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INHIBITION OF PURIFIED HUMAN POSTHEPARIN LIPOPROTEIN LIPASE BY BETA-ADRENERGIC BLOCKERS *IN VITRO*

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Abstract—We examined the effects of five beta-adrenergic blockers on the hydrolysis of phosphatidylcholine-stabilized triolein particles by purified human postheparin lipoprotein lipase (PHLpL) in order to evaluate the possible role of direct inhibition as a mechanism of drug-induced hypertriglyceridemia. The relative inhibitory potencies were observed in the following order: propranolol ⩾ pindolol > metoprolol > atenolol > nadolol. There was a positive correlation between the octanol/water partition coefficients of these agents and their inhibition of lipoprotein lipase, suggesting that hydrophobicity may be one of the major determinants for PHLpL inhibition. The amount of the beta-adrenergic blockers required to produce 50% inhibition of human PHLpL was much greater than that required to inhibit purified bovine lipoprotein lipase.

Beta blockers are widely used for the treatment of hypertension and angina pectoris. It is well known that there is an enormous number of hypertensive persons in the world. The 1984 report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure showed that more than 60 million persons are hypertensive in the United States; beta blockers have been recommended as initial treatment especially in patients less than 50 years old [1]. However, clinical studies have shown that beta blockers cause an increase of plasma triglyceride levels and a reduction in high density lipoprotein (HDL) levels [2–26] which may increase the coronary risk factors, potentially offsetting their favorable effects.

Plasma triglycerides are present mainly in chylomicrons and very low density lipoprotein (VLDL) and are hydrolyzed by lipoprotein lipase (LpL) as the first step in their metabolism. Nascent HDL is thought to be produced during hydrolysis of triglyceride-rich lipoprotein [27, 28] as well as by direct secretion from liver. Tanaka *et al.* [29] reported a decrease in LpL activity of postheparin plasma during propranolol treatment *in vivo*, suggesting that the elevation of plasma triglyceride levels caused by beta blockers may be due to an impaired hydrolysis of triglyceride-rich lipoprotein by LpL, whereas another group of investigators concluded that there was no change in postheparin plasma LpL (PHLpL) [30].

A number of beta blockers are now in widespread clinical use. Thus, it seems important to evaluate the effects of beta blockers on lipoprotein metabolism. Kubo and Hostetler [31] demonstrated direct inhibitory effects of several beta blockers on LpL from bovine milk *in vitro*, suggesting that this may be a mechanism of drug-induced diminution of triglyceride-rich lipoprotein catabolism. To examine this possibility further, we purified LpL from human postheparin plasma and examined the effects of five beta blockers on the LpL-catalyzed hydrolysis of phospholipid-stabilized triolein emulsions containing human apolipoprotein C-II.

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MATERIALS AND METHODS

Isolation of lipoprotein lipase from human postheparin plasma. LpL was isolated as described by Hayashi *et al.* [32]. Briefly, postheparin plasma was obtained from heparinized blood left in the extracorporeal circuit after open heart surgery. The plasma was stirred with heparin-Sepharose, the gel was packed into a column, and LpL was eluted with 1.5 M NaCl in sodium barbital buffer. The fraction was then subjected to chromatography with heparin-Sepharose, hydroxylapatite, and concanavalin A-Sepharose. The fraction containing LpL activity was stored at −80°.

Purification of apolipoprotein C-II. Apolipoprotein C-II (apoC-II) was prepared from plasma of human subjects with Type V and Type I hyperlipoproteinemia by Dr. S. Tajima as previously described [33]. The delipidated apolipoproteins from a lipoprotein fraction (density <1.019 g/ml) isolated by ultracentrifugation were dissolved in 6 M urea containing 10 mM Tris–HCl (pH 8.0) and applied to a Sephadex G-200 column. The apoC-II enriched fraction was applied to DEAE-cellulose and eluted with a 10–120 mM gradient of Tris–HCl (pH 8.0). The final preparation of apoC-II was dissolved in 3 M guanidine hydrochloride and stored at −20°.

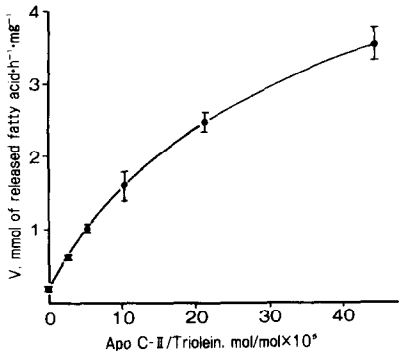


Fig. 1. Effects of apoC-II concentration on the activity of human LpL. Data in the figure are the rate of hydrolysis of eggPC stabilized triolein particles activated by various amounts of apoC-II. The incubation mixture contained 285 ng of human LpL in 0.1 M sodium phosphate buffer, pH 7.4, containing 5% fatty acid free bovine serum albumin and 0.05 M NaCl. Incubations and assays were done as described in Materials and Methods. The data have been corrected for control incubations and are the mean ± SD of triplicate experiments.

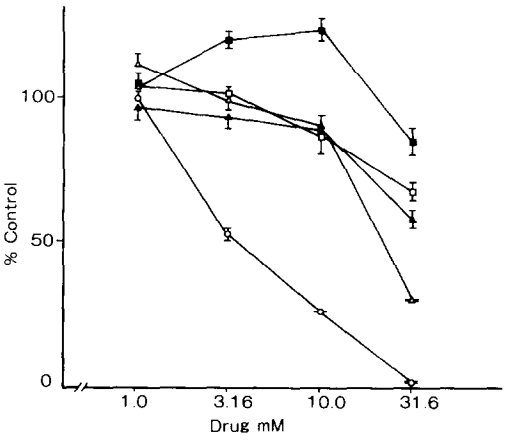


Fig. 2. Comparison of the effects of various beta blockers on the activity of human PHLpL *in vitro*. The incubation mixture contained 0.6 mM tri[1-^{14}C]olein particles stabilized with egg phosphatidylcholine with 14.3×10^{-5} mol of apo-C-II/mol of triolein, 285 ng human LpL, 0.1 M sodium phosphate buffer, pH 7.4, 5% fatty acid free bovine serum albumin and 0.05 M NaCl and beta blockers as indicated. Incubations were done at 30° for 20 min, and the ^{14}C-fatty acid released was measured. Results are expressed as mean percent (±SD) of the uninhibited control rate for triplicate experiments. Control values were 929 ± 59 μmol FFA/mg/hr. Symbols: (○)propranolol; (△) pindolol; (▲) metoprolol; (□) atenolol; and (■) nadolol.

The final apoC-II preparation was shown to migrate as a single band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and in isoelectric focusing electrophoresis.

Preparation of phospholipid-stabilized triolein particles. Triolein particles stabilized with egg phosphatidylcholine (eggPC) were prepared as previously described by Tajima *et al.* [34]. One hundred micromoles of the labeled trioleoylglycerol (tri[1-^{14}C]oleoylglycerol, sp. act. 73.5 mCi/mol), and 12.5 μmol of eggPC were taken to dryness under a nitrogen stream and suspended in 5 ml of distilled water. The suspension was sonicated under nitrogen for 1 hr on ice with a microtip of a Branson Sonifier model 200 cell disruptor at a setting of 3. The sonicated [1-^{14}C]triolein/PC emulsion was centrifuged in an AH-650 swinging bucket rotor (Sorvall OTD-65B) at 27,000 *g* for 20 min at 4°. The floating cream layer was collected, and 3-ml aliquots were used for incubation with apoC-II as indicated. ApoC-II was added to [^{14}C]triolein/PC particles. After incubation at 30° for 90 min, the mixtures were adjusted to 10% sucrose, overlaid with distilled water and centrifuged in an AH-650 rotor at 27,000 *g* for 30 min at 4°. Approximately half of the top cream layer containing the apoC-II phospholipid-stabilized [1-^{14}C]triolein was collected and used as substrate. The concentration of [1-^{14}C]triolein was determined by liquid scintillation counting, and appropriate amounts of the substrates were transferred to incubation tubes and assayed for LpL activity as described below.

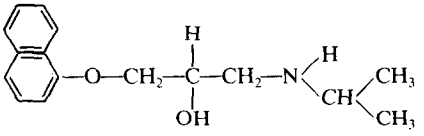
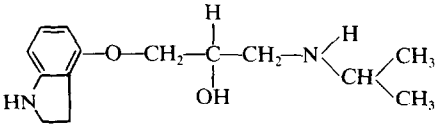
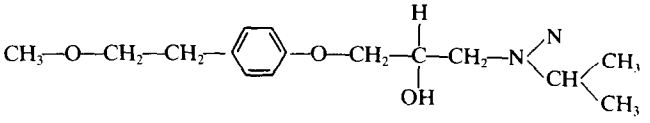
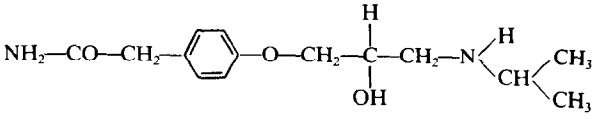
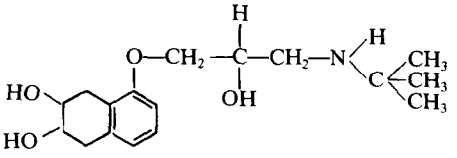
Assay of lipoprotein lipase. EggPC-stabilized tri[1-^{14}C]olein particles containing apoC-II at 14.3×10^{-5} mol/mol triolein (2.15×10^{-3} mol/mol eggPC) were prepared as noted above and used as a substrate at the indicated concentrations. The incubation medium contained triolein substrate, 0.1 M sodium phosphate buffer, pH 7.4, 5% fatty acid free bovine serum albumin, and 0.05 M NaCl in a final volume of 0.400 ml. LpL protein (285 ng) was added to start the reaction. Control incubations were done without enzyme. After incubation for 20 min at 30° in a shaking water bath, the reaction was stopped by adding 3 ml of benzene/chloroform/methanol (20/10/24) containing 0.2 mM oleic acid as a carrier and 0.1 ml of 1 N NaOH as described by Vance *et al.* [35]. Released [1-^{14}C]oleic acid in the upper phase was counted by scintillation, and the activity of LpL was calculated. Under these conditions, the generation of [1-^{14}C]oleic acid was linear for at least 30 min.

Materials. Sodium heparin from porcine intestinal mucosa and triolein were purchased from the Sigma Chemical Co., St. Louis, MO. Sepharose 4B, CNBr-activated Sepharose, concanavalin A-Sepharose, Sephedex G-200 and DEAE Sephadex A-25 were obtained from Pharmacia, Piscataway, NJ. Hydroxylapatite gel was obtained from the Clarkson Chemical Co. α-Methyl-D-glucopyranoside was from Wako Pure Chemical Industries Ltd., Osaka, Japan. Tri[1-^{14}C]olein was from Amersham, Arlington Heights, IL, and egg phosphatidylcholine was from Avanti, Birmingham, AL. Pindolol was a gift from Sandoz Pharmaceuticals Ltd., East Hanover, NJ. *d,l*-Propranolol was provided by Ayerst Laboratories, New York, NY; atenolol was provided by Stuart Pharmaceuticals Division of ICI Americas, Inc., Wilmington, DE. Metoprolol was obtained from the Ciba Geigy Corp., Summit, NJ, and nadolol was provided by E. R. Squibb, Inc., Princeton, NJ.

RESULTS

Figure 1 shows the effect of apoC-II on the activity

Table 1. The IC_{25} values, log octanol/water partition coefficients and structures of beta blockers

Drug	IC_{25}* (mM)	Log octanol/water partition coefficient†	Structure
Propranolol	2	3.65	
Pindolol	16	1.75	
Metoprolol	20	2.15	
Atenolol	24	0.23	
Nadolol	—	0.71	

* Concentration of a drug required to reduce the rate of hydrolysis by 25%.
† Adapted from Ref. 36.

of purified human postheparin LpL *in vitro* expressed as the initial reaction velocity versus the apoC-II/triolein ratio. The ratio of apoC-II/triolein needed to give half-maximal activation was 14.3×10^{-5} mol of apoC-II/mol of triolein (2.15×10^{-3} mol of apoC-II/mol of PC).

Figure 2 shows the effects of several beta-adrenergic blockers on the activity of human PHLpL at a 0.6 mM triolein concentration. The phospholipid-stabilized triolein particles contained enough apoC-II to provide half-maximal activation. Under these conditions, propranolol caused the strongest suppression of human PHLpL activity. Propranolol reduced the rate of [1-^{14}C]oleic acid release by 25% (IC_{25}) at 2 mM, and at 31.6 mM LpL activity was essentially nil. Pindolol was not greatly inhibitory at concentrations below 10 mM, but stronger inhibition was observed between 10 and 31.6 mM. The IC_{25} for pindolol was 16 mM. Metoprolol and atenolol suppressed the activity of human PHLpL moderately with IC_{25} values of 20 and 24 mM respectively. Nadolol was not effective as an inhibitor of human PHLpL, and the agent even activated LpL at low concentrations.

DISCUSSION

In the present study, we evaluated the effects of several beta blockers on purified human PHLpL and demonstrated that propranolol is an effective inhibitor. Pindolol, metoprolol and atenolol also inhibited human PHLpL but the inhibitory effects were much weaker than that of propranolol. Nadolol had no inhibitory effects on LpL activity under the conditions of this study. The inhibitory effects of these beta blockers on PHLpL seem to be closely related to the hydrophobicity of the drugs expressed by the octanol/water partition coefficient as shown in Table 1. Previous studies with purified lysosomal phospholipase A showed that beta-adrenergic blockers were inhibitory. Similar to the findings with human PHLpL, there was a strong negative correlation between the octanol/water partition coefficients and IC_{50} (the concentration of a drug required to reduce the rate of hydrolysis by 50%) values for phospholipase A inhibition [37–39].

In the present studies with purified human PHLpL, the concentrations of the various beta blockers required to produce inhibition were greater than

those reported earlier with bovine milk LpL [31]. For example, to produce a 25% inhibition of human PHLpL, about ten times more propranolol was required whereas nearly thirty times more metoprolol was needed to produce 25% inhibition of human PHLpL than that required to produce a similar degree of inhibition of bovine milk LpL.

Although the specific activity, optimum pH and molecular weight of LpL from human postheparin plasma have been reported to be similar to those of bovine milk LpL [40–42], the K_m values for apoC-II of human LpL observed in the present study were about ten times higher than those of bovine milk LpL. The ratio of apoC-II/triolein which gives half-maximal activation of bovine milk LpL is 2.1×10^{-5} mol of apoC-II/mol of triolein (3.2×10^{-4} mol of apoC-II/mol of PC) versus 14.3×10^{-5} mol of apoC-II/mol of triolein (2.15×10^{-3} mol of apoC-II/mol of PC) for human PHLpL (Fig. 1). Hayashi *et al.* [32] also reported that the apparent K_m value of human PHLpL for apoC-II is larger than that of bovine milk LpL, using triolein-gum arabic emulsion as substrate.

Propranolol has consistently been reported to raise serum triglyceride and reduce HDL-cholesterol [4–13]. Atenolol, metoprolol and pindolol have also been reported to raise serum triglyceride and reduce HDL-cholesterol levels [11–17], but there are several reports showing no significant change on the serum lipid and lipoproteins [18–20]. Nadolol, on the other hand, has been reported not to raise triglyceride levels [26]. These reports are generally consistent with the order of effectiveness of the various beta blockers on purified human PHLpL activity which we found *in vitro*. Thus, their effects on lipoprotein metabolism *in vivo* could conceivably be related to the inhibition of LpL, the key enzyme involved in hydrolysis of the triglyceride-rich lipoproteins. The amounts of these agents required for inhibition *in vitro* are greater than the plasma levels in patients [36]. However, during chronic administration, propranolol can concentrate in the phospholipid surface coat of lipoproteins and cell surface membranes achieving higher local concentrations which could lead to LpL inhibition, as discussed earlier [31, 43]. Based on our present knowledge, it seems possible that the inhibition of PHLpL observed *in vitro* may be at least partially responsible for the clinically observed tendency of some of these agents to produce hypertriglyceridemia in humans.

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