PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

RECIPROCITY OF CHANGES IN LYSOSOMAL ENZYME ACTIVITY OF RABBIT CARDIOMYOCYTES AND HEPATOCYTES AFTER INJECTION OF DIPHTHERIA TOXIN

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KEY WORDS: lysosomes; heart; liver.

The writers showed previously [3, 4] that in various pathological processes and also in some physiological states, accompanied by increased permeability of lysosomal membranes of cardiomyocytes and hepatocytes, mechanisms leading to intensification of fixation of their enzymes inside the lysosomes begin to function in these organelles, and in this way autolytic cell damage is prevented. The present study is devoted to further investigation of these protective autoregulatory mechanisms, using a method of correlation analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male chinchilla rabbits weighing 2.5-3.5 kg. In an experimental model of diphtheria 30 animals were given a single intravenous injection of 0.4 MLD/kg of diphtheria toxin. Ten rabbits served as the control. In the control and also 24 and 72 h and 5 days after injection of the toxin thoracotomy was performed under superficial hexobarbital anesthesia, after which the heart and liver were removed. Venous blood was taken simultaneously. By traditional methods [5] the lysosomal fraction of the cardiomyocytes and hepatocytes was isolated by differential centrifugation on an 18-M centrifuge ("Beckman," USA). Nonsedimented (free) activity (NSA) on β-D-glucuronidase (EC 3.2.1.31) and N-acetyl-β-D-galactosaminidase (EC 3.2.1.53) was determined in the lysosomal supernatant in cardiomyoctyes of the left and right ventricles and also in the hepatocytes. Activity of these enzymes also was determined in the blood plasma. The determination was made colorimetrically by the usual methods [1, 2] on DU 8 M spectrophotometer ("Beckman," USA). Enzyme activity was expressed in nanomoles of substrate hydrolyzed in 1 min, per milligram protein or per milliliter blood plasma. In the course of correlation analysis, using the program devised by ourselves on an IBM PC/XT personal computer, correlation was estimated as strong if the absolute value of the coefficient of correlation was $\rho \geq 0.7$, as moderately strong if $\rho = 0.69$-0.3, and as weak if $\rho \leq 0.29$. The significance of the coefficient of correlation was determined by the use of appropriate equations, included in our computer program. Pairs of values for all animals and at all times of experimental diphtheria were subjected to combined correlation analysis.

EXPERIMENTAL RESULTS

The time course of activity of the lysosomal enzymes which we studied in blood plasma, ventricles of the heart, and liver in the course of experimental diphtheria is illustrated in Fig. 1. At all points other than that marked by an asterisk in fragment a the difference between the mean values of the present and previous determinations was significant; differences between mean values and the control were significant at all points.

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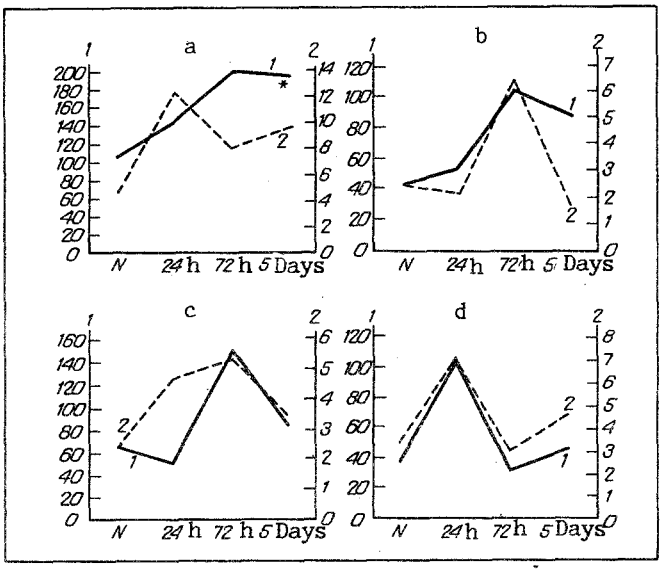

Fig. 1. Changes in N-acetyl-β-D-galactosaminidase (ADGase, 1) and β-D-glucuronidase (DGLase, 2) in blood plasma (a) and NSA of these enzymes in lysosomal fraction of cardiomyocytes from left (b) and right (c) ventricles, and hepatocytes (d) in course of experimental diphtheria. Numbers 1 and 2 mark not only corresponding curves, but also ordinate; asterisk indicates a mean value which does not differ significantly from the mean value at the previous time of investigation Remainder of explanation in text. Abscissa, period of process; ordinate, activity, nmoles/ml (a) or nmoles/mg (b-d).

A marked increase in activity of both enzymes was observed in the blood plasma (Fig. 1a) 24 h after injection of diphtheria toxin, and after 72 h, activity of N-acetyl-β-D-galactosaminidase (ADG) activity was increased even more compared with the previous time, but activity of 3-D-glucuronidase (DGLase) was reduced, although it still remained higher than initially. By the 6th day after injection of diphtheria toxin, activity of the first enzyme was at the level of the previous time, whereas activity of the second was higher than previously.

In the left ventricle (Fig. 1b) NSA of ADGase after 24 h showed a very small increase, but that of DGLase showed an equally insignificant fall. After 72 h, NSA of both enzymes was sharply increased, whereas after 5 days it was lower than at the previous time for ADGase, although still remaining significantly higher than initially. Meanwhile NSA of DGLase showed a sharp decrease (below the control).

NSA of ADGase in the right ventricle of the heart (Fig. 1c) fell 24 h after injection of the toxin, but NSA of DGLase rose sharply. After 72 h, just as in the left ventricle, NSA of both enzymes rose, and by the 6th day of the pathological process both showed a decrease, which was equal in degree.

In the liver (Fig. 1c) the trend of changes in NSA of the two enzymes was discordant: a sharp rise after 24 h, a fall after 72 h, and another, smaller rise after 5 days.

To sum up the results described above, in experimental diphtheria phasic changes take place in nonsedimented lysosomal enzyme activity in all the tissues studied.

Correlation analysis revealed the following situation (Table 1). Since strong positive correlation was observed between NSA both of the same and of different enzymes (taken in all possible combinations) in cardiomyocytes of the left and right ventricles and also between NSA of the different enzymes in the liver, it can be tentatively suggested that this depends on common regulatory mechanisms, characteristic of each organ as a whole.

TABLE 1. Correlation of NA of Various Lysosomal Enzymes in Ventricles of the Heart and Liver

Pairs of parameters	ρ
ADGase of left ventricle – DGLase left ventricle	+0.66
ADGase of right ventricle – DGLase of right ventricle	+0.70
ADGase of left ventricle – AGDase of right ventricle	+0.89
DGLase of left ventricle – DGLase of right ventricle	+0.86
NGDase of left ventricle – DGLase of right ventricle	+0.69
ADGase of right ventricle – DGLase of left ventricle	+0.87
DGLase of liver – ADGase of liver	+0.96
ADGase of left ventricle – ADGase of liver	−9.0
ADGase of right ventrilce – ADGase of liver	−0.83
ADGase of left ventricle – DGLase of liver	−0.93
ADGase of right ventricle – DGLase of liver	−0.92
DGLase of left ventricle – DGLase of liver	−0.83
DGLase of right ventricle – DGLase of liver	−0.55
DGLase of left ventricle – ADGase of liver	−0.66
DGLase of right ventricle – ADGase of liver	−0.47

Legend. All values of ρ are significant.

TABLE 2. Correlation between Plasma DGLase and Its NSA in the Ventricles of the Heart and in the Liver

Pairs of parameters	ρ
DGLase of plasma – DGLase of left ventricle	−0.59
DGLase of plasma – DGLase of right ventricle	−0.74
DGLase of plasma – DGLase of liver	+0.79

The strong negative correlation between enzymes of the ventricles of the heart and of the liver, relating both to the same and to different enzymes are very interesting. The reciprocity of activity of the same enzymes in cardiomyocytes and hepatocytes can be explained by inhibition of the enzyme reaction by the concentration of its end product. That this conclusion is correct is shown by the results in Table 2 (using the enzyme DGLase, which is specific for hepatocyte lysosomes).

Strong positive correlation was found between NSA of DGLase in the liver and its activity in the plasma, but strong and significant negative correlation between the plasma and ventricles. Strengthening of activity of the enzyme in the liver evidently leads to elevation of its plasma level, which in turn causes inhibition of its activation in the cardiomyocytes.

So far as the data in Table 1, indicating reciprocity of dissimilar lysosomal enzymes of the hepatocytes and cardiomyocytes, are concerned, they suggest the existence of a completely different mechanism, possibly connected with the influence of the extralysosomal activity of the enzyme on permeability of the lysosomal membranes and determined by their biophysical characteristics.

The investigation thus demonstrated the presence of yet another protective self-regulating mechanism in the lysosomes and aimed at preventing enzymic destruction of the cell when lysosomal membrane permeability is sharply increased, and based on the principle of reciprocity of activity of the enzymes of these organelles.

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ADAPTATION TO STRESS PREVENTS THE CARDIOTOXIC EFFECT OF RIFAMPICIN BUT NOT THAT OF POLYMYXIN B

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Adaptation to stress has been shown to increase the resistance of the isolated heart to reperfusion injury and heat shock [4], to high Ca^{2+} concentrations and toxic doses of catecholamines [1, 8], and cellular structures (elements of the sarcoplasmic reticulum of the mitochondrion and nucleus), isolated from the myocardium of adapted animals, have been found to differ from the controls in having high resistance to damaging factors [5, 9]. This combination of phenomena has been called adaptive stabilization of structures (ASS) [3, 9]. An important role in the mechanism of the ASS phenomenon has been shown to be played by accumulation of heat shock proteins (hsp 70) in the cells of the organ [10]. Investigations have shown [11, 15] that hsp 70, by their disaggregating effects, protect cell proteins and, in particular, enzymes against damaging factors. However, the problem whether the resistance of the heart to the toxic effects of enzyme inhibitors is increased during adaptation to stress has not previously been studied.

The aim of this investigation was to assess, on the basis of physiological criteria, the effect of adaptation to stress on the resistance of the heart to the toxic action of the RNA-polymerase inhibitor rifampicin [13] and the protein kinase C inhibitor polymyxin B [14].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. Adaptation to stress was produced by immobilizing the rats in the supine position for 1 h on alternate days, for a total of eight procedures [4]. To reproduce the toxic effects of the antibiotics rifampicin was given in a dose of 7 mg/kg and polymyxin B intramuscularly in a dose of 0.12 mg/kg for 8 days. Experiments were carried out on six groups of animals: 1) control, 2) adapted rats, 3) rats receiving rifampicin, 4) adapted rats receiving rifampicin, 5) rats receiving polymyxin B, and 6) adapted rats receiving polymyxin B

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