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# NONRECEPTOR INTERACTION OF LOW DENSITY LIPOPROTEINS WITH HUMAN FIBROBLASTS

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According to views on receptor-mediated endocytosis, low-density lipoproteins (LDL), on penetrating into the cell, are transported in "coated" vesicles into lysosomes, where degradation of the protein and hydrolysis of the cholesterol esters of the LDL take place [3]. If the LDL concentrations in the extracellular medium exceed the limits of receptor interaction, they may be taken up by the cell even without receptor participation. This method of LDL catabolism, independent of controlled receptors, may assume great importance in the development of hypercholesterolemia and of atherosclerotic changes in the arterial wall.

The object of the present investigation was to study catabolism of [$^{125}$I]-protein- and [$^{3}$H]-lipid-labeled LDL by human fibroblasts under conditions when the participation of specific receptors for LDL was suppressed.

## EXPERIMENTAL METHOD

Experiments were carried out on cultures of human embryonic lung fibroblasts at the 10th passage. The cells were cultured in Pavitskii flasks under conditions of restricted receptor-mediated LDL uptake by the cells. For this purpose fibroblasts were used in the experiment after monolayer formation in 10% bovine serum made up in Eagle's medium (without preincubation of the cells in medium not containing lipoproteins), and high concentrations of LDL (50-100 μg/ml as protein), suppressing receptor synthesis, were used. The duration of the experiments (24 h) also was much longer than the time required to inhibit synthesis of LDL receptors (with LDL present in the medium). To study the rate of intracellular LDL degradation under conditions of nonreceptor interaction, the "pulse labeling" method was used. In these experiments fibroblasts were incubated in medium containing [$^{125}$I]-LDL in a concentration of 100 μg/ml as protein for 24 h, after which the medium was poured off and the cell monolayer was washed with the solution five times [2], after which the cells were flooded with medium containing unlabeled LDL in the same concentration. In parallel experiments the cells were flooded with medium containing $^{125}$I-labeled LDL (100 μg/ml), and 24 h later extra-

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TABLE 1. Radioactivity of [$^{14}$C]-Lipids (in cpm) of LDL of Incubation Medium and Fibroblasts after Incubation with [$^{14}$C]-, Lipid-Labeled LDL for 24 h

| Material | Intracellular radioactivity, cpm/$10^6$ cells | PL | CH | TG | ECH |
|---|---|---|---|---|---|
| LDL of medium | — | 483±71 (8,23) | 386±51 (6,65) | 2641±198 (45,33) | 2292±116 (39,60) |
| Fibroblasts | 762±81 | 139±27 (18)* | 52±20 (7) | 229±21 (30)* | 338±16 (44) |
| Fibroblasts + chloroquine | 1041±113 | 86±13 (8) | 93±9 (9) | 316±12 (30)* | 543±16 (52)* |

Legend. Specific radioactivity of LDL was 2.3 × $10^5$ cpm/mg protein. Mean results of five experiments. Mean percentage distribution of radioactivity given in parentheses. *) Differences in percentage distribution of radioactivity relative to distribution in medium are significant ($P < 0.05$). PL) Phospholipids, CH) nonesterified cholesterol, TG) triglycerides, ECH) esterified cholesterol.

and intracellular radioactivity was analyzed. The radioactivity of the TCA-supernatant of the medium was used as indicator of the rate of degradation of the [$^{125}$I]-LDL. The radioactivity of the protein was determined after delipidization of the TCA-residue of the cells or proteins of the medium with choloroform–methanol (2:1). To study interaction with cells containing [$^3$H]-lipid-labeled LDL, fibroblasts after monolayer formation were flooded with medium containing [$^3$H]-LDL (50 μg/ml) and were incubated for 24 h. After incubation the medium was poured off and the cells washed five times with buffer solution [2]. The cell lipids and LDL of the incubation medium were extracted with choloroform–methanol (2:1) and, after separation of the lipids by thin-layer chromatography, their radioactivity was measured. Distribution (in %) of radioactivity of protein of [$^{125}$I]-LDL and their degradation products in the cells and incubation medium 24 h after replacement of medium containing labeled [$^{125}$I]-LDL by medium containing unlabeled LDL was as follows: cell protein 34.0 ± 1.7%; TCA supernatant 19.1 ± 1.1%; TCA residue of the medium (mean experimental data) 46.3 ± 2.6%. In parallel experiments chloroquine (50 μM) was added to the medium to inhibit lysosomal enzyme activity. LDL (d = 1.019-1.055 g/ml) were isolated from human blood plasma taken from blood donors, and from blood serum of a rabbit with experimental hypercholesterolemia, by the ultracentrifugation method [4]. Human LDL were labeled for protein-$^{125}$I by the iodine monochloride method [5]. LDL labeled for [$^3$H]-lipid were separated by ultracentrifugation of blood serum from a rabbit with experimental hypercholesterolemia, into which 5 mCi of [$^3$H]-oleic acid, solubilized with Tween-20 in physiological saline, was injected intravenously 1 h before the blood was taken. Protein was determined by Lowry's method [6].

## EXPERIMENTAL RESULTS

The experiments showed that the rate of degradation of [$^{125}$I]-LDL by fibroblasts, in incubation medium containing lipoproteins in a concentration of 100 μg/ml as protein, averaged 250.5 ± 13 ng/h/$10^6$ cells (h = 6). The rate of degradation of [$^{125}$I]-LDL remaining in the cells after replacement of the medium containing [$^{125}$I]-LDL by medium containing unlabeled LDL (100 μg/ml) averaged 1.12 ± 0.09 ng/h/$10^6$ cells, or about 1/200th of the rate of degradation in incubation medium containing [$^{125}$I]-LDL in the same concentration. The total radioactivity of medium and cells (32,349 ± 1212 cpm) at the end of the experiment was taken as 100. This radioactivity corresponds to the initial intracellular radioactivity immediately after the change of medium. The specific radioactivity of LDL was 23 × $10^6$ cpm/mg protein.

The appearance of radioactivity in the TCA-precipitated fraction after replacement of the labeled LDL of the medium by unlabeled is evidence that during nonreceptor interaction some of the LDL of the intracellular pool, present in the composition of uncoated vesicles, can

enter the extracellular space by exocytosis (regurgitation). Lowering the intracellular concentration of [$^{125}$I]-LDL in these experiments, which occurred after replacement of the labeled LDL of the medium by unlabeled, explains the cause of the much lower rate of degradation of [$^{125}$I]-protein of LDL under these conditions than in the presence of [$^{125}$I]-LDL in the medium.

The results of experiments to study uptake of [$^{14}$C]-lipid-labeled LDL by human fibroblasts are given in Table 1. As Table 1 shows, the radioactive LDL which were used contained label mainly in TG and ECH. As a result of incubation of these LDL for 24 h with fibroblasts, the latter took up part of the LDL in the form of intact particles, as shown by the distribution of radioactivity in the cell (the overwhelming majority of it, just as in LDL of the medium, belonged to TG and ECH). At the same time, there were some differences in the distribution of radioactivity in the cell compared with its distribution in lipoproteins of the medium. The percentage of radioactivity belonging to TG was reduced in the cells, evidently because of their partial oxidation, and there was a corresponding increase in the percentage of radioactivity belonging to PL and ECH.

Under conditions of inhibition of lysosomal enzymes by chloroquine, hydrolysis of TG and ECH was inhibited to some extent. It was on their account that the total intracellular radioactivity rose (by 1.4 times compared with the experiments without addition of chloroquine). However, the radioactivity of CH in the cell did not fall, as might be expected, but on the contrary it rose, and on the whole the ratio between the radioactivities of ECH and CH in the cell decreased in the presence of chloroquine. The absence of any clear general rule for the change in distribution of radioactivity in the cell in the experiments with and without chloroquine can perhaps be explained on the grounds that during nonreceptor uptake of LDL by the cell degradation of the lipid components of the lipoproteins took place at a low rate, while exocytosis of LDL was in progress. It can tentatively suggested that the ability of the cell to accumulate large quantities of native LDL under conditions of nonreceptor interaction (in the composition of uncoated vesicles) was due to the absence of coupling between the processes of lipoprotein uptake and transport into lysosomes.

Hence, unlike receptor-mediated uptake and catabolism of LDL, when receptor activity of the fibroblasts is suppressed the uncontrolled (nonreceptor) uptake of LDL is accompanied by exocytosis of these lipoproteins from the cell into the surrounding medium. Under these circumstances only part of the LDL taken up by the cells is catabolized in the lysosomes. On the whole the results of these experiments agree with the principal rules governing plasmalemmal transport [1, 7, 8].

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