HDL SUBFRACTIONS, HDL RECEPTORS AND HDL TURNOVER

A. van Tol, F.M. van 't Hooft, T. van Gent and G.M. Dallinga-Thie

Department of Biochemistry I, Erasmus University Rotterdam
P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

INTRODUCTION

Plasma lipoproteins can be classified according to density, size or electrophoretic mobility. The latter is based on the apolipoprotein composition. Alaupovic proposed a distinction between so-called simple lipoproteins, containing only one apolipoprotein, and complex lipoproteins, containing more than one apolipoprotein. Recent publications show that isolation of plasma lipoproteins by ultracentrifugation may alter the apolipoprotein composition of the isolated lipoprotein complexes. Due to the high sheering forces and salt concentrations encountered during ultracentrifugation, part of the lipoproteins will be "stripped" from their native lipoprotein and redistributed among the various lipoprotein complexes. In this paper we analyzed rat lipoproteins by gel filtration followed by specific immunoprecipitation, describe the interaction of rat HDL* subclasses with lipoprotein receptors and discuss the consequences of receptor interaction for the turnover of the most important HDL protein: apo A-I.

MATERIALS AND METHODS

Treatment of rats

Male rats of the Wistar strain, weighing 300-400 g, were used. Where indicated 1 ml of 17α-EE, dissolved in 1,2-dihydroxypropane, was administered subcutaneously in a concentration of 4 mg/kg for five consecutive days. All animals had free access to normal rat chow and water.

Isolation and labelling of lipoproteins for in vivo turnover studies

Rat HDL was labelled by incubation of rat serum with pure rat 131I-apo A-I and subsequently isolated by gel filtration as described before. Unlabelled human LDL and rat HDL were isolated, by sequential ultracentri-
fugation of serum in a Beckman 50.2 Ti rotor, from the density intervals 1.019–1.063 g/ml and 1.063–1.21 g/ml, respectively. The labelled HDL was administered in a dose of 0.05–0.15 mg cholesterol/rat. The unlabelled HDL was administered in a dose of 5–8 mg cholesterol/rat, sufficient to raise the serum cholesterol concentration in estrogen-treated rats to physiological levels. Unlabelled LDL was injected in a dose of 10–15 mg cholesterol/rat.

Isolation and labelling of rat HDL subclasses for in vitro membrane binding studies

Total rat HDL was isolated, by sequential ultracentrifugation of serum in a Beckman 50 H rotor, from the density interval 1.05–1.21 g/ml. Rat HDL₂ was isolated from the density interval 1.05–1.125 g/ml. Apo A-I and apo E mass were measured by electroimmunoassay as described before. The ratio of apo E/A-I was 0.3 in total rat HDL and as high as 1.3 in rat HDL₂. The isolated lipoproteins were iodinated using the ICl method. The specific radioactivities of the labelled lipoproteins varied between 200 and 300 cpm/ng protein.

Preparation of kidney and liver membranes

Freshly isolated rat kidneys were homogenised in 0.15 M NaCl, using a Potter Elvehjem type B homogeniser. The membranes sedimenting between centrifugation for 30 min at 32,000 x gav and 60 min at 100,000 x gav had the highest specific activity of the plasma membrane marker enzyme 5'-nucleotidase and were used for the binding experiments. Liver membranes were isolated according to Kovanen et al.

Binding of ¹²⁵I-lipoproteins to liver and kidney membranes

The binding assay consisted of a 60 min incubation at 0°C of about 0.04 mg of membrane protein with labelled rat HDL (25 µg protein/ml), in the presence or absence of an excess of unlabelled HDL protein (1 mg/ml). The incubation medium contained 125 mM NaCl, 50 mM Tris-HCl, 1.25 mM CaCl₂, 0.25 mM EDTA and 20 mg/ml BSA. The pH was 7.5. After incubation the membrane-bound ¹²⁵I-HDL was separated from the bulk of unbound ¹²⁵I-HDL by centrifugation through a layer of 10% Ficoll, containing 50 mM Tris-HCl (pH 7.4), 1.25 mM CaCl₂ and 100 mM NaCl. Both radioactivity and protein were assayed in the membrane pellets.

RESULTS AND DISCUSSION

Lipoprotein subclasses

The chemical composition of rat HDL, isolated by ultracentrifugation from the density range 1.05–1.21 g/ml, is given in Table 1. Note the