EFFECT OF RAT LIVER GANGLIOSIDES ON INTERACTION BETWEEN LIPOSOMES AND RAT HEPATOCYTES IN VITRO

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Phospholipid vesicles, or liposomes, have been extensively used as carriers for transporting biologically active substances in the body [3]. The effectiveness of a substance, when incorporated into liposomes, in reaching target cells largely depends on the degree of affinity of the liposomes for these cells. Attachment of ligands, which have receptors on the surface of the target cell, to the surface of liposomes is one of the most researched methods of increasing affinity of liposomes for cells. In some investigations, carbohydrate-containing compounds have been used as such ligands [6, 9]. The writers showed previously that incorporation of total gangliosides from rat liver into the liposomal membrane increases uptake of liposomes by hepatocytes [2].

This paper gives the results of investigations into the effect of individual rat liver gangliosides on interaction of liposomes with rat hepatocytes in culture.

EXPERIMENTAL METHOD

Adult male Wistar rats were used. Liver lipids were extracted by the method in [5]. The gangliosides were fractionated by thin-layer chromatography on plates (13 × 18 cm) covered with a mixture of silica-gels L and LS (Chemapol, Czechoslovakia) in the ratio of 1:1 in a solvent system of chloroform–methanol–concentrated ammonia–water (60:35:1:7). Zones containing gangliosides were located by the use of resorcinol reagent and an iodine cell. Resorcinol-positive zones were scraped into test tubes and gangliosides eluted from the silica-gel. The ganglioside content was determined by the resorcinol method [1]. Individual fractions of gangliosides were identified by comparison with bovine gangliosides.

Small single-layered liposomes were prepared from phospholipids, cholesterol, and gangliosides in the molar ratio of 10:5:1.5, with the addition of cholesteryl-[^{14}C]-oleate (Amersham International, England). The mixture of lipids was dried on a rotary evaporator, and the resulting film was covered with phosphate buffer (pH 7.4) in the ratio of 1.7 mg of lipids to 1 ml of buffer. After shaking, the emulsion was sonicated on an ultrasonic disintegrator at room temperature until the solution became clear.

Hepatocytes were isolated by the method described previously [2]. Liposomes (20 µl) were added to wells containing 2×10^5 cells, and incubated at 37°C in an atmosphere of 5% CO_2 and 95% air. After incubation of the cells with liposomes, any unbound liposomes were removed by washing the cells three times with physiological saline. Cells with bound liposomes, after lysis, were transferred into scintillation flasks and their radioactivity measured on a RackBeta counter (LKB, Sweden). Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

To study the role of individual fractions of rat liver gangliosides in interaction between liposomes made from liver phospholipids and rat hepatocytes, the gangliosides were fractionated by thin-layer chromatography. The following basic fractions of rat liver

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TABLE 1. Uptake of Liposomes (in nmoles lipid/mg protein) with Different Gangliosides by Rat Hepatocytes (M ± m)

Composition of liposomes	Incubation temperature, °C		Uptake of liposomes
	37	4	
Phospholipids	0,74±0,02	0,55±0,03	0,19±0,03
Phospholipids + total liver gangliosides	3,88±0,04	1,16±0,08	2,72±0,35
Phospholipids + G_{M1}	9,25±0,31	3,74±0,11	5,51±2,17
Phospholipids + G_{M3}	0,70±0,06	0,33±0,01	0,37±0,03
Phospholipids + G_{D1}	1,04±0,07	0,78±0,04	0,26±0,09
Phospholipids + G_{T1}	7,03±0,26	2,48±0,11	4,55±1,41

Legend. Incubation time 1 h. Number of experiments was four.

TABLE 2. Effect of Carbohydrates on Uptake of Liposomes (in %) with Different Gangliosides by Hepatocytes

Experimental conditions	Composition of liposomes		
	phospholipids + total liver gangliosides	phospholipids + G_{M1}	phospholipids + G_{T1}
Control *	100	100	100
N-acetylglucosamine	75,6	55,5	85,7
D-galactose	84,1	78,0	77,0

Legend. Uptake of liposomes in medium without carbohydrates (control) taken as 100%. Carbohydrates were added before liposomes; concentration of carbohydrates in medium 50 mM. Incubation time 30 min (4°C).

gangliosides were obtained for the work: G_{M1}, G_{M3}, G_{D1}, and G_{T1}. Uptake of liposomes with different gangliosides by hepatocytes at 37°C was shown to differ significantly (Table 1). Liposomes containing gangliosides G_{M1} and G_{T1} bound most effectively. Gangliosides G_{M3} did not affect uptake of liposomes by hepatocytes, whereas ganglioside G_{D1} increased it but not significantly.

It is nowadays considered that there are four basic mechanisms of interaction between liposomes and cells: 1) adsorption of liposomes on the cell surface; 2) fusion of the liposomal membrane with the cell membrane; 3) endocytosis; 4) lipid exchange between liposomes and cells. The principal method whereby the intraliposomal contents enter the cell is by endocytosis of the liposomes. We therefore studied the effect of individual liver gangliosides on endocytosis of liposomes by hepatocytes. To determine the fraction of liposomes undergoing endocytosis compared with the total uptake of liposomes by the hepatocytes at 37°C, uptake at 4°C was subtracted, for endocytosis does not take place at that temperature. Liposomes containing gangliosides G_{M1} and G_{T1} were found to be assimilated most effectively (Table 1).

At 4°C liposomes with gangliosides G_{M1} and G_{T1} bound most effectively with the hepatocytes. This points to the existence of specific binding sites for gangliosides on the surface of hepatocytes. We know that carbohydrate-binding proteins are present on the surface of hepatocytes [4]. In the next series of experiments we therefore studied inhibition of binding of liposomes by carbohydrates. It was found that at 4°C D-galactose inhibits binding of all the liposomes investigated by hepatocytes to a very slight degree. N-Acetylglucosamine inhibited uptake of liposomes with G_{T1} by the cells by almost 50%, and inhibited uptake of other liposomes only slightly (Table 2).

It can be concluded from these data that binding sites (receptors) on the hepatocyte surface for different gangliosides differ from one another.

The existence of receptors for particular gangliosides has been demonstrated for alveolar macrophages [8]. Gangliosides G_{M2} and G_{D1}, i.e., different gangliosides from those which we showed to have high affinity for hepatocytes, had the highest affinity in the investigation cited. Only further experiments will show whether this means that receptors specific for each type of cell exist on the cell surface for a particular ganglioside (or set of gangliosides).

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MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF THE THYROID GLAND OF INTACT AND PARTIALLY DESYMPATHIZED RATS IN DIFFERENT AGE GROUPS

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It has now been shown that changes in the pattern of function of the sympathetic nervous system causes readjustment of the morphological and physiological parameters of the endocrine organs. Data on the response of the thyroid gland (TG) to desympathization are few in number and contradictory in nature. Besides a definite reduction of TG functional activity in response to depression of the adrenergic innervation [4, 5], a significant increase in the height of the follicular epithelium and a decrease in the diameter of the follicles are observed [7], evidence of hyperfunction of the gland. Finally, in some investigations no morphological changes whatsoever could be found in the gland as a result of desympathization [2, 10]. In most investigations the question of the role of sympathetic impulses in the regulation of TG activity has been tackled mainly by means of surgical methods of desympathization which, as a rule, lead to mixed denervation of the gland. There have been few investigations of a comprehensive nature into the morphological and functional parameters of TG [2, 15], especially from the age standpoint [5, 7].

The aim of this investigation was to study morphological and functional parameters of TG in rats of different age groups, developing normally and after partial chemical desympathization.

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