SLOWING OF INTRALYSOSOMAL PROTEOLYSIS AND LABILIZATION OF LYSOSOMES IN THE RAT LIVER AFTER ADMINISTRATION OF SURAMIN

T. A. Korolenko, A. B. Pupyshev, and A. E. Malygin

UDC 612.351.11-014.21.014.46:615.275

KEY WORDS: lysosomotropic agents; suramin; protein catabolism; rat liver lysosomes.

Lysosomotropic agents (LTA), if administered in vivo accumulate selectively in lysosomes; the chemical nature of the substance and the mechanism of its uptake may differ [9]. The LTA include certain drugs used in clinical practice; lysosomotropism, moreover, is a property with which most drugs can be endowed by injecting them together with a suitable carrier or in the form of liposomes. Because of these prospects for clinical use of different types of LTA, their detailed study has become necessary. It is particularly important to know the characteristics of interaction between LTA and lysosomes, in which these substances accumulate.

The object of this investigation was to study the effect of the LTA suramin on the structural integrity of rat liver lysosomes, on activity of hydrolytic enzymes, and on the catabolic function of the particles (proteolysis).

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g (13 experimental and eight control animals) were used in the experiments. Suramin* (Bayer-205, germanin – the symmetrical ureide of m-aminobenzoyl-m-amino-p-methylbenzoylnaphthylamino-1-trisulfonate-4,6,8-sodium) was injected intraperitoneally in the form of a solution in 0.9% NaCl in a dose of 250 mg/kg. The animals were killed 24 and 48 h after injection of the preparation. Modified bovine serum albumin labeled with ^{14}C (BSA-^{14}C, specific radioactivity 0.18 μCi/mg protein)† was injected into the caudal vein of the control and experimental animals 30 min before sacrifice in a dose of 1 mg/100 g body weight. The degree of uptake and digestion of the labeled protein was estimated by the method of Davies et al. [8]. Radioactivity was counted in a Mark I scintillation counter (USA). Preparative and analytical procedures were carried out as described previously [1], with minor modifications. During differential centrifugation of the liver homogenate, intact cells were removed by preliminary low-speed centrifugation at 30g for 15 and 10 min. Total activity of lysosomal enzymes was determined after freezing (in liquid nitrogen) and thawing seven times. When activity of acid hydrolases was measured the recommendations of Barrett [4] were followed. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The content of BSA-^{14}C in the liver was determined from the level of acid-insoluble (in TCA) radioactivity; acid-soluble radioactivity is due to products of intralysosomal hydrolysis of labeled protein [8, 12]. An increase in the content of BSA-^{14}C in the rat liver, calculated in absolute values of acid-insoluble radioactivity, and also relative to the injected dose of labeled protein (in the latter case, a tendency toward an increase was observed after 48 h; Table 1), was observed 24 and 48 h after injection of suramin. Since the

*The suramin was generously provided by Dr. D'Arcy Hart, National Institute for Medical Research, Mill Hill, London, England.

†The authors are grateful to V. G. Budker, working at the Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR, for help with preparing the labeled BSA and counting radioactivity.

Central Research Laboratory, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 686-688, December, 1980. Original article submitted February 4, 1980.

TABLE 1. Content of BSA-^{14}C in Rat Liver after Injection of Suramin (M ± m)

Index	Intact rats	Rats receiving suramin	
		24 h	48 h
BSA content, cpm/g wet weight of tissue	27 015±2519	40 943±951	35 611±2772
BSA-^{14}C content (acid-insoluble radioactivity), % of injected dose	17,7±1,6	29,5±1,2	21,6±2,5

Legend. Here and in Table 2, results calculated by methods of Davies et al. [8] and Mego [12].

TABLE 2. Digestion of BSA-^{14}C in Rat Liver Homogenate and Its Fractions (M ± m)

Index	Intact rats	Rats receiving suramin	
		24 h	48 h
Acid-soluble radioactivity of rat liver homogenate, % of total radioactivity of homogenate	50,1±3,3	26,7±3,8*	37,8±4,0*
Increase in acid-soluble radioactivity of homogenate, % of acid-insoluble radioactivity	26,2±4,2	15,9±4,8*	17,6±3,5
Digesting activity of lysosomal fraction (acid-soluble radioactivity, % of total values for heterolysosomes of nuclear heavy and light mitochondrial fraction)	30,8±3,8	10,7±2,0*	12,8±2,1*

*$P < 0.05$.

uptake of protein evidently was not increased after administration of suramin in this dose [6], it can be considered that the accumulation of protein was largely due to a decrease in the rate of its proteolysis.

The rate of intralysosomal digestion of BSA-^{14}C was reduced after injection of suramin (Table 2).

A sharp decrease in specific activity of acid phosphatase (to 35% of the corresponding values in intact animals) and an increase in acid RNase activity were observed in the liver homogenate (Fig. 1). Nonsedimented activity of all lysosomal enzymes studied, except acid phosphatase, was increased (Table 3), evidence of labilization of the liver lysosomes [2].

The results confirm the observations of Smeesters and Jacques [13], who found a reduction in acid phosphatase, β-glucuronidase, and β-N-acetylaminodeoxyglucosidase activity in liver homogenate 48 h after injection of the same dose of suramin into rats, but the workers cited found no change in β-galactosidase, α-glucosidase, or cathepsin D activity. According to other workers, the same dose of suramin inhibited cathepsin D activity [5, 6].

Although suramin has been used for a fairly long time for the treatment and prevention of parasitic diseases [6], the mechanism of its action has been insufficiently studied. Suramin is a urea derivative with six sulfonic acid residues, which give the compound its polyanionic character. These particular features of

TABLE 3. Nonsedimented Activity of Lysosomal Enzymes of Rat Liver Homogenate (in % of total activity) after Injection of Suramin (M ± m)

Enzyme	Intact rats	Rats receiving suramin	
		24 h	48 h
Acid phosphatase	6,1±0,2	7,5±0,9	6,3±0,9
β-galactosidase	9,5±0,7	17,4±1,2*	19,4±1,4*
Acid RNase	4,6±0,4	8,4±0,7*	9,8±0,9*
Cathepsin D	11,7±1,2	15,5±0,4*	17,8±1,5*

*P < 0.05.

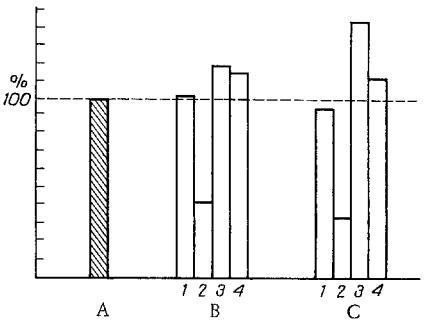

Fig. 1. Specific activity of lysosomal enzymes of rat liver homogenate after injection of suramin. A) Control (100%); B and C) specific activity of lysosomal enzymes 24 and 48 h respectively after injection of suramin (in % of control). 1) β-Galactosidase; 2) acid phosphatase; 3) acid RNase; 4) cathepsin D.

the compound make complex formation possible with proteins, including with enzymes. For instance, in the case of albumin, binding sites for this compound with high affinity have been found [6]. After parenteral injection, suramin readily forms complexes with blood plasma proteins and reaches the liver by endocytosis. It enters the lysosomes, and the main effect of the compound is evidently its action as an intralysosomal modifier. Suramin accumulates in vivo in the lysosomes of Kupffer cells and hepatocytes, where its concentration may reach 1-10 mM [6]. Intralysosomal accumulation of the compound is accompanied by inhibition of activity of certain acid hydrolases (acid phosphatase, for example). Slowing of proteolysis after injection of suramin in a dose of 250 mg/kg may be associated with inhibition of lysosomal enzyme activity, but no changes in specific activity of cathepsin D, which plays an important role in the degradation of modified proteins, was found in this particular investigation.

It must also be recalled that suramin disturbs fusion of lysosomes with phagosomes, and this may be accompanied by slowing of intralysosomal digestion of protein [8, 11]. Disturbance of protein catabolism causes the accumulation of unhydrolyzed substances in the lysosomes. This state to some degree resembles in some features the lysosomal accumulation diseases. Meanwhile injection of suramin can be used to "control" the function of lysosomes and, for example, to depress the catabolic function of the lysosomes of liver cells. Depression of proteolysis may be accompanied by labilization of lysosomes, as has been demonstrated in ischemia and anoxia [7], and may give rise to disorders of cell function (for example, it may slow the secretion of albumin by hepatocytes [3]).

Labilization of liver lysosomes is observed after administration of various LTA, including Triton WR 1339 [1], sodium aurothiomalate, and also suramin. These substances, which cannot be hydrolyzed in lysosomes, overload the vacuolar apparatus to a varied degree.

Labilization of particles following injection of suramin, it must be noted, is connected with the formation of autophagosomes, which have greater "fragility" [10]. Autophagy is observed after injection of Triton WR 1339, suramin, and some other LTAs and may be a manifestation of a nonspecific cellular reaction to injection of these substances.

LITERATURE CITED

1. T. A. Korolenko and V. G. Titova, Byull. Éksp. Biol. Med., No. 7, 816 (1976).
2. A. A. Pokrovskii and V. A. Tutel'yan, Lysosmes [in Russian], Moscow (1976).
3. I. D. Algranati and D. D. Sabatini, Biochem. Biophys. Res. Commun., 90, 220 (1979).
4. A. J. Barrett, in: Lysosomes: A Laboratory Handbook, J. T. Dingle, ed., North-Holland, Amsterdam (1972).
5. C. H. C. M. Buys, M. G. L. Elferink, J. M. W. Bouma, et al., J. Reticuloend. Soc., 14, 209 (1973).
6. C. H. C. M. Buys, J. M. W. Bouma, M. Gruber, et al., Naunyn-Schmiederberg's Arch. Pharmacol., 304, 183 (1978).
7. B. Chua, R. L. Kao, E. Rannels, et al., J. Biol. Chem., 254, 6617 (1979).
8. M. Davies, J. B. Lloyd, and F. Beck, Biochem. J., 121, 21 (1971).
9. C. De Duve, T. De Barsy, B. Poole, et al., Biochem. Pharmacol., 23, 2495 (1974).
10. P. Harikumar and V. Ninjoor, Biochim. Biopphys. Acta, 587, 60 (1979).
11. P. D'Arcy Hart and M. R. Young, Nature, 256, 47 (1975).
12. J. L. Mego, in: Lysosomes in Biology and Pathology, Vol. 3, North-Holland, Amsterdam (1973), p. 138.
13. C. Smeesters and P. J. Jacques, Abstracts of Papers at the XII International Congress of Cell Biology, No. 143, Brussels (1968). Excerpta Medica International Congress Series, No. 166.

ACTION OF PREDNISOLONE ON ^{3}H-CATECHOLAMINE SYNTHESIS IN RAT ADRENALS DURING PHYSICAL FATIGUE

E. V. Volina, É. Sh. Matlina, and G. D. Kurbanova

UDC 612.014.32-08:612.452.014.46:615.357.453

KEY WORDS: glucocorticoids; synthesis of catecholamines; adrenals; physical exertion.

It was shown previously that during severe physical fatigue noradrenalin and adrenalin synthesis in the adrenals from tyrosine and dopa is inhibited in rats [3]. Glucocorticoids both in vivo and in vitro normalize catecholamine synthesis in such animals but do not affect the synthesis of these amines in intact rats. It has been found that the stimulating effect of glucorticoids on depressed catecholamine synthesis is manifested only if tyrosine is used as the precursor [5]. Under the influence of hydrocortisone, synthesis of ^{3}H-noradrenalin and ^{3}H-dopamine from ^{3}H-tyrosine also is activated in the rat CNS [6]. These facts suggest that glucocorticoids are evidently universal regulators of the intensity of catecholamine synthesis both in the central and in the peripheral regions of the sympathico-adrenal system.

In the present investigation, which was aimed at studying the effect of the glucorticoid prednisolone on the ability of the adrenals to synthesize catecholamines during physical fatigue in animals, a radioisotopic method was used. By so doing, by contrast with previous studies [1, 2], it was possible to use the principal precursor of these amines, namely tyrosine, in low physical concentrations and also to investigate dopamine synthesis under these conditions. Animals in a state of severe physical fatigue after swimming for 8 h served as the experimental model.

Laboratory of Physiology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR. Laboratory of Sport Endocrinology, All-Union Research Institute of Physical Culture, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 688-690, December, 1980. Original article submitted December 24, 1979.