EFFECT OF CYCLOHEXIMIDE ON LYSOSOMAL PROTEINASE ACTIVITY IN VARIOUS RAT ORGANS

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Recent investigations have conclusively demonstrated the leading role of lysosomal proteinases in intracellular protein catabolism and also their participation in specific functions connected with organic proteolysis reactions [3, 10]. Meanwhile the character of interrelations maintaining dynamic equilibrium of protein synthesis and breakdown processes in the body is still far from clear. Since, on the one hand, correlation has been established between the level of lysosomal proteolytic activity and the rate of breakdown of intracellular proteins and, on the other hand, direct dependence of the intensity of protein biosynthesis on total protein degradation has been discovered [1, 8], the question of the role of lysosomal proteinases in the realization of regulatory mechanisms maintaining a stable relationship between protein biosynthesis and catabolism in the cell must be considered.

The aim of the present investigation was accordingly to study the character of changes in activity of lysosomal hydrolases of the rat liver, kidney, and spleen under conditions where protein synthesis was inhibited to a varied degree.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 100-150 g, divided into five groups. Rats of the four experimental groups received cycloheximide by intraperitoneal injection in a dose of 0.1 mg/kg once, twice, or three or four times at intervals of 6 h respectively. Control animals received an injection of the equivalent volume of physiological saline. The rats were killed 6 h after the last injection. Homogenates of liver, kidneys, and spleen were prepared by the standard method [5] in a Potter–Elvehjem glass homogenizer (1200 rpm, 90 sec), using 0.25 M sucrose, pH 7.4, containing 1 mM EDTA as the suspending medium. All procedures were carried out at 0-2°C.

Total activity of 7 lysosomal enzymes (cathepsins A, B, C, and D, aryl sulfatases A and B, and β-galactosidase) was determined in tissue homogenates treated beforehand with Triton X-100.

Activity of cathepsins B and C was determined spectrofluorometrically by the method in [12] in our own modification, using benzoyl-DL-arginine-β-naphthylamide and glycyl-L-phenalanine-β-naphthylamide respectively as the substrates. Activity of cathepsins A and D was determined by the method developed previously [2], based on spectrofluorometric determination of free ε-NH_2-groups in amino acids liberated by hydrolysis of the corresponding substrates (hemoglobin for cathepsin D and N-carbobenzoyl-L-glutamyl-L-tyrosine for cathepsin A). Activity of aryl sulfatases A and B and of β-galactosidase was determined spectrophotometrically, using the dipotassium salt of 2-hydroxy-5-nitrophenyl sulfate (from Sigma, USA) and o-nitrophenyl-β-D-galactopyranoside (from Chemapol, Czechoslovakia) respectively as substrates [4, 6, 7].

Enzyme activity was expressed in micromoles of products formed by hydrolysis of the substrates per minute per gram wet weight of tissue or per gram protein. The protein concentration was determined by the method in [11] after preliminary solution of the protein in 0.25 M NaOH.

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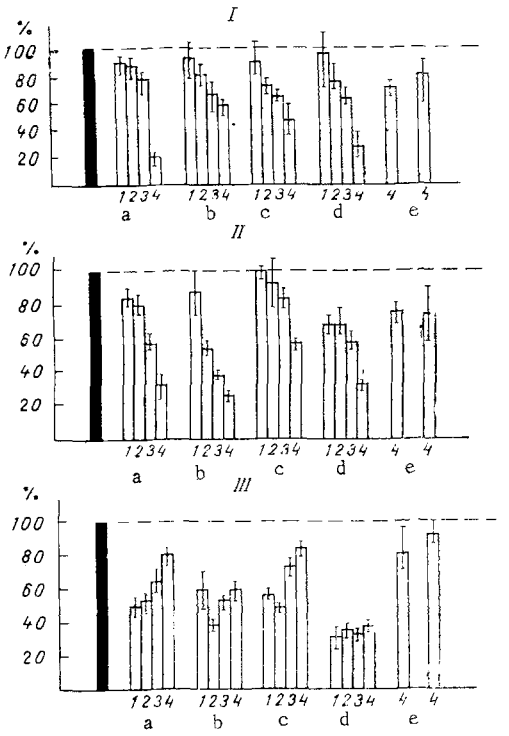

Fig. 1. Activity of lysosomal enzymes in liver, kidneys, and spleen of rats after injection of cycloheximide (in percent of control). I) Liver, II) kidneys, III) spleen. a, b, c, d) Cathepsins A, B, C, and D respectively; e, f) aryl sulfatases A and B and β-galactosidase. 1, 2, 3, and 4) Doses of cycloheximide injected (0.1, 0.2, 0.3, and 0.4 mg/kg respectively).

EXPERIMENTAL RESULTS

Injection of cycloheximide led to a significant fall in activity of all enzymes studied in the liver, kidneys, and spleen of the animals (Fig. 1). The marked dose-dependent effect will be noted: An increase in concentration of cycloheximide was accompanied by a greater decrease in lysosomal proteinase activity, especially in the liver and kidneys. For instance, whereas the minimal (0.1 mg/kg) dose of cycloheximide caused a very small decrease in enzyme activity, with the exception of cathepsin D in the kidneys whose activity was lowered by 32%, a subsequent increase in the dose of cycloheximide caused a progressive fall in proteinase activity. In particular, with a dose of 0.2 mg/kg cycloheximide activity of cathepsins A, B, and D in the liver fell on average by 10-15% and activity of cathepsin C fell by 22%. In the kidneys, activity of cathepsin B fell sharply (by 45%). An increase in the total dose of cycloheximide to 0.4 mg/kg led to a decrease in activity of all proteinases – by 64, 33, 42, and 57% respectively in the liver and by 67, 75, 42, and 66% in the kidneys for cathepsins A, B, C, and D.

Unlike in the liver and kidneys, activity of most of the enzymes tested fell sharply in the spleen after injection of the smallest dose of cycloheximide; the levels of their activity, moreover, did not change appreciably with an increase in dose of the drug. The exception was cathepsin C, whose activity was reduced by 54% after injection of the minimal dose of cycloheximide, but an increase in its dose caused no further decline in activity of this enzyme. Conversely, when cycloheximide was injected in doses of 0.3 and 0.4 mg/kg cathepsin C activity in the spleen fell by a lesser degree to 73 and 85% of the control. By contrast with proteinases, activity of other lysosomal enzymes, notably aryl sulfatases A and B and β-galactosidase, fell by a much lesser degree (by not more than 10-25% after the maximal dose of cycloheximide).

The reaction of lysosomal enzymes to injection of cycloheximide is thus characterized by a decrease in activity of all acid hydrolases studied, with a more marked decrease in proteolytic enzyme activity.

So far as the sharp (compared with other hydrolases) decline in lysosomal proteinase activity in the spleen even after the minimal dose of cycloheximide is concerned, this is evidently associated with the particularly

rapid exchange of cell populations in this organ. The results suggest that the functional state of the lysosomal proteolytic system is indissolubly connected with all stages of intracellular protein metabolism, and the decline in lysosomal cathepsin activity during inhibition of protein biosynthesis may be a regulatory mechanism, aimed at preserving the protein reserves of the cell. Since cathepsins have the shortest half-life of all the lysosomal hydrolases [9], there is reason to suppose that participation of lysosomal proteinases in the maintenance of stable equilibrium between protein biosynthesis and catabolism is a genetically determined mechanism.

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ISOLATION OF TISSUE-SPECIFIC INHIBITORS OF DNA SYNTHESIS (CHALONES) FROM RAT LIVER

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Several methods of obtaining and purifying chalones from mammalian liver homogenate have been described [6-9], but none of them is universally suitable and can yield the inhibitor in a pure form. The molecular weight of such preparations as have been obtained varies within wide limits (from 1000 to 40,000 daltons), and their preliminary characteristics are not identical. In the investigation described below an attempt was made to isolate and purify G_1-chalone from an aqueous extract of normal rat liver, possessing chalone activity, and partially purified by alcoholic fractionation. The writers showed previously [2] that such an extract is heterogeneous in composition and contains several inhibitors of DNA synthesis and mitosis whose action is exhibited in an in vivo system.

EXPERIMENTAL METHOD

Water-soluble liver proteins from noninbred albino rats obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR, precipitated with ethanol in a saturation of between 55 and 81%, were used as the original material [2, 3]. After lyophilization the resulting chalone-containing liver extract was fractionated on Sephadex G-75 (column 19 × 460 mm), and the sample applied contained 100-300 mg protein in 4 ml water.

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