A PROTECTIVE LYSOSOMAL SELF-REGULATING MECHANISM AND ITS DISTURBANCE BY DIPHTHERIA TOXIN

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Any considerable increase in permeability of lysosomal membranes may be accompanied by the release of a flood of lysosomal enzymes into the hyaloplasm, which lead to profound alteration of the cells [6, 8]. However, electron-microscopic investigations often reveal lysosomes with a completely destroyed membrane and with the absence of any serious injury to intracellular structures in nearby areas. On the basis of investigations by biochemical methods on lysosomes of hepatocytes and by electron microscopy on cardiomyocytes, the writers postulated that a self-regulating mechanism is present in lysosomes which increases the degree of fixation of hydrolases in these organelles when their membranes are damaged [7], and this prevents damage to the cells when there is an increased possibility of the escape of enzymes from lysosomes.

The aim of this investigation was to discover the presence or absence of a self-regulating mechanism in cells of the contractile myocardium, for when the heart is damaged by a pathogenic factor the lysosomal apparatus is activated and the degree of permeability of the lysosomal membranes is increased [2-4].

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Chinchilla rabbits weighing 2.5-3.5 kg. A model of diphtheria toxemia was created in 30 animals by means of a single intravenous injection of 1 MLD/kg of diphtheria toxin. The quantity of toxin which, when given as a single intraperitoneal injection into guinea pigs weighing 250 g, caused death of 40% of the animals on the 3rd day with evidence of damage to the adrenals, was taken as 1 MLD. Extirpation of the heart was carried out on 10 control animals and also on the experimental animals 1 and 3 h and 6 days after injection of the toxin, under superficial hexobarbital anesthesia. The ventricles were washed in ice-cold buffer solution for homogenization: 0.6 M KCl (chemically pure), 0.25 M sucrose (chemically pure), 10 mM imidazole ("Serva," West Germany), and 1 mM EDTA ("Serva"), pH 7.25. Tissue from the left and right ventricles was then cut into small pieces with scissors at 0-4°C and then homogenized in a Potter–Elvehjem glass homogenizer with Teflon pestle (gap 0.21 mm) for 180 sec at 40 rpm. A homogenate of 1:10 of weight of tissue per volume of medium was subjected to differential centrifugation [9] on an L8-M ultracentrifuge ("Beckman"). The lysosomal supernatant and lysosome-enriched fraction (residue) were obtained and the latter was resuspended in the ratio 1:0.5 in a buffer solution of 0.7 M sucrose (chemically pure) and 1 mM EDTA ("Serva") at pH 7.0. Total, nonsedimented, accessible, and lysosomal-bound activity of N-acetyl-β-D-galactosaminidase, an enzyme with high activity in cardiomyocyte lysosomes [10], was investigated colorimetrically [1] at 405 nm, as the rate of release of 4-nitrophenol from a substrate consisting of 4-nitrophenyl-N-acetyl-β-D-galactosamine ("Sigma," USA). Enzyme activity was expressed in units of enzyme activity per milligram protein, determined by Lowry's method. The ratio of nonsedimented to total enzyme activity, expressed in per cent, was determined as the coefficient of permeability of the lysosomal membranes (CPLM).

All the numerical data were subjected to statistical analysis and correlation analysis on a "Commodore 64" personal computer, by a program written by ourselves.

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TABLE 1. Dependence of CPLM on Total N-Acetyl-β-D-Galactosaminidase Activity (here and in Table 2, coefficients of correlation are given)

Group of animals	Ventricle	
	left	right
Control	−0,84	−0,39
Receiving diphtheria toxin		
1 day	−0,33	+0,94
3 days	−0,96	−0,74
6 days	−0,74	+0,73

TABLE 2. Dependence of Lysosome-Bound N-Acetyl-β-D-Galactosaminidase Activity on Total Activity of this Enzyme

Group of animals	Ventricle	
	left	right
Control	+1,0	+0,39
Receiving diphtheria toxin		
1 day	+0,75	+0,77
2 days	+0,97	+0,96
6 days	+0,62	+0,97

EXPERIMENTAL RESULTS

The results of correlation analysis are given in Tables 1 and 2.

The results show strong and significant negative correlation in the left ventricle of the intact animals between CPLM and total enzyme activity: the more enzyme is present in the lysosomes, the more permeable their membrane (since total activity includes both that contained in the lysosomes and nonsedimented activity, the negative correlation is illogical). This dependence is weaker in the right ventricle but is in the same direction. This relationship became much weaker in the left ventricle 1 day after injection of diphtheria toxin, but in the right ventricle correlation changed to positive. In other words the enzymes began to move out into the cytoplasm more rapidly. According to our previous data [5], it is on the 1st day of the process that the most significant disturbances of contractility of the heart are observed in diphtheria toxemia. After 3 days, when the contractile function of the heart is stabilized, the mechanism of fixation of the enzyme in the lysosomes of both ventricles, characteristic of the normal state, was restored. After 6 days of the process this correlation in the left ventricle weakened again, but in the right it again became positive, in agreement with the newly developing worsening of the contractile function of the heart muscle.

Relations between bound and total enzyme activity in the lysosome were similar in character (Table 2). Under normal conditions strong and significant positive correlation was observed in both ventricles between these parameters: the greater the total activity of the enzyme, the more strongly it was fixed in the lysosome. This binding weakened in both ventricles toward the 3rd day, and after 6 days it reached its lowest value in the left ventricle.

Consequently, a self-regulating mechanism functions in the lysosomes, whereby the degree of fixation of the enzyme in the organelle is enhanced, and this undoubtedly protects the myocardium against damage by the enzyme. Under the influence of diphtheria toxin, on the 1st and 6th days of the process this mechanism is disturbed, and this is immediately reflected in weakening of the contractile function of the heart, evidently due to destruction of its contractile elements by the enzyme. The period of stabilization of functions of the heart muscle, namely on the 3rd day, coincides with definite recovery of the mechanism discussed above.

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