A SPECIFIC APOPROTEIN ACTIVATOR FOR LIPOPROTEIN LIPASE

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SUMMARY

These studies were designed to determine which of the apoproteins of high density lipoprotein (HDL) function as the cofactor for lipoprotein lipase (LPL). ApoLP-gln and apoLP-thr, the major HDL apoproteins, as well as apoLP-val, a minor apoprotein constituent, are inactive as cofactors even in the presence of phospholipid. ApoLP-ala, another minor constituent, is inactive alone but in the presence of phospholipid stimulates lipase activity twofold. Only apoLP-glu is able to stimulate LPL activity in the absence of phospholipid and, in the presence of phospholipid, increases activity twelvefold over baseline levels. It is possible that apoLP-glu and perhaps apoLP-ala are obligatory "co-factors" for the hydrolytic step required for normal clearing of triglyceride from the plasma.

INTRODUCTION

It has been earlier demonstrated that high density lipoprotein (HDL) is an activator of lipoprotein lipase (LPL) (EC3.1.1.3) (1), and that the delipidated apolipoprotein of HDL (apoHDL) is apparently inactive in the absence of phospholipid (2). ApoHDL is now known to be heterogeneous, containing at least five proteins (3). At least three of these are also major constituents of the apoproteins of very low density lipoproteins (VLDL). As identified by their carboxyterminal residues, these proteins are apoLP-valine (apoLP-val), apoLP-alanine (apoLP-ala) and apoLP-glutamic acid (apoLP-glu) (4). They comprise about 10% of the HDL apoproteins; most of apoHDL consists of two other proteins, apoLP-glutamine (apoLP-gln) and apoLP-threonine (apoLP-thr) (3, 5). It has been demonstrated recently that the latter two proteins do not account for the

activation of LPL by HDL and that such activity resides in one or more of the minor apoHDL components (6). The present studies were designed to identify which of the several lesser apoproteins are activators of LPL.

MATERIALS AND METHODS

Preparation of Protein Co-factors: Lipoprotein apoproteins were isolated from VLDL or HDL prepared in the ultracentrifuge and delipidated with 3:1 ethanol: ether (7). The specific apoproteins were separated on Sephadex G-150 or G-200 and DEAE-cellulose as previously described (3-5). Each of the purified apoproteins migrated as a single band on agarose and all but one as a single band on polyacrylamide gel (8). ApoLP-ala yielded two bands on polyacrylamide consistent with prior findings that this protein is subject to polymorphism due to small differences in carbohydrate content (9). Anti-sera specific for apoLP-gln, apoLP-thr, apoLP-ala and apoLP-val were used to demonstrate the purity of these apoprotein preparations. All apoprotein fractions were examined immunochemically and contained no detectable albumin, globulin or other serum nonlipoprotein protein contaminents (10).

A mixture of phospholipids (PL) was prepared from a low density lipoprotein preparation by chromatography on silicic acid (11) and stored at -10° in benzene. The mixture was protein-free and contained approximately 10 mg/ml of phospholipid. It contained the following compounds separable by two-dimensional thin-layer chromatography: sphingomyelin, phosphatidyl choline, lysophosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and cardiolipin (12). One-tenth ml of this PL solution was pipetted into a 5 cc test tube and the benzene was removed under nitrogen. Approximately 1.0 mg of pure apoprotein in 1.0 ml of distilled water was added. When PL was assayed

in the absence of apoprotein, PL was taken up in 1.0 cc of distilled water.

Preparation of Lipoprotein Lipase: Acetone-ether powders of testicular fat pads from NIH Mendel rats (250-300 gm) were prepared as previously described (13). Powders were either stored in vacuo at 4°C for no more than 72 hours or were used immediately. Five mg of powder were homogenized in 1.0 ml of 1.25 M NH_4OH, pH 8.6 in a Potter-Elvehjem homogenizer for four minutes at 4°C. The resultant suspension was centrifuged and 0.5 cc aliquots of the supernatant solution used as enzyme source.

Triglyceride Lipase Assay: Substrates were prepared by adding 14 nmoles of glyceryl-1-C^{14}-trioleate (5.5×10^5 dpm, Amersham-Searle) and 125 nmoles of glyceryl trioleate (Hormel) in benzene to a 20 cc counting vial. The solvent was removed under nitrogen and to each vial were added 6 ml of 0.01 M Tris buffer, pH 8.6 in 0.15 M NaCl, 0.2 ml 1% albumin solution, 0.1 ml of heparin, 100 units ml, 0.1 ml of 1:100 Triton X-100 solution and varying amounts of apoproteins or apoproteins and phospholipids. The mixture was sonified for one minute on a Branson sonifier and 3.0 cc pipetted into each of two duplicate vials. All incubations were carried out in a Dubnoff shaking water bath at 27°C for one hour. After lipolysis, fatty acids were extracted from 1.0 cc of the incubate (14). Fatty acids were isolated from 3.0 cc of the iso-octane phase by the method of Kelley (15). Labeled fatty acid was counted in a Packard liquid scintillation counter. All results were expressed in terms of nmoles of labeled fatty acid liberated per hour per ml of LPL extract.

RESULTS

Activation of LPL with Apoproteins Alone: Of the five apoproteins tested, only apoLP-glu activated LPL in the absence of phospholipid

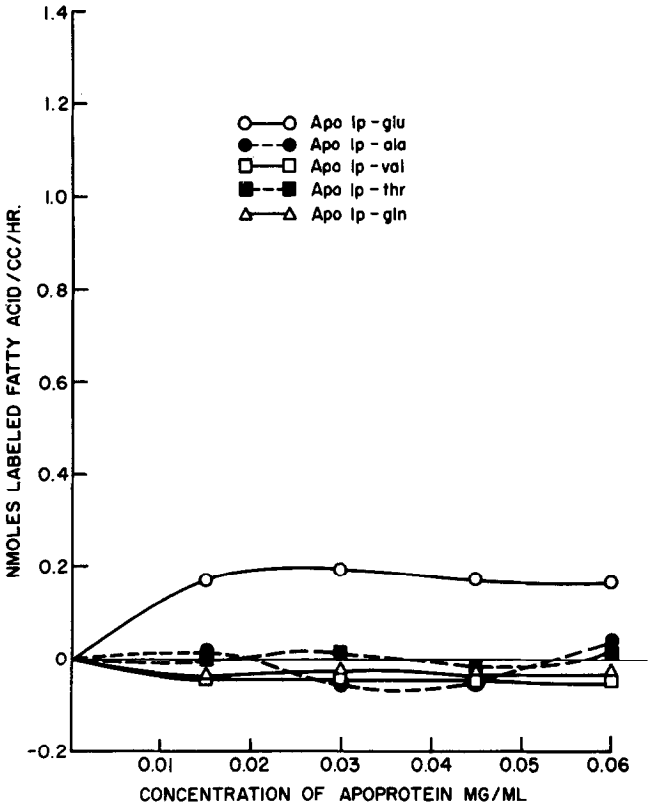

Figure 1. Effect of apolipoprotein fractions on LPL activity in the absence of phospholipid. In order to compare experiments, the activity present with no added apolipoprotein is corrected to zero and the changes in activity obtained with increasing levels of apolipoprotein are plotted.

(Figure 1). In all instances, a small amount of activity was present in the absence of any apoprotein.

Activation of LPL with Apoprotein and Phospholipid: When an equal weight of the crude phospholipid mixture (PL) was added along with each of the apoproteins, apoLP-val, apoLP-thr, and apoLP-gln did not enhance LPL activity nor did PL added alone. The addition of apoLP-ala and PL increased activity approximately twofold over the baseline. ApoLP-glu and PL increased activity twelvefold over baseline levels (Figure 2). The maximum stimulation was achieved with a concentration of 0.045 mg of

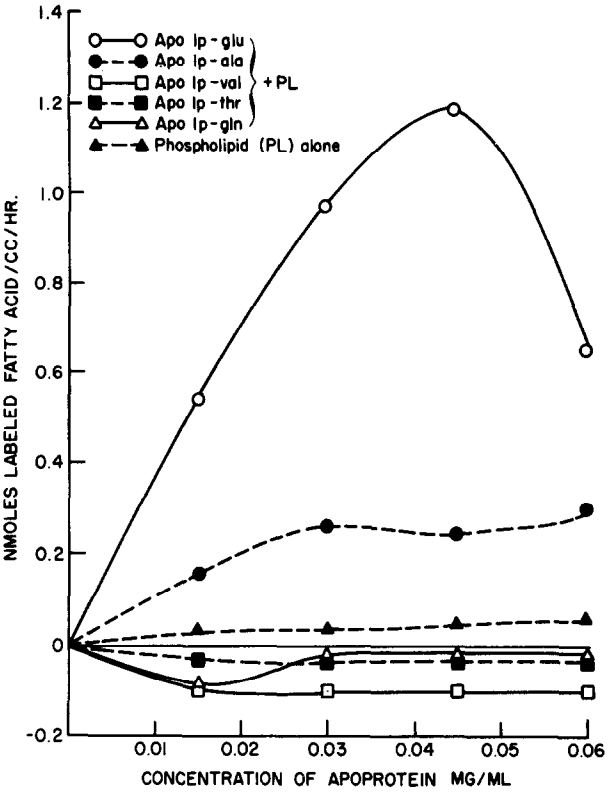

Figure 2. Effect of apolipoprotein fractions on LPL activity in the presence of phospholipid. As in Fig. 1, activity present with no added apolipoprotein or phospholipid is corrected to zero. Phospholipid is present in weight equal to the weight of apolipoprotein.

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apoLP-glu per ml of incubation medium. Stimulation was less with a slightly higher concentration (0.06 mg/ml).

DISCUSSION

These studies provide strong evidence that activation of lipoprotein lipase is a property limited only to certain apoproteins. The two major apoproteins of HDL, apoLP-thr and apoLP-gln, are incapable of activating LPL even in the presence of phospholipid, and this is equally true of apoLP-val, one of the minor constituents of apoHDL. ApoLP-ala, another minor constituent, has some ability to activate LPL but only in the presence

of phospholipid. Only apoLP-glu is able to stimulate LPL activity in the absence of phospholipid and this property is greatly enhanced in the presence of phospholipid.

The mechanism by which these apoproteins activate LPL remains unknown. The observation that phospholipid enhances activation suggests that in the presence of triglyceride, phospholipid and apoprotein, an artificial "lipoprotein" is formed in which the triglyceride is more available for LPL hydrolysis than it is in a simple emulsion. It is possible that apoLP-glu and perhaps apoLP-ala are obligatory "co-factors" for the hydrolytic step required in the normal clearing of triglyceride from the plasma.

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