EFFECT OF SUBEXTREMAL AND EXTREMAL FACTORS ON LIVER LYSOSOMES

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The state of the lysosomal apparatus of the liver in rats was studied under the influence of subextremal and extremal factors (intensive physical exertion, starvation, and a combination of the two). When extremal stimuli act on the body, acid hydrolase activity in the liver rises and labilization of the lysosomal membranes takes place. Activation of the lysosomal apparatus corresponds to the degree of stress on the body and is an advantageous process aimed at the adaptive reorganization of the structures and metabolism of the cells.
KEY WORDS: subextremal and extremal factors; adaptation; lysosomes; enzymes; membranes.

Lysosomes are among the first of the ultrastructures of the cell to be implicated in the response reactions of the body to extremal stimuli. Under these circumstances the number and size of the lysosomes are increased, lysosome populations are redistributed, their localization in the cell changes relative to the nucleus, the permeability of their membranes is increased, and hydrolytic enzymes are activated [3-5, 7, 9, 12, 14, 15]. This high reactivity of the lysosomes suggests an important role for them in the adaptive reorganization of cell function and cell metabolism.

In this investigation the state of the liver lysosomes was studied during adaptation to subextremal and extremal factors.

EXPERIMENTAL METHOD

Male Wistar albino rats were used. All the animals were divided into four groups with 10 rats in each group. The intact animals of group 1 acted as the control. The rats of group 2 underwent intensive physical exertion (swimming in a tank for 3.5 h carrying a load of 4 g/100 g body weight). The animals of group 3 were deprived of food for 3 days. The rats of group 4 swam with the load after preliminary starvation for 3 days.

After decapitation of the rats the livers were quickly removed and washed with cold 0.25 M sucrose solution containing Tris-HCl, pH 7.4, and then dried with filter paper and minced. The liver was homogenized in a glass homogenizer with a Teflon pestle at 2000 rpm for 20 sec. The fraction of lysosomes was obtained from the homogenate by differential centrifugation in a sucrose density gradient [1].

Changes in the activity of three lysosomal hydrolases were studied: acid phosphatase, acid ribonuclease (RNase), and cathepsin. Total and nonsedimented (20,000g) enzyme activity were determined by the addition of the nonpolar detergent Triton X-100 to the incubation mixture in a final concentration of 0.1% (w/v). Free activity was determined in freshly prepared homogenates and lysosomes. Activity of RNase was detected by Bening's method [2] and expressed as percentages of acid-soluble products formed during hydrolysis of the nucleic acid per gram of protein per minute. Cathepsin activity was determined by hydrolysis of hemoglobin and expressed in optical density units (E_{280}) per gram of protein per 30 minutes. Acid phosphatase activity was determined from the hydrolysis of p-nitrophenyl phosphate and expressed in Bessey's units per milligram protein per minute. The protein content was determined by Lowry's method.

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TABLE 1. Changes in Activity of Lysosomal Enzymes under Influence of Subextremal and Extremal Factors (M ± m, n = 10)

Experimental conditions	Material	Acid RNase			Acid phosphatase			Cathepsin		
		free activity	total activity	ratio of free to total activity	free activity	total activity	ratio of free to total activity	free activity	total activity	ratio of free to total activity
Control	Homogenate	4,0±0,5	14,0±0,8	0,286	0,36±0,2	1,47±0,15	0,245	32±3	141±7	0,227
	Lysosomes	13,0±1,2	49,0±3,2	0,265	1,38±0,09	3,22±0,31	0,430	161±10	260±17	0,619
Swimming	Homogenate	4,0±0,5	19,0±1,4*	0,222	0,65±0,13*	1,88±0,09*	0,345	50±7*	165±9	0,303
	Lysosomes	17,0±1,6	47,0±3,8	0,360	1,89±0,13*	1,98±0,20	0,955	190±12*	298±22	0,637
Starvation	Homogenate	5,0±0,8	18,0±1,0*	0,278	0,79±0,07*	1,63±0,18*	0,481	38±4	166±8*	0,231
	Lysosomes	16,0±1,5	57,0±4,8	0,380	1,75±0,12*	2,85±0,33	0,610	197±13*	301±21	0,654
Swimming after starvation	Homogenate	6,0±0,7*	20,0±1,5*	0,300	1,02±0,17*	1,98±0,11	0,515	55±8*	221±13*	0,249
	Lysosomes	16,0±1,5	49,0±4,0	0,326	2,09±0,14*	2,97±0,42	0,703	201±12*	311±20*	0,647

Legend: Units of enzyme activity given in section "Experimental Method." *) $P < 0.05$ (compared with control), n) number of experiments.

EXPERIMENTAL RESULTS

As Table 1 shows, there was a definite tendency for the total acid RNase activity in the liver homogenate of animals exposed to stress to be increased. The most marked increase was observed in the case of combined exposure, i.e., swimming after starvation (Table 1). Free RNase activity also was moderately increased in the starved animals but it remained within normal limits in liver homogenate from rats subjected to physical exertion. In all the animals exposed to stress an increase was observed in the nonsedimented RNase activity. Under the influence of intensive physical exertion the nonsedimented RNase activity was increased on average by 4 units, and after starvation by 3 units.

Total acid phosphatase activity, like RNase activity, showed a particularly marked increase in the animals exposed to a combination of extremal factors. On average the total acid phosphatase activity rose by 28% after physical exertion, by 11% after starvation, and by 35% after swimming preceded by starvation. A moderate increase in nonprecipitated acid phosphatase activity was observed in the experimental animals of all groups. Unlike acid RNase, measurement of free acid phosphatase activity showed it to be increased in all rats exposed to stress. By contrast, the total activity in the suspension of lysosomes was actually slightly reduced compared with the lysosomal fraction isolated from the liver of intact animals.

Determination of cathepsin activity revealed an increase in all types of activity both in the homogenate and in the suspension of lysosomes isolated from the liver of rats exposed to stress.

An increase in the ratio of free to total enzyme activity after exposure to all extremal factors will be noted. It has not yet been firmly established whether this increase is an indication of the duration of enzymes in vivo or whether it is the result of the fact that the lysosomal membranes become more labile and, consequently, break up more easily during the technical manipulations of isolation and purification [11]. Hence the difficulty of interpreting the results: Are these changes attributable to alterations in the membrane or matrix or both? In the writers' view, they indicate modification of the lysosomes, in agreement with the views of other workers [10, 11].

In the cases now observed all types of stress were accompanied by changes in the liver lysosomal apparatus of the experimental rats. These changes consisted of activation of lysosomal enzymes, as shown by an increase in the total activity of the enzymes in the homogenates and labilization of the lysosomal membranes with liberation of hydrolases into the cytoplasm. Evidence of this is given by the increase in the ratio of free to total enzyme activity and the increase in the nonsedimented enzyme activity.

The loss of acid phosphatase from the lysosomes evidently exceeded that of the other enzymes, so that during isolation and purification of the lysosomal fraction this enzyme appeared in the supernatant. This could probably explain why, together with an increase in total acid phosphatase activity in the homogenate, there was a decrease in this type of activity in the lysosomal suspension. Other evidence of increased permeability of the lysosomal membranes was given by the increase in free and nonsedimented acid phosphatase activity in the homogenate.

The total acid RNase activity in the purified fraction of lysosomes from the liver of the experimental rats was almost unchanged, but cathepsin activity was actually increased, as it was also in the homogenate. This result can evidently be explained by the closer binding of these enzymes with the membranes of the lysosomes, so that more vigorous action is required for their solubilization.

The character of the response of the lysosomal apparatus in these experiments, incidentally, corresponded to the degree of stress to which the animal was exposed through the action of the extremal stimulus. The changes in the lysosomes observed in the extremal situation were presumably biologically advantageous and were directed toward the adaptive reorganization of the structures and metabolism of the cell. This hypothesis is confirmed by investigations showing the role of lysosomes in the compensatory increase in energy production by the mitochondria of the rabbit's myocardium under the influence of intensive physical exertion [6, 8], and also by observations on specific activation of fructose-1,6-diphosphatase (an enzyme of gluconeogenesis) by lysosomal cathepsins under conditions of starvation [13]. There is no doubt that the activity of the lysosomal enzymes is very finely controlled in the body. During extremal stress and exhaustion of these regulators a lysosomal cytolytic chain reaction may develop, leading to the onset of necrobiotic and necrotic changes in the tissues, such as are known to be observed when the body is exposed to the action of many unfavorable factors [3, 4, 14].

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