8. S. Nakamura, Brain Res., 93, 140 (1975).
9. Z. Olson and K. Fuxe, Brain Res., 43, 289 (1972).
10. J. C. Rose and W. F. Ganong, Current Developments in Psychopharmacology, No. 4, New York (1976), p. 87.
11. U. Ungerstedt, Acta Physiol. Scand., Suppl. 367, 1 (1971).
12. P. Vanhoutte, Trends Pharm. Sci., 3, 370 (1982).
13. J. Vernikos-Danellis, K. Kellar, D. Kent, et al., Ann. N.Y. Acad. Sci., 297, 518 (1977).
14. T. Yao, S. Andersson, and P. Thoren, Brain Res., 244, 295 (1982).

EFFECT OF PROTEOLYSIS OF LOW-DENSITY SERUM LIPOPROTEINS ON THEIR INTERACTION WITH MACROPHAGES

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KEY WORDS: lipoproteins; atherogenesis.

The writers previously postulated, on the basis of changes observed in the structural stability of low-density lipoproteins (LDL) during treatment with pepsin or aortic cathepsin D, that enzymatic modifications may lead to potentiation of the atherogenic properties of LDL [1, 2]. We know that treatment of LDL with trypsin causes an increase in their binding with aortic glycosaminoglycans [5] and to an increase in degradation by fibroblasts of patients with hereditary hypercholesterolemia [7]. Limited proteolysis of LDL with pepsin facilitated their binding with fibronectin [4]. In recent years, to assess the atherogenicity of lipoproteins, their interaction with macrophages has been studied [6]. Popov [3], who used this model, found more rapid uptake of trypsin-treated LDL by macrophages.

In the present investigation uptake and degradation of LDL by macrophages were studied after their limited hydrolysis by pepsin — an analog of tissue cathepsin D.

EXPERIMENTAL METHOD

LDL ($1.019 < d < 1.063$ g/ml) were isolated from serum of healthy blood donors by ultracentrifugation [10]. Iodination of LDL with ^{125}I was carried out by the iodine monochloride method [14] and the reaction products were removed by dialysis. Specific radioactivity of the resulting preparations was 80-150 cpm/ng protein. Hydrolysis of native and radioiodinated LDL was carried out as described previously [1]. Low-molecular-weight products of proteolysis of LDL by pepsin were removed by gel-filtration on a column with Sephadex G-75. The efficiency of removal of low-molecular-weight products was estimated by precipitation with 10% TCA and measurement of radioactivity and of protein in the material dissolved in TCA. Protein in the samples was determined by Lowry's method [13]. To obtain human lipoprotein-deficient serum (LDS) preparative ultracentrifugation at a density of 1.21 g/ml was used [11]. The isolated LDS was dialyzed against 0.15 M NaCl in 0.02 M Na-phosphate buffer, pH 7.4, sterilized by filtration through a filter with pore diameter of 0.22 μ, and kept at −20°C.

To obtain macrophages, 5 ml of a 3% solution of peptone in Hanks' medium was injected into noninbred male albino mice. The peritoneal cavity of the mice was flushed out 2 days later with 7 ml of medium A (Eagle's medium with 10% bovine serum, 290 μg/ml of L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin), containing 85 U/ml of heparin. The peritoneal cells were collected by centrifugation of the washings (900 rpm, 5 min, 4°C) and the cells were washed once with medium A, resuspended in the same medium, and diluted to a density of 10^6-$2 \cdot 10^6$ cells/ml. Aliquots of the suspension (2 ml) were poured into plastic Petri dishes (40 × 10 mm) and incubated in an atmosphere of 5% CO_2 and 95% O_2 at 37°C. Each dish was washed twice after 2 h with 2 ml of warm medium A and incubated overnight at 37°C in an atmosphere of 5% CO_2 and 95% O_2.

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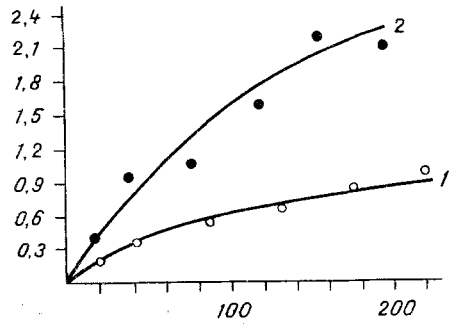

Fig. 1

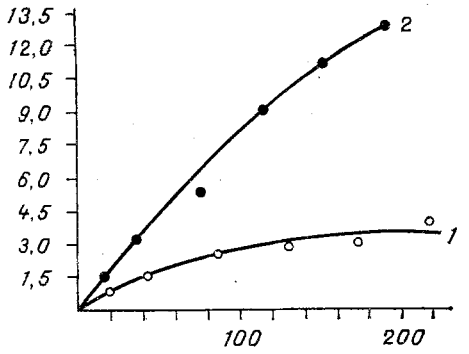

Fig. 2

Fig. 1. LDL uptake by cultured macrophages. Abscissa, concentration (in μg protein/ml) of ^{125}I-LDL, native (1) and after limited hydrolysis with pepsin (2); ordinate, uptake of LDL by cells (in μg/ml cell protein). Each point shows average of two parallel tests.

Fig. 2. Degradation of LDL by cultured macrophages. Ordinate, degradation of LDL (in μg/mg cell protein) by cells. Remainder of legend as to Fig. 1.

Before the experiments the cells were washed twice with 2 ml of medium A (without serum), after which 1 ml of medium A with LDS (3% by volume) and the necessary quantity of radioiodinated LDL were added. The cells were incubated with LDL at 37°C for 5 h in an atmosphere of 5% CO_2 and 95% O_2. After the end of incubation samples of culture medium were taken into test tubes with TCA to determine degradation (see below), the dishes were washed [9] three times with 0.15 M NaCl (2 ml each time) in 0.05 M Tris-HCl buffer, pH 7.4, washed twice with the same solution with the addition of albumin (2 mg/ml), and finally twice with the same solution but without albumin. The cells were dissolved in 1 ml of 0.1 M NaOH overnight at room temperature and their radioactivity was determined on a gamma-counter. After measurement of radioactivity, the protein concentration was determined in the same samples. The quantity of ingested LDL was calculated from the results of measurement of radioactivity of the cells and specific radioactivity of the added LDL preparations. The value thus obtained reflected not only the binding of LDL with the cell surface, but also penetration of LDL inside the macrophages. The results were expressed in micrograms ingested protein of LDL per milligram cell protein.

To determine degradation of LDL the culture medium was collected after incubation and precipitated with 10% TCA (final concentration); the supernatant was removed by centrifugation at 1000g for 15 min, incubated with H_2O_2 in the presence of KI, and extracted with chloroform to remove free iodine, after which the radioactivity of the aqueous phase was measured [8]. Petri dishes not containing cells served as the control. Lipoproteins were added to them, after which they were incubated and treated in the same way as the experimental samples. The results were subtracted from those of the experimental samples. The final results were expressed in micrograms degraded LDL protein per milligram cell protein.

EXPERIMENTAL RESULTS

Dependence of ingestion of ^{124}I-LDL (both native and treated with pepsin) by macrophages at 37°C on their concentration in the culture medium is shown in Fig. 1. In the experiments with partially hydrolyzed LDL the degree of proteolysis was 8-10%. The writers showed previously that with this degree of proteolysis, appreciable aggregation and denaturation of LDL does not yet take place [1]. It will be clear from Fig. 1 that ingestion of LDL by the cells was considerably increased after their partial hydrolysis by pepsin. The course of the uptake curves (of native and enzymatically modified LDL) is evidence that there are two ways of lipoprotein uptake. One is characterized by high affinity and the presence of saturation, the other by low affinity and absence of saturation. Partially hydrolyzed LDL interact with higher affinity with macrophages than native LDL. This difference applies to pathways both with and without the presence of saturation.

The increased uptake of LDL, partially hydrolyzed by pepsin, was accompanied by their more intensive degradation by macrophages, as shown by the accumulation of degradation products of ^{125}I-LDL, soluble in TCA, in the culture medium.

The increase in uptake and degradation of pepsin-modified ^{125}I-LDL by macrophages may indicate their greater atherogenicity. This conclusion is supported by the parallel, described in the literature, between protein degradation processes and intracellular accumulation of cholesterol esters in macrophages incubated with acetylated LDL [9].

The use of pepsin to modify LDL is convenient in the respect that it is inactivated by simple neutralization of the medium; consequently, there is no need to remove it before adding the LDL to the cell culture. At the same time, as had already been stated, pepsin is a close analog of cathepsin D. We know that cathepsin D acts on LDL [2] and may modify lipoproteins when cells are destroyed as a result of inflammatory and other pathological processes [12].

The results are thus evidence in support of the view that enzymatically modified LDL possess higher atherogenicity than native.

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LITERATURE CITED

1. I. M. Karmanskii, Vopr. Med. Khim., 23, 530 (1977).
2. I. M. Karmanskii, A. L. Pichugin, and V. O. Shpikiter, Vorp. Med. Khim., 25, 738 (1979).
3. A. V. Popov, in: High-Density Lipoproteins and Atherosclerosis [in Russian], Moscow (1983), p. 159.
4. T. M. Chulkova, Byull, Éksp. Biol. Med., No. 9, 301 (1985).
5. M. Bihari-Varga, S. Goldstein, D. Lagrange, and E. Gruber, Int. J. Biol. Macromol., 4, 438 (1982).
6. M. S. Brown and J. L. Goldstein, Annu. Rev. Biochem., 52, 223 (1983).
7. T. E. Carew, M. J. Chapman, S. Goldstein, and D. Steinberg, Biochim. Biophys. Acta, 529, 171 (1978).
8. J. L. Goldstein, S. K. Basu, G. Y. Brunschede, and M. S. Brown, Cell, 7, 85 (1976).
9. J. L. Goldstein, Y. K. Ho, S. K. Basu, and M. S. Brown, Proc. Natl. Akad. Sci. USA, 76, 733 (1979).
10. R. J. Havel, H. A. Eder, and J. H. Bragdon, J. Clin. Invest., 34, 1345 (1955).
11. B. L. Knight and A. K. Soutar, Biochem. J., 202, 145 (1982).
12. D. S. Leak and T. J. Peters, Biochim. Biophys. Acta, 664, 108 (1981).
13. O. H. Lowry, N. J. Rosebrough. A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
14. A. S. MacFarlane, Nature, 182, 53 (1958).

ANTENATAL PREVENTION BY LITHIUM HYDROXYBUTYRATE OF DEVELOPMENTAL CHANGES INDUCED IN THE RAT BRAIN BY SERUM FROM SCHIZOPHRENIC PATIENTS

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KEY WORDS: lithium hydroxybutyrate; serum from schizophrenic patients; antenatal period; rat brain.

The antenatal effects of most drugs are generally assessed from the standpoint of their possible adverse effects on the pregnant mother and fetus. Data on the stimulating effect of lithium hydroxybutyrate (LHB), administered in the antenatal period, on ontogeny of the CNS [1], have suggested the possible use of LHB in the prevention of dysfunction and dysregulation of maturation of the CNS associated with the action of endotoxins and exotoxins.

The specific disturbance of metabolic mechanisms in schizophrenia is responsible for the appearance of factors of toxic nature in the blood [3, 14]. The so-called "maternal effect" — the significant increase in the number of children affected with schizophrenia in the progeny of an affected mother compared with the frequency of affected children in fami-

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