Cholesterol uptake of isolated rat hepatocytes is accelerated by several kinds of phosphatidylcholine

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Uptake of cholesterol by isolated rat hepatocytes in a serum-free medium was remarkably enchanced by dispersion with several kinds of phosphatidylcholine. Of the various phosphatidylcholines tested, dilinoylphosphatidylcholine had the strongest accelerating effect, while dipalmitoylphosphatidylcholine was the weakest. The abilities to accelerate cholesterol uptake were in proportion to the content of unsaturated fatty acid in the phosphatidylcholine used. It was confirmed by electron microscopy that there is no relation between the size of the cholesterol-phosphatidylcholine complex and uptake. These data suggest that recognition of unsaturated fatty acids in phosphatidylcholine by isolated cells enhances uptake of cholesterol.

Cholesterol uptake *(Isolated rat hepatocyte)* *Phosphatidylcholine* *Unsaturated fatty acid* *Dioleoylphosphatidylcholine* *Dipalmitoylphosphatidylcholine*

1. INTRODUCTION

Cholesterol by itself is practically insoluble in water, but can be dispersed by mixing with phosphatidylcholine and sonication. We investigated the effects of phosphatidylcholine on cholesterol uptake by isolated rat hepatocytes and found that it was remarkably enhanced by dispersing cholesterol with several kinds of phosphatidylcholine. The participation of a receptor-like protein seems likely since trypsin treatment of isolated rat hepatocytes reduced its uptake activity.

Abbreviations: DLPC, dilinoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PSPC, palmitoylstearoylphosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; S-PC, soybean phosphatidylcholine; Y-PC, yolk phosphatidylcholine; HBSS, Hank's balanced salt solution

2. MATERIALS AND METHODS

2.1. *Materials*

Over 99.9% purified DLPC, DOPC, POPC, PSPC, DSPC, DPPC and S-PC were purchased from Nippon Oil & Fat (Tokyo), Y-PC from Sigma (St. Louis), [1,3-^{3}H(n)]cholesterol (55.0–60.0 Ci/mmol) from New England Nuclear (Boston) and Hepes and Scintisol EX-H (scintillation cocktail for aqueous solution) from Dojindo (Kumamoto). Collagenase for the hepatocyte preparation and other reagents of analytical grade were purchased from Wako (Osaka).

2.2. *Preparation of cholesterol solution*

An appropriate amount of a lipid mixture of tritiated cholesterol (specific activity adjusted to 0.5 Ci/mol with cold cholesterol) and each phosphatidylcholine (1:1, mol/mol) was used to form a lipid film on the walls of a flask by evaporation of an organic solvent. After adding HBSS to the flask, the film was sonicated for

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10 min using a UR-200P ultrasonic disruptor (Tomy Seiko, Tokyo). The clarified solution was then centrifuged at 10000 × *g* for 10 min to remove small lumps. The cholesterol concentration of each solution was adjusted to 0.1 mM with HBSS.

2.3. *Isolation of rat hepatocytes*

Rat hepatocyte was isolated from male Wistar rats (200–250 g) using Seglen's collagenase perfusion method [1] with a minor modification [2] and suspended in HBSS at 10^6 cells/ml. Isolated cells used for the experiments exhibited more than 85% cell viability by the trypan blue exclusion test.

2.4. *Assay of cholesterol uptake*

0.25 ml cholesterol solution was added to 0.5 ml isolated rat hepatocytes, then incubated at 37°C and gassed with a mixture of 95% O_2/5% CO_2. After 30 min, 0.5 ml of the reaction mixture was added to 0.5 ml ice-cold HBSS and centrifuged at 500 × *g* for 0.5 min with a Fisher model 59 centrifuge. The supernatant was discarded and the precipitated cells washed with HBSS 4 times. The fully washed cells were resuspended in 1.1 ml HBSS, then sonicated for 0.5 min with a Handy Sonic UR-20P (Tomy Seiko). 1 ml sonicated sample was mixed with 10 ml Scintisol EX-H and its radioactivity assayed.

2.5. *Electron microscopy of the cholesterol solution*

The cholesterol complexes were observed under an electron microscope according to Inoue et al. [3] and their diameters measured.

3. RESULTS AND DISCUSSION

As shown in fig.1, cholesterol was taken up by isolated rat hepatocytes in a time-dependent manner and phosphatidylcholines promoted cholesterol uptake by dispersing it in the following order: DLPC, DOPC, S-PC, POPC, Y-PC, DSPC, PSPC, DPPC. Cholesterol uptake with Y-PC (similar to POPC) was greater than that with DSPC or DPPC, but lesser than those with DLPC or DOPC. Cholesterol with S-PC, rich in DLPC, was more readily taken up than that with POPC or Y-PC. Fig.2 shows the effect of the phospholipid ratio of DOPC to DPPC on cholesterol uptake.

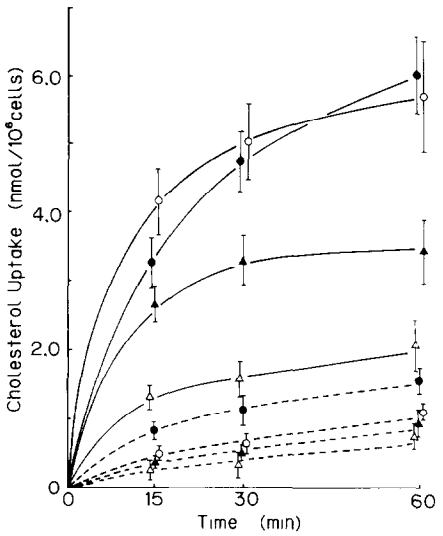

Fig.1. Effect of phosphatidylcholine on cholesterol uptake by isolated rat hepatocytes. Cholesterol was dispersed with DLPC (○—○), DOPC (●—●), S-PC (▲—▲), POPC (△—△), Y-PC (●---●), DSPC (○---○), PSPC (▲---▲), and DPPC (△---△). Preparation of cholesterol solution with several kinds of phosphatidylcholine and its uptake by isolated rat hepatocytes were carried out as described in section 2. Vertical bars indicate means ± SD. All experiments were repeated at least 5 times with different cell preparations.

The amount of cholesterol taken up was in proportion to that of DOPC in the cholesterol solution, while the amount of unsaturated phospholipids in the solution was in direct proportion to that of cholesterol taken up. Thus, the uptake rate was closely related to the fatty acid composition of the phosphatidylcholine used for dispersion. An increment of unsaturated fatty acids in the phosphatidylcholine resulted in an increase in cholesterol uptake.

To elucidate whether the acceleration of cholesterol uptake by unsaturated phospholipids was true uptake or merely an affinity to the hepatocyte surface, subcellular fractionation of hepatocytes was carried out. After incubating hepatocytes and the cholesterol solution with DOPC, about 70% of the cholesterol taken up was found in the cytoplasm, especially in lysosomes and the soluble fraction, the remainder being in the nuclear and membrane fractions. The effect of temperature on uptake was also investigated and it

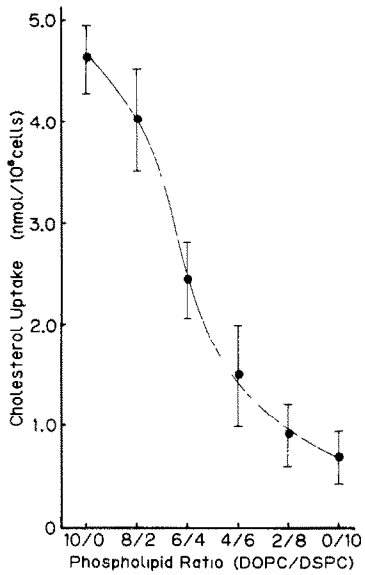

Fig.2. Effect of phospholipid ratio of DOPC to DPPC on cholesterol uptake. Preparation of cholesterol solution containing the indicated ratio of phosphatidylcholine and its uptake by isolated rat hepatocytes were carried out as described in section 2. Vertical bars indicate means ± SD. All experiments were repeated at least 5 times with different cell preparations.

Table 1

Size of cholesterol-phosphatidylcholine complexes

Cholesterol with	Size of complexes (nm ± SD)
DLPC	72.1 ± 17.2
DOPC	97.1 ± 47.6
S-PC	111.6 ± 20.0
POPC	77.7 ± 19.2
Y-PC	105.5 ± 25.6
DSPC	94.3 ± 38.9
PSPC	88.4 ± 24.7
DPPC	112.1 ± 37.2

Cholesterol solution was prepared as described in section 2. Sizes of cholesterol-phosphatidylcholine were measured with more than 200 particles in a definite view of the electron micrograph

was confirmed that a low temperature (4°C) completely inhibited uptake. Thus cholesterol was not bound to the cell surface but taken up into the cytoplasm.

It is suspected that when cholesterol is dispersed with phosphatidylcholine, a cholesterol-phosphatidylcholine complex is formed in the aqueous phase and that this complex forms single unilamellar vesicles since the method for cholesterol dispersion is a modification of liposome preparation [4]. Therefore, it is possible that the size of the complex in the solution affects uptake, since it was reported in liposome experiments that the size of the liposome may affect uptake [5,6]. In our experiment, electron microscopy showed that many cholesterol-phosphatidylcholine complexes formed single unilamellar vesicles. In addition, measurement of their size showed no considerable difference (table 1). Thus we were not able to determine any correlation between the size of complex and uptake rate, i.e. the effect of phosphatidylcholine on cholesterol uptake does not seem to be caused by the size of the complex, but rather by the unsaturated fatty acid content in the phosphatidylcholine. Gregoriadis and Senior [4] have investigated the effects of composed phospholipids on liposome clearance in mice and concluded that "there is no obvious relationship between clearance rates of liposomal dye and physical characteristics of the component phospholipid". However, our data indicate a strong relationship between cholesterol uptake and the structural characteristics of the component phospholipid used for dispersing it. In our experiments cholesterol also formed single unilamellar vesicles, as described above. The reason for this discrepancy is that their liposome clearance test in vivo was affected by many factors, such as stability of liposomes, effect of blood, and uptake by various organs. In fact, the data of Senior et al. [7] indicate that hepatic uptake of Y-PC liposomes was greater than that of DSPC liposomes in vivo, which corresponds to our data on isolated rat hepatocytes.

This uptake must involve some protein factor (carrier protein?) on the cell surface since tryptic digestion of hepatocytes impaired the incorporation of cholesterol in a dose-dependent manner. Inhibition by trypsin was not due to reduction of cell viability since the conditions for tryptic treatment were the same as for the digestion of lectin-like receptors reported by Kolb et al. [8].

Thus, this uptake may be a kind of carrier-mediated endocytosis. Our findings suggest that

the differences in cholesterol uptake must be due to fatty acid recognition in phosphatidylcholines on the hepatocyte cell membrane.

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