EFFECT OF ADRENALIN ON FUNCTIONAL STATE OF LIVER LYSOSOMES AND SERUM HYDROLASE ACTIVITY IN RATS AFTER SEVERE MECHANICAL TRAUMA

L. N. Kobylyanskii

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Adrenalin, called the "emergency hormone," under extremal conditions induces an urgent reorganization of various systems aimed at increasing the resistance of the organism. It is considered that this sympathomimetic amine affects the plasma membranes of cells and organelles through cyclic 3',5'-AMP (cAMP). cAMP is synthesized in the cell by adenylate cyclase, whose activity is modified by catecholamines [1, 6]. In the course of our investigations to study lysosomal enzyme activity after mechanical trauma, the need arose to discover the effect of catecholamines on enzyme activity of the above-mentioned cell organelles in stress. This subject is not discussed in the literature.

The aim of the present investigation was to study the role of adrenalin in the mechanism of changes in liver lysosome function and serum hydrolytic enzyme activity in rats after severe mechanical trauma caused by crushing of the soft tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 130 male Wistar rats divided into 13 groups with 10 animals in each group. Two groups served as the control: a) intact rats, b) rats receiving an intraperitoneal injection of adrenalin in a dose of 0.1 mg/kg body weight. Parenteral injection of adrenalin was used as the model simulating excitation of the sympathicoadrenal system. The animals of the remaining 11 groups were killed soon after trauma (fixation of forceps without crushing) and at various times after application of the forceps. The whole period of injury was 5 h in duration. The experimental rats were traumatized by crushing the soft tissues of the hind limbs with special forceps [2]. For a more complete appraisal of the effect of adrenalin on rat liver cell lysosomes the forceps were not removed before the end of the experiment, thus restricting generalization of the toxic products of the traumatized tissues.

At the end of the period of soft tissue crushing the rats were decapitated, the liver was immediately removed, and washed in cold physiological saline, and weighed samples of liver tissue were homogenized in a Potter—Elvehjem glass homogenizer with Teflon pestle in 0.25 M sucrose with 1 mM EDTA [4]. To determine total lysosomal enzyme activity the liver homogenate was incubated for 3 min at 0°C in Triton X-100 in a final concentration of 0.1%. To study unsedimented enzyme activity the liver homogenate was centrifuged for 30 min at 105,000 g on a Superspeed-65 ultracentrifuge (from MSE, England), after which the transparent cytosol was separated. To determine enzyme activity in the serum, blood collected after decapitation was centrifuged for 30 min at 800-1000 g.

Activity of four lysosomal enzymes was determined in the homogenate, supernatant, and blood serum of the rats: acid DNase, acid RNase, acid phosphatase, and aryl sulfatases A and B. Activity of the enzymes was determined by spectrophotometric micromethods, using as the substrates high-polymer DNA (from Koch—Light, England), high polymer RNA (from Sigma, USA), sodium β-glycerophosphate (from Merck, West Germany), and p-nitrocatechol sulfate (from Sigma, USA) [3, 7].

The numerical results were subjected to statistical analysis [8].

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TABLE 1. Total (in μmole substrate/g tissue/min) and Unsedimented (in % of total) Activity of Liver Lysosomal Enzymes of Rats with Crushed Soft Tissues after Injection of Adrenalin (M ± m, n = 10)

Enzyme	Control (intact animals)	Control + adrenalin	Short-term fixation	Duration of crushing, h		
				½	1	1½
Total activity						
Acid DNase	0,217±0,013	0,630±0,020**	0,410±0,010*	0,335±0,26	0,408±0,022*	0,484±0,021*
Acid RNase	0,717±0,040	1,230±0,065†	0,900±0,010	0,991±0,084	1,232±0,007**	1,123±0,024*
Acid phosphatase	0,380±0,024	0,250±0,016†	0,210±0,004†	0,190±0,011†	0,250±0,012†	0,290±0,050
Aryl sulfatases A and B	0,474±0,023	0,679±0,023†	0,610±0,025	0,516±0,024*	0,445±0,044	0,429±0,025
Unsedimented activity						
Acid DNase	14,1±0,7	26,3±0,8	20,6±0,7	15,8±0,7	15,0±0,9	14,9±0,6
Acid RNase	9,5±0,8	10,0±0,7	11,1±0,7*	10,1±0,8	10,0±0,7	10,1±0,5
Acid phosphatase	6,7±0,2	12,3±0,4**	12,4±0,6**	11,8±0,5**	11,7±0,6**	11,8±0,8**
Aryl sulfatases A and B	1,5±0,1	7,5±0,3**	7,4±0,7**	6,7±0,6**	6,4±0,7**	6,1±0,8**

Duration of crushing, h						
2	2½	3	3½	4	4½	5
Total activity						
0,442±0,022*	0,499±0,012*	0,431±0,012*	0,415±0,015	0,418±0,014	0,440±0,064	0,444±0,013*
1,177±0,042†	1,142±0,041*	1,091±0,042	1,108±0,066	0,984±0,027	1,103±0,064	1,210±0,029†
0,300±0,003	0,310±0,012	0,330±0,011	0,340±0,011	0,350±0,021	0,340±0,021	0,350±0,023*
0,476±0,025	0,481±0,023†	0,514±0,022*	0,501±0,025	0,494±0,028*	0,467±0,025*	0,472±0,023*
Unsedimented activity						
15,3±0,8	15,0±0,5	15,2±0,8	15,1±0,7	14,9±0,6	15,0±0,8	14,7±0,7
10,2±0,9	10,2±0,8	10,3±0,6	10,7±0,7	10,5±0,9	10,6±0,7	10,9±0,8
14,7±0,5**	14,8±0,8**	14,3±0,9**	18,8±0,7**	16,9±0,6**	16,8±0,8**	16,5±0,9**
6,1±0,5**	6,2±0,7**	6,5±0,4**	6,9±0,5**	7,2±0,4**	7,1±0,5**	7,3±0,7**

*P < 0.05.
†P < 0.01.
**P < 0.001.

TABLE 2. Lysosomal Enzyme Activity in Blood Serum of Rats (in μmoles substrate/ml/min) during Crushing of Soft Tissues Preceded by Injection of Adrenalin (M ± m, n = 10)

Enzyme	Control (intact animals)	After injection of adrenalin	Short-term fixation	Duration of crushing, h		
				½	1	1½
Acid DNase	0,121±0,013	0,605±0,014**	0,260±0,006**	0,145±0,003	0,136±0,002	0,137±0,004
Acid RNase	0,531±0,035	0,539±0,006	0,420±0,012†	0,478±0,005	0,452±0,007*	0,473±0,006
Acid phosphatase	0,055±0,002	0,096±0,003†	0,120±0,003**	0,091±0,002*	0,112±0,004†	0,109±0,003†
Aryl sulfatases A and B	0,142±0,006	0,366±0,005	0,300±0,005	0,308±0,005	0,252±0,005	0,208±0,002

Duration of crushing, h						
2	2½	3	3½	4	4½	5
0,255±0,002**	0,244±0,003**	0,275±0,015**	0,201±0,003†	0,226±0,004†	0,222±0,002†	0,160±0,008*
0,495±0,008	0,454±0,009*	0,479±0,011	0,487±0,007	0,433±0,013†	0,455±0,007*	0,535±0,007
0,089±0,003*	0,094±0,002†	0,094±0,001†	0,146±0,003**	0,121±0,003**	0,121±0,002**	0,099±0,002†
0,187±0,003	0,183±0,004	0,192±0,002	0,225±0,012	0,252±0,005	0,214±0,003	0,242±0,020

*P < 0.05.
†P < 0.01.
**P < 0.001.

EXPERIMENTAL RESULTS

Table 1 shows that injection of adrenalin into intact animals caused marked activation of all liver lysosomal hydrolases studied with the exception of acid phosphatase. The sharpest increase was observed in acid DNase activity (almost threefold compared with the intact control). Acid RNase activity also was significantly increased (by 1.7 times), activity of aryl sulfatases A and B less so (by 1.4 times). Meanwhile, injection of adrenalin caused considerable inhibition of acid phosphatase activity, which was depressed by 34% compared with the control. These data agree with modern views on adrenalin as a powerful inducer of various enzyme systems and, in particular, of lysosomal enzymes [5]. At the same time they demonstrate marked selectivity of the induction effects of adrenalin, and are thus evidence that these effects are realized not through an increase in the total lysosome population, but through intervention in regulation of the catalytic activity of individual enzymes of these organelles.

Parenteral injection of adrenalin into animals with severe mechanical trauma also caused significant changes in total liver lysosomal enzyme activity (Table 1). For instance, exogenous adrenalin induced a considerable increase in acid DNase and RNase activity at all times of the experiment. The increase in activity of these acid nucleases was most marked in the case of DNase after 1.5-2.5 h (by 2-2.3 times compared with the intact control) and RNase after 1-2 h of crushing of the soft tissues (by 1.6-1.7 times compared with the intact control). Later the degree of nuclease activation fell somewhat, although it still remained high. Despite the marked activating action of adrenalin on rat liver DNase during crushing of the soft tissues, at no time during the experiment did activity of the enzyme reach its level in rats receiving adrenalin intraperitoneally. Injection of adrenalin into rats with severe mechanical trauma also led to an increase in RNase activity; the activating action of adrenalin was manifested at all times of the experiment, moreover, except during the period of short-term fixation of the forceps.

The response of the other two lysosomal hydrolases, namely aryl sulfatases A and B and, in particular, acid phosphatase, to injection of adrenalin differed significantly from the reaction of the nucleases. In particular, no "extra" activation of the rat liver aryl sulfatases was observed under these circumstances except during the period after short-term fixation of the forceps. Consequently, crushing of the soft tissues prevents the action of adrenalin on the increase in activity of liver aryl sulfatases, which was well marked in the intact control rats. Differences in the action of adrenalin on DNase and RNase and on the acid phosphatase level were even more marked. As already stated, similar differences in enzyme activity occurred also in the control rats, in which exogenous adrenalin lowered acid phosphatase activity. The inhibitory action of adrenalin on acid phosphatase activity was manifested during the first 2 h after crushing of the soft tissues. During this period activity of the enzyme was substantially reduced (to 50% of the intact control during the first 30 min of crushing and to 66-79% during the next 1.5 h of the experiment — Table 1). With an increase in the period of crushing the action of adrenalin on acid phosphatase activity was weakened.

The results of investigation of unsedimented rat liver lysosomal enzyme activity are also given in Table 1. They show that injection of adrenalin into rats not subjected to crushing was followed by a marked increase in unsedimented activity of all the enzymes tested except acid RNase, activity of which increased only a little. Unsedimented activity of aryl sulfatases A and B was significantly increased (fivefold compared with the initial level) and also of acid phosphatase (which was almost doubled) — readily solubilized lysosomal enzymes. Meanwhile, under the influence of adrenalin, the release of DNase — an enzyme more closely connected than the previous enzymes with lysosomal structures — from the lysosomes was considerably (by 1.8 times) increased. The above description suggests greater labilization of the lysosomal membranes as a result of parenteral administration of adrenalin. The results of these experiments agree with data in the literature [9, 10].

Crushing of the soft tissues after preliminary injection of adrenalin also was accompanied by a marked increase in unsedimented liver lysosomal enzyme activity. The most marked increase in unsedimented activity was observed in the case of acid phosphatase and, in particular, of aryl sulfatases A and B, i.e., enzymes loosely bound with lysosomal membranes. For instance, during short-term fixation of the forceps unsedimented liver aryl sulfatase A and B activity increased fivefold compared with the intact control, whereas acid phosphatase activity at this time amounted to 183% of the intact control. The high level of activity of these enzymes in the liver cytosol of rats during crushing of the soft tissues preceded by administration of adrenalin persisted until the end of the experiment. Consequently, preliminary injection of adrenalin into rats increased the labilization of liver lysosomal membranes of the rats caused by crushing of the soft tissues.

So far as the investigations of serum enzyme activity are concerned (Table 2) short-term crushing of the soft tissues preceded by administration of adrenalin to the rats led to a marked increase in activity of DNase, acid phosphatase, and aryl sulfatase; the degree of elevation of activity of all the enzymes was similar (activity was more than doubled compared with the control). With an increase in the period of crushing to 30 min activity of the acid DNase and phosphatase in the blood fell considerably (to 121 and 166% of the intact control, respectively), whereas aryl sulfatase activity remained at its previous level. With a further increase in the period of crushing of the soft tissues the dynamics of changes in the activity of these enzymes in the blood differed. DNase activity reached almost the control level after 1 and 1.5 h of crushing (113% of the control), but rose sharply again after crushing for 2 h (to 212%) and remained close to this level until the end of the investigation, when it fell again to 133%. Activity of aryl sulfatases A and B fell to 146% of the intact control during crushing of the tissues for 1.5 to 3 h, and then rose again after 3.5 h, to reach a maximum (258%) at the end of the experiment. Blood acid phosphatase activity remained high throughout the experiment and reached its highest peak 3.5-4 h after application of the forceps (258% of the control). Unlike the other two enzymes, blood acid RNase activity of rats traumatized after injection of adrenalin not only was not increased but, on the contrary, it was reduced by 21% below the control level after short-term crushing and remained somewhat reduced (by 10-15%) throughout the experiment, but returned to the control level at the end of the investigation.

The experiments thus showed that parenteral injection of adrenalin before infliction of trauma on animals does not cause any qualitative changes in the response of the liver lysosomes to trauma. This response was manifested as quantitative changes — activation of the lysosomal hydrolases studied and disturbance of stability with release of the enzymes into the cytoplasm of the hepatocytes, and from them into the systemic circulation.

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EFFECT OF ELECTRICAL STIMULATION OF THE DENTATE NUCLEUS ON CORTICAL EPILEPTIC FOCI

G. N. Kryzhanovskii,* R. F. Makul'kin, A. A. Shandra, and L. S. Godlevskii

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Suppression of neuropathological syndromes on activation of corresponding brain structures is highly relevant to the understanding of activity both of pathological systems, which

*Corresponding Member, Academy of Medical Sciences of the USSR.

Department of Pathophysiology, N. I. Pirogov Odessa Medical Institute. Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

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