Biochemical Pharmacology, Vol. 28, pp. 1015–1016.
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0006-2952/79/0401-1015 $02.00/0

INCREASED LYSOSOMAL ENZYME ACTIVITIES IN TISSUES OF RATS SUFFERING FROM CHLORPHENTERMINE INDUCED LIPIDOSIS

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(*Received* 27 *April* 1978; *accepted* 5 *October* 1978)

Abstract—The activities of four lysosomal enzymes, β-D-glucuronidase, β-D-galactosidase, α-D-mannosidase and acid phosphatase, were determined in tissue homogenates of rats suffering from drug-induced lipidosis. The lysosomal storage disease was produced by daily oral administration of 30–40 mg chlorphentermine/kg b.wt. for 12 weeks. The enzyme activities were found either increased or unchanged. A rise of about 2.5 fold was displayed by the β-D-glucuronidase in liver and kidneys and of 1.85 fold by the α-D-mannosidase in kidneys. The activity of the acid phosphatase was only slightly enhanced in the adrenals. The finding that a drug-induced lysosomal lipid storage is accompanied by an increase of some enzyme activities, corresponds to observations made on human cases of inherited storage diseases, and to experimental results obtained by overloading lysosomes with exogeneous material.

Inherited lipid storage diseases are caused by a defect of one particular lysosomal enzyme. Concomitantly, several other lysosomal enzymes may display enhanced activities as compared with healthy controls [1]. An increased level of lysosomal enzyme activity can also be induced experimentally by overloading the lysosomal system of phagocytic cells with indigestible materials [2]. A lysosomal lipid storage can be induced by certain drugs and is characterized by the occurrence of inclusion bodies resembling those found in some inherited storage diseases. There is experimental evidence that drug-induced lipidosis results from direct interaction between the drug and polar lipids rather than by a drug–enzyme interaction [3, 4]. Therefore the question arose whether or not the lysosomal enzyme activities become altered in drug-induced lipidosis. We investigated four lysosomal enzymes in tissues of rats which had developed generalized lipidosis due to chronic administration of chlorphentermine. This drug was chosen because the ultrastructural changes and the lipid storage upon chronic treatment had been documented before [5–9]. The results obtained indicate that some lysosomal enzyme activities are increased also in drug-induced lipidosis.

METHODS

Male Sprague–Dawley rats with an initial body weight of about 300 g were used. The animals were kept at a temperature of 25° and had free access to food (Altromin 1321) and drinking water in which chlorphentermine* was dissolved in a concentration of 0.5 g/l., resulting in a daily intake of 30–40 mg chlorphentermine/kg. The rats were treated for 12 weeks and killed 24 hr after discontinuation of the drug treatment. Samples of liver and renal cortex were dissected and both adrenals obtained. The tissues were weighted and homogenized in the 50 fold volume of distilled water for 30 sec at 14,500 r.p.m. (Ultra-Turrax).

The activities of β-D-glucoronidase, β-D-galactosidase and α-D-mannosidase were estimated by the following substrates: 4-methyl-umbelliferone-β-D-glucoronide-trihydrate, 4-methyl-umbelliferone-β-galactopyranoside-monohydrate and 4-methyl-umbelliferone-α-D-mannopyranoside, respectively (Koch-Light, Colubrook, England). Upon enzymatic degradation, the free 4-methyl-umbelliferone will exhibit a characteristic fluorescence [10, 11] which was determined by fluorescence-spectrophotometry. The activity of acid phosphatase was spectrophotometrically determined by means of 4-nitrophenyl-phosphate (E. Merck, Darmstadt, West Germany) as described by Walter and Schütt [12]. All substrates were applied to yield a final concentration of 0.5 per cent. The protein concentrations of the tissue homogenates were estimated by the methods of Lowry *et al.* [13]. The enzyme activities were expressed as mU/mg protein.

RESULTS

The activities of four lysosomal enzymes of liver, kidneys and of adrenals of controls and of lipidotic rats are summarized in Table 1. A marked rise of enzyme activity of the β-glucuronidase was found in liver and kidneys, and of the α-mannidose in kidneys, a small increase could be demonstrated for the β-galactosidase in liver and kidneys, for the α-mannosidase in the liver, and for acid phosphatase in kidneys and adrenals. No change could be detected of acid phosphatase activity in the liver, or of the β-galactosidase and α-mannosidase in the adrenals. Thus, the determined enzyme activities displayed no uniform pattern in lipidotic organs.

DISCUSSION

By purpose, the enzymes have been determined in tissue homogenates and not in isolated lysosomes, since it is already a difficult task to quantitatively separate

* 4-Chloro-α,α-dimethyl-phenethylamine; the compound was kindly supplied by Tropon-Werke, Köln-Mülheim (Germany), Lucofen®, Prc-Sate®.

Table 1. Activities of four lysosomal enzymes of controls and of lipidotic rats

	Control rats $n = 6$	Lipidotic rats $n = 6$	Ratio Lipidosis/Controls
	mU/mg protein of homogenate		
Liver			
β-D-Glucuronidase	6.3 ± 0.6	15.1 ± 1.1*	2.40
β-D-Galactosidase	1.8 ± 0.1	2.4 ± 0.1*	1.35
α-D-Mannosidase	1.9 ± 0.2	2.4 ± 0.3	1.26
Acid phosphatase	18.7 ± 0.6	18.4 ± 1.3	0.99
Renal Cortex			
β-D-Glucuronidase	1.5 ± 0.2	3.7 ± 0.4*	2.46
β-D-Galactosidase	9.8 ± 0.5	13.0 ± 1.2*	1.33
α-D-Mannosidase	3.4 ± 0.2	6.3 ± 0.5*	1.85
Acid phosphatase	28.5 ± 0.5	34.5 ± 2.5	1.21
Adrenals			
β-D-Glucuronidase	3.5 ± 0.3	4.4 ± 0.5	1.25
β-D-Galactosidase	4.3 ± 0.2	4.2 ± 0.6	0.98
α-D-Mannosidase	1.2 ± 0.1	1.3 ± 0.1	1.08
Acid phosphatase	16.4 ± 1.1	22.3 ± 1.1*	1.36

Values are expressed as means ± S.E.M.
* Indicates significance ($P < 0.05$).
The lipidosis was induced by chronic treatment with chlorphentermine.

normal lysosomes in a pure state from tissue homogenates of untreated animals. It will be even more difficult to separate lipidotic lysosomes displaying different and inhomogeneous properties with respect to size and density. The use of tissue homogenates, on the other hand, requires cautious interpretation of the reported results because of the possibility that "lysosomal" enzymes might also be located at other cellular sites than lysosomes [14–16].

Keeping this reservation in mind, the present findings can be considered to indicate that the enzymatic ability of the lysosomal apparatus is not generally impaired by a considerable degree of lipid storage as revealed by ultrastructural and biochemical observations. On the contrary, some of the enzyme displayed higher activities than controls. The increase in lysosomal enzyme activities differed between the organs studied. The relatively small changes observed in adrenals as compared with liver and kidneys might be explained by ultrastructural observations: in rats subjected to the present treatment, all cell types of the adrenal gland contain many giant residual bodies devoid of a limiting membrane and presumably no longer possessing considerable enzymatic activities. In contrast, in liver and kidneys the degree of lysosomal storage is rather inhomogeneous, depending on the cell types; thus many cells may have an overloaded, but yet functioning and hypertrophied lysosomal system.

The present findings are in line with previous reports that lysosomal overloading leads to an increase of several lysosomal enzyme activities. For such an unspecific increase it appears to be of little importance whether lysosomal overloading results either from inherited enzyme deficiency [1], from over supply of exogeneous substances [2] or from drug induced lipid accumulation as shown presently for chlorphentermine and previously for two other amphiphilic drugs [17, 18].

Acknowledgements—We are grateful to Drs. M. Cantz and J. Gehler for the methodical advice. E.U.M. was the recipient of a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg.

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