. N., Lindgren, F. T., & Brown, W. V. (1982) *J. Clin. Invest. 70*, 1184–1192.

Groot, P. H. E., Jansen, H., & van Tol, A. (1981) *FEBS Lett. 129*, 269–272.

Groot, P. H. E., Scheek, L. M., & Jansen, H. (1983) *Biochim. Biophys. Acta 751*, 393–400.

Grosser, J., Schrecker, O., & Greten, H. (1981) *J. Lipid Res. 22*, 437–442.

Jackson, R. L. (1983) *Enzymes (3rd Ed.) 16*, 141–181.

Jackson, R. L., Pattus, F., & deHaas, G. (1980) *Biochemistry 19*, 373–378.

Jahn, C. E., Osborne, J. C., Schaefer, E. J., & Brewer, H. B., Jr. (1981) *FEBS Lett. 131*, 366–368.

Jahn, C. E., Osborne, J. C., Schaefer, E. J., & Brewer, H. B., Jr. (1983) *Eur. J. Biochem. 131*, 25–29.

Jansen, H., van Tol, A., & Hulsmann, W. C. (1980) *Biochem. Biophys. Res. Commun. 92*, 53–59.

Kinnunen, P. K. J. (1983) in *Lipases* (Bergstrom, B., & Brockman, H. L., Eds.) p 301, Elsevier, Amsterdam.

Kinnunen, P. K. J., & Ehhholm, C. (1976) *FEBS Lett. 65*, 354–357.

Kinnunen, P. K. J., Virtanen, J. A., & Vainio, P. (1983) *Atheroscler. Rev. 11*, 65–105.
Kubo, M., Matsuzawa, Y., Yokoyama, S., Tajima, S., Ishikawa, K., Yamamoto, A., & Tarui, S. (1982) *J. Biochem. (Tokyo) 92*, 865–870.
Kuusi, T. (1979) Doctoral Dissertation, University of Helsinki.
Kuusi, T., Kinnunen, P. K. J., & Nikkila, E. A. (1979) *FEBS Lett. 104*, 384–387.
Kuusi, T., Saarinen, P., & Nikkila, E. A. (1980) *Atherosclerosis (Shannon, Irel.) 36*, 589–593.
Landin, B., Nilsson, A., Twu, J.-S., & Schotz, M. C. (1984) *J. Lipid Res. 25*, 559–563.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. L. (1951) *J. Biol. Chem. 193*, 265–275.
Matsuoka, N., Shirai, K., & Jackson, R. L. (1980) *Biochim. Biophys. Acta 620*, 308–316.
Mendelsohn, R., Sander, S., & Bernstein, H. J. (1976) *Biochim. Biophys. Acta 419*, 563–569.
Miller, K. W., & Small, D. M. (1983) *J. Biol. Chem. 258*, 13772–13784.
Murase, T., & Itakura, H. (1981) *Atherosclerosis (Shannon, Irel.) 39*, 293–300.
Musliner, T. A., Herbert, P. N., & Kingston, M. J. (1979) *Biochim. Biophys. Acta 575*, 277–288.
Nikkila, E. A. (1983) in *The Metabolic Basis of Inherited Diseases* (Stanbury, J. B., Wyngaarden, J. B., Fredrickson, P. S., Goldstein, J. L., & Brown, M. S., Eds.) 5th ed., pp 622–642.
Quinn, D., Shirai, K., & Jackson, R. L. (1983) *Prog. Lipid Res. 22*, 35–78.
Rao, S. N., Cortese, C., Miller, N. E., Levy, Y., & Lewis, B. (1982) *FEBS Lett. 150*, 255–259.
Reardon, M. F., Sakai, H., & Steiner, G. (1982) *Arteriosclerosis (Dallas) 2*, 396–402.
Shinomiya, M., Sasaki, N., Barnhart, R. L., Shirai, K., & Jackson, R. L. (1982) *Biochim. Biophys. Acta 713*, 292–299.
Shirai, K., Barnhart, R. L., & Jackson, R. L. (1981) *Biochem. Biophys. Res. Commun. 100*, 591–599.
Smith, L. C., & Pownall, H. J. (1983) in *Lipases* (Bergstrom, B., & Brockman, H. L., Eds.) p 26, Elsevier, Amsterdam.
Suurkuusk, J., Lentz, B. R., Barenholz, Y., Biltonen, R. L., & Thompson, T. E., (1976) *Biochemistry 15*, 1393–1401.
Van Tol, A., van Gent, T., & Jansen, H. (1980) *Biochem. Biophys. Res. Commun. 94*, 101–108.
Yamada, N., Murase, T., Akanuma, Y., Itakura, H., & Kosaka, K. (1981) *Clin. Chim. Acta 110*, 45–49.

Androgen Induction of Messenger RNA Concentrations in Mouse Kidney Is Posttranscriptional†

Franklin G. Berger*,‡
Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263
David Loose and Herman Meisner
Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106
Gordon Watson
Department of Genetics, University of California at Berkeley, Berkeley, California 94720
Received April 24, 1985; Revised Manuscript Received October 1, 1985

ABSTRACT: The concentrations of several mRNAs in mouse kidney increase in response to testosterone. To determine if the increases are generated at the level of gene transcription, we have assayed transcription rates for several androgen-inducible mRNAs in kidney nuclei in vitro. No significant changes were found in the synthesis of three mRNAs whose concentrations increase 10–20-fold during testosterone treatment. Kinetic analysis of changes in transcript levels after testosterone administration and withdrawal suggests that mRNA stabilization is a major factor in the inductions. Thus, the androgen-mediated induction of these kidney mRNAs is generated predominantly at the posttranscriptional level.

Several studies indicate that steroid hormone induction of specific mRNA concentrations occurs at the level of gene transcription (Ringold et al., 1977; McKnight & Palmiter, 1979; Brock & Shapiro, 1983a). In a number of cases, the inductions have been shown to be mediated by specific DNA elements that are located in the immediate vicinity of the modulated genes and that have strong affinities for hormone–receptor complexes (Chandler et al., 1983; Karin et al., 1984; Renkawitz et al., 1984; Moore et al., 1985). However, marked alterations of mRNA stability in response to steroids have also been observed (Wiskocil et al., 1980; Brock & Shapiro, 1983b; Vannice et al., 1984), indicating that steroids function at multiple levels.

Androgenic steroids have profound effects on the mouse kidney (Bardin & Catterall, 1981); these effects, which are primarily exerted on proximal tubule cells, include inductions of a number of specific gene products. Treatment of female

† Supported by NIH grants to F.G.B. (AM-31818) and H.M. (ES-01170). D.L. is a postdoctoral trainee of the NIH.
‡ Present address: Department of Biology, University of South Carolina, Columbia, SC 29208.

0006-2960/86/0425-1170$01.50/0