CHARACTERISTICS OF BINDING AND TRANSPORT OF BENZ[α]PYRENE WITH BLOOD SERUM LIPOPROTEINS

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One of the principal functions of lipoproteins (LP) of the blood serum is lipid transport. However, LP also bind and transport steroid hormones [1], certain xenobiotics including therapeutic preparations [5, 9], and carcinogens [4, 10].

In the investigation described below ultracentrifugation and gel-filtration methods were used to study binding of benz[α]pyrene (BP), a xenobiotic of the methylcholanthrene series, with the blood serum LP. To establish the degree and character of the affinity of BP with the protein component of LP, the method of quenching of tryptophan fluorescence was used. Using LP of different classes as transport forms, we studied the character of distribution of labeled BP among rat organs and tissues.

EXPERIMENTAL METHOD

A solution of ^{3}H-BP (20-MBq pack) in benzene and ethanol was evaporated in a current of nitrogen, dissolved in dimethyl sulfoxide, and added to rat serum in a concentration of 0.2 MBq/ml serum. Preparative isolation of LP was carried out by ultracentrifugation in KBr solution [7] on a "Beckman L-75" centrifuge with 75 Ti rotor. The very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins thus obtained were assessed for radioactivity. Gel-filtration of LP was carried out on Sephadex G-25 ("Pharmacia").

The in vitro experiments were conducted on male Wistar rats weighing 160-180 g. LP loaded with ^{3}H-benz[α]pyrene were injected into a caudal vein of the animals in a dose of 30-50 kBq. The animals were killed 30 min after injection of LP. The tissues were homogenized and applied to GF/C glass filters ("Whatman"). Radioactivity was counted on a Mark III liquid counter.

Interaction of tryptophan-containing proteins of LP with BP was analyzed on an MPF-4 spectrofluorometer ("Hitachi") with excitation wavelength of 280 nm and emission wavelength of 340 nm. Quenching of fluorescence was recorded on the addition of aliquots of BP ($2 \cdot 10^{-4}$ M) to 0.5 ml of the solution of LP. The protein concentration in the cuvette was 0.04-0.1 mg/ml. The dissociation constant (K_d) for the LP—BP complex was calculated by the method in [3].

EXPERIMENTAL RESULTS

Addition of BP to blood serum followed by ultracentrifugation showed that most of the label (77.3%) was bound with the LP fractions and only 22.7% remained in the infranatant. The greatest radioactivity was found in the HDL fraction (40.1%), there was only half as much in VLDL (23.4%), and only 13.8% in LDL. After ultracentrifugation, the LP fractions were subjected to gel-filtration on Sephadex G-25 (Fig. 1). In all cases the peak of radioactivity of BP coincided with the elution volume of the LP fraction. The relative levels of the label in HDL, LDL, and VLDL were virtually unchanged after gel-filtration, although some decrease in radioactivity was observed due to adsorption of ^{3}H-BP by Sephadex granules.

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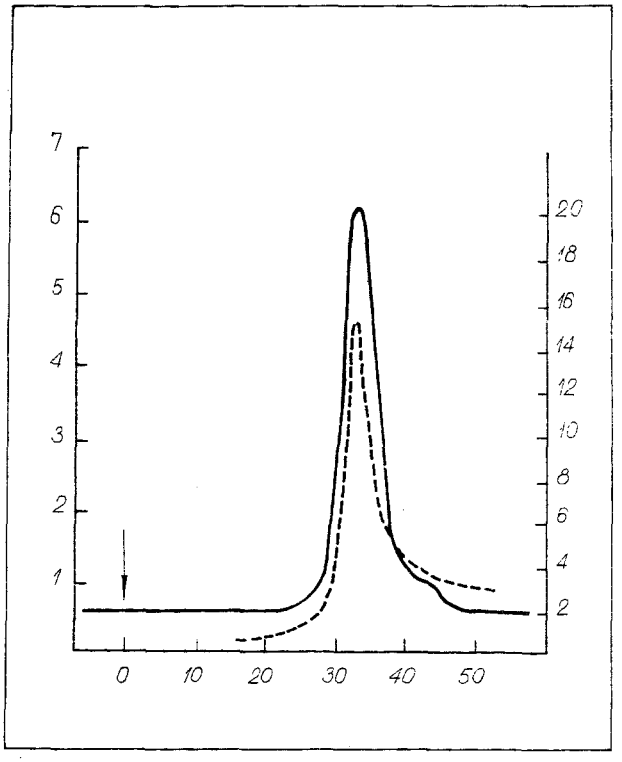

Fig. 1. Typical elution profile of LP (the one shown is for HDL) and radioactivity of ^{3}H-BP (in cpm × 10^6) on Sephadex G-25. Abscissa, volume of fractions (in ml); absorption expressed as protein (ordinate; on left, 280 nm) continuous line. Distribution of radioactivity shown by broken line.

TABLE 1. Characteristics of Binding of BP with LP of Rat Blood Serum

LP	K_d for BP – LP complex, M	Number of BP molecules to one LP particle
VLDL	$1.5 \cdot 10^{-6}$	23
LDL	$6.6 \cdot 10^{-7}$	19
HDL	$4.2 \cdot 10^{-6}$	10

Legend. Mean values of three parallel measurements are given.

To determine the character and degree of binding of BP with LP we used the method of quenching of tryptophan fluorescence [3]. The cause of the reduction of tryptophan fluorescence may be the formation of an apoprotein — BP complex, and also a change in polarity of the lipid environment of the tryptophanyl groups through interaction of the ligand with the lipid phase of the lipoprotein particle. Table 1 gives values of K_d of complexes of BP with HDL, LDL, and VLDL. The highest value of K_d was obtained for LDL. A closely similar value of K_d was obtained by an analogous method for the 5-methoxypsoralen—LDL complex [12]. For every LDL particle there were about 45 molecules of the preparation. In our experiments the number of binding sites in LP was rather lower.

To evaluate the role of LP in BP transport, the latter was injected into a caudal vein of rats. The animals were killed and radioactivity measured 30 min after injection of the preparation. When VLDL was used as the transport form the largest quantity of label was found in the liver and adrenals, followed by the kidneys and lungs (Table 2). We observed previously a high degree of absorption of protein-labeled VLDL by the liver and adrenals [2]. The use of LDL did not significantly change the distribution of label between the tissues compared with VLDL.

TABLE 2. Absorption of ^{3}H-Benz[α]pyrene by Rat Organs and Tissues 30 min After Intravenous Injection as a Component of VLDL, LDL, and HDL

Organs and tissues	Radioactityn imp./min for for 1 mg of tissue		
	VLDL	LDL	HDL
Liver	27,9±4,1	25,9±2,6	40,8±9,5
Lungs	6,6±1,6	5,9±1,0	9,6±1,1
Heart	3,1±0,1	2,51±0,2	2,3±0,7
Spleen	1,9±0,3	3,7±0,5	5,9±1,0
Kidneys	11,5±2,0	9,1±1,0	26,3±3,8
Adrenals	24,7±3,3	29,1±3,7	47,5±9,4
Thymus	4,1±0,9	3,8±0,4	6,4±1,3
Adipose tissue	4,8±0,3	5,4±1,0	6,8±0,8

Legend. In each group number of animals was five.

The high degree of absorption of BP by the adrenals when all classes of LP were used, but especially when HDL were administered, is noteworthy. This fact can easily be understood, for cholesterol of HDL is utilized in the rat adrenals for steroid hormone synthesis [8]. There is evidence that the predominant binding of ^{14}C-chlorodecane with HDL is responsible for its absorption by the adrenals and testes — organs most exposed to the toxic action of foreign compounds [11]. The presence of high radioactivity in the liver can be explained by the leading role of this organ both in synthesis and in catabolism of LP [13].

The high level of ^{3}H-BP in the kidneys must be noted, especially when HDL were used as the transport form. This is confirmed by a series of interesting investigations [6, 15], in which the most important role of the kidneys in the catabolism of apoproteins A-I, A-IV, and E, when components of HDL, was demonstrated.

It can accordingly be concluded from these results that LP are involved in the binding and oriented transport of xenobiotics of the methylcholanthrene series (BP) in the body. These facts must be taken into account during the development of ideas regarding the transport of xenobiotics into microsomes and the mechanisms of induction of enzymes of the microsomal oxidation system.

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