EFFECT OF SEROTONIN ON THE LYSOSOMAL APPARATUS OF LIVER CELLS AND ON BLOOD HYDROLASE ACTIVITY AFTER SEVERE MECHANICAL TRAUMA

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After trauma, the serotonin content in the hepatocytes increases sharply (eightfold) [2]. On entering the intracellular organelles, serotonin is able to affect the course of various biochemical processes participating in the realization of protective and adaptive processes at the cellular level [5, 9]. Since serotonin has an inhibitory action on protein biosynthesis, the study of its effect on subcellular systems which participate in the catabolism of biopolymers, namely the lysosomal system of the cell, is of definite interest.

This paper describes an attempt to determine the effect of serotonin on the activity of certain enzymes of rat liver lysosomes and to differentiate between its possible direct action and the action of the stress factor on the functional state of the lysosomal system.

EXPERIMENTAL METHOD

Experiments were carried out on 130 male Wistar rats weighing 250-280 g. Of the 13 groups of animals (10 rats in each group) the first three were controls: 1) intact animals, 2) rats receiving serotonin by intraperitoneal injection in a dose of 0.003 mg/kg body weight, 3) animals to which a vise was applied 15 min after injection of serotonin, and killed 2-3 min later. Trauma was caused by crushing of the soft tissues of the hind limbs of the rats with a special vise [6]. To assess the effect of serotonin on the functional state of the lysosomal system of the liver cells more completely, a modified model of long-term crushing of the soft tissues was used — the vise was not removed until the end of the experiments, so that the majority of the traumatized thigh muscles were isolated from the rest of the body. The duration of tissue traumatization was increased by 30 min in each consecutive series of experiments. The total duration of traumatization was 5 h. At the end of the period of soft tissue crushing, the rats were decapitated, blood was immediately collected, the liver removed and carefully washed in cold physiological saline, after which it was weighed and homogenized in a glass Potter-Elvehjem homogenizer with Teflon pestle in 0.25 M sucrose solution with 0.001 M EDTA [8]. All operations were conducted at a temperature of 0-2°C. Changes in the functional state of the lysosomal system of the liver cells during crushing of the soft tissues of the rats' hind limbs after injection of serotonin were judged by determination of total and nonsedimented activity of acid DNase (EC 3.1.4.5), acid RNase (EC 2.7.7.16), acid phosphatase — AcP (EC 3.1.3.2), and aryl sulfatases A and B (EC 3.1.6.1), and also activity of these enzymes in the blood serum. To determine total enzyme activity, the liver homogenate was preincubated for 3 min at 37°C in Triton X-100 in a final concentration of 0.1%. Nonsedimented enzyme activity was investigated in supernatants obtained after centrifugation of the liver homogenate at 105,000g for 30 min on a "Superspeed-65" ultracentrifuge (from MSE, England). Activity of the enzymes was determined in the homogenate of the resulting supernatant and rat blood serum by a spectrophotometric micromethod [7]. The results were subjected to statistical analysis and Van der Waerden's nonparametric criterion was used for the evaluation of differences [12].

EXPERIMENTAL RESULTS

The total activity of all lysosomal hydrolases studied 15 min after intraperitoneal injection of serotonin into the rats was found to be significantly reduced in the liver compared with activity in intact animals (Table 1). The sharpest fall in activity was found with acid phosphatase and aryl sulfatases A and B (by 62 and 48%, respectively). After injection of

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TABLE 1. Total Activity of Lysosomal Enzymes in Liver (in μmoles/min/g wet weight of tissue) and Blood Serum (in μmoles/min/ml) at Different Times after Crushing of Soft Tissues of Rats Treated with Serotonin ($\overline{X} \pm S_{\overline{X}}$)

Test object	Enzyme	Intact animals	Control with serotonin	Fixation with vise (2-3 min)	Duration of crushing, h		
					½	1	1½
Liver	DNase	0,217±0,033	0,204±0,010	0,411±0,008	0,189±0,007*	0,187±0,003	0,212±0,002
	RNase	0,717±0,040	0,551±0,012*	0,789±0,010*	0,530±0,013*	0,709±0,008*	0,663±0,005*
	AcP	0,380±0,024	0,244±0,006*	0,197±0,001*	0,187±0,004*	0,283±0,004*	0,311±0,002*
	Aryl sulfatases A and B	0,474±0,023	0,248±0,003*	0,345±0,009*	0,348±0,006*	0,406±0,004*	0,392±0,008*
Blood	DNase	0,121±0,013	0,291±0,033*	0,398±0,003	0,337±0,022	0,324±0,017	0,271±0,011
	RNase	0,531±0,035	0,506±0,003*	0,583±0,015*	0,438±0,008*	0,358±0,012*	0,395±0,007*
	AcP	0,055±0,002	0,153±0,002*	0,190±0,003*	0,123±0,003*	0,134±0,004*	0,147±0,001*
	Aryl sulfatases A and B	0,142±0,006	0,187±0,003*	0,227±0,008*	0,239±0,003	0,270±0,003*	0,201±0,003*

Test object	Enzyme	Duration of crushing, h						
		2	2½	3	3½	4	4½	5
Liver	DNase	0,214±0,006	0,269±0,003*	0,328±0,001*	0,344±0,002*	0,315±0,005*	0,347±0,003*	0,360±0,003*
	RNase	0,787±0,013*	0,859±0,008*	0,947±0,013*	0,905±0,007*	0,909±0,008*	0,952±0,014*	0,956±0,007*
	AcP	0,326±0,002*	0,323±0,031*	0,321±0,001*	0,311±0,001*	0,295±0,010*	0,314±0,002*	0,312±0,004*
	Aryl sulfatases A and B	0,407±0,011*	0,443±0,001*	0,431±0,008*	0,482±0,002*	0,523±0,003	0,555±0,007*	0,556±0,001*
Blood	DNase	0,300±0,017	0,297±0,005	0,215±0,009	0,247±0,006	0,304±0,004	0,221±0,006	0,216±0,004
	RNase	0,422±0,004*	0,465±0,008*	0,421±0,002*	0,455±0,006*	0,556±0,011*	0,507±0,017*	0,598±0,020*
	AcP	0,125±0,004*	0,094±0,002*	0,086±0,002*	0,084±0,003*	0,095±0,002*	0,113±0,003*	0,113±0,001*
	Aryl sulfatases A and B	0,185±0,003*	0,313±0,006*	0,258±0,002*	0,249±0,011*	0,296±0,008*	0,286±0,005*	0,249±0,005*

Legend. Mean data of 10 experiments shown; *P < 0.01

TABLE 2. Nonsedimented Activity of Lysosomal Enzymes in Liver (in % of total activity) at Different Times after Crushing Soft Tissues of Rats Treated with Serotonin

Enzyme	Intact animals	Control with serotonin	Fixation with vise (2-3 min)	Duration of crushing, h		
				1/2	1	1 1/2
DNase	14,1±0,7	27,6±0,5*	28,4±0,6*	27,9±0,5*	28,1±0,7*	26,1±0,8*
RNase	9,5±0,8	9,9±0,6	10,2±0,7	10,0±0,6	9,9±0,7	9,8±0,4
AcP	6,7±0,2	12,9±0,4*	13,1±0,5*	13,0±0,6*	13,2±0,8*	13,4±0,7*
Aryl sulfatases A and B	1,5±0,1	4,4±0,7*	5,6±0,4*	5,4±0,2*	6,0±0,4*	5,9±0,6*

Enzyme	Duration of crushing, h						
	2	2 1/2	3	3 1/2	4	4 1/2	5
DNase	26,5±0,3*	26,7±0,8*	24,5±0,6*	25,1±0,4*	26,0±0,7*	24,8±0,5*	24,5±0,7*
RNase	9,4±0,7	9,5±0,4	9,9±0,5	9,4±0,7	10,0±0,6	10,0±0,7	10,1±0,8
AcP	13,0±0,6*	11,5±0,8*	11,2±0,4*	11,3±0,7*	11,8±0,6*	12,0±0,8*	12,1±0,6*
Aryl sulfatases A and B	5,4±0,1*	6,0±0,7*	5,7±0,6*	5,8±0,2*	6,1±0,9*	6,2±0,8*	6,0±0,7*

serotonin, the permeability of the lysosomal membranes in the liver increased, incidentally, as shown by the substantial rise in nonsedimented AcP and DNase activity (Table 2).

Short-term application of the vise to the rats' limbs (for 2-3 min) after preliminary injection of serotonin caused a marked increase in total activity of acid DNase, acid RNase, AcP, and aryl sulfatases A and B in the liver (by 150, 65, 37, and 164%, respectively) compared with the activity of these enzymes in rats receiving serotonin without traumatization, whereas the level of nonsedimented activity of all the enzymes except AcP was raised a little. The level of acid DNase and AcP activity in the blood serum (Table 1) was more than doubled, whereas activity of aryl sulfatases A and B amounted to 132% of their activity in the blood serum of intact animals.

It can thus be concluded that even short-term traumatization of the tissues is accompanied by active release of lysosomal hydrolases into the systemic circulation. In this connection, the regulatory role of serotonin in the mechanism of secretion of lysosomal enzymes into the extracellular medium cannot be ruled out.

The results are evidence that 30 min after compression of the soft tissues of the rat thigh after administration of serotonin, the total activity of acid nucleases in the liver was reduced, whereas the AcP level was substantially increased, and activity of aryl sulfatases A and B remained the same as in rats receiving serotonin alone. In the subsequent periods after trauma (1-5 h) total activity of all enzymes tested in the liver rose progressively, and toward the 5th hour of tissue crushing it was 1.5-2 times higher than in the control animals. The increase in acid nuclease activity found in the liver was evidently connected with a decrease in the degree of their involvement in nucleotide degradation, for an increase in the serotonin content in hepatocytes leads to a decrease in their content of DNA and RNA and in the intensity of protein synthesis [4, 11].

Under the influence of serotonin, destabilization of the lysosomal membranes was more marked in the early stages of traumatization (Table 2). In particular, in the period from 30 min to 2 h 30 min of tissue compression, nonsedimented DNase activity was around 203%. Later (3-5 h) intensive release of hydrolases from the hepatocyte cytoplasm took place into the systemic circulation. Nonsedimented activity of acid nucleases and of aryl sulfatases A and B rose most sharply. The intensive release of these enzymes through the lysosomal membranes into the hepatocyte cytoplasm, and then into the systemic circulation may perhaps take place through an increase in hypoxia of the liver tissue and a decrease in the energy reserves of the cells under the influence of serotonin. An increase in its content in the hepatocytes is known to inhibit respiration, oxidative phosphorylation, and electron transport in the cytochrome system [1, 3, 10, 13].

The results suggest that serotonin may have a significant effect on the biochemical status of the lysosomal system of the cell. Intraperitoneal injection of serotonin into rats reduces total and increases nonsedimented activity of hydrolytic enzymes of the lysosomes, with a simultaneous characteristic release of these enzymes into the systemic circulation. Under conditions of severe mechanical trauma serotonin increases the lability of the lysosomal membranes in the early period of traumatization and increases total activity of the enzyme during long-

term crushing of soft tissues in rats. The high blood enzyme levels were due mainly to activity of acid nucleases and aryl sulfatases A and B starting from the 3rd hour of tissue crushing and until the end of the experiment.

LITERATURE CITED

1. A. I. Basaeva, I. N. Pidevich, and M. F. Vyalykh, Byull. Éksp. Biol. Med., No. 10, 35 (1970).
2. V. N. El'skii, Vopr. Med. Khim., 23, 365 (1977).
3. A. M. Zubovskii, Vopr. Med. Khim., 14, 152 (1978).
4. V. I. Kocherga, "Effect of serotonin on nucleic acid metabolism in the brain of animals," Candidate's Dissertation, Kiev (1963).
5. G. N. Kryzhanovskii and S. Ya. Ostryi, Patol. Fiziol., No. 5, 24 (1967).
6. V. K. Kulagin, The Pathological Physiology of Trauma and Shock [in Russian], Leningrad (1978).
7. A. A. Pokrovskii, in: The Chemical Basis of Physiological Processes [in Russian], Moscow (1962), p. 311.
8. A. A. Pokrovskii and V. A. Tutel'yan, Biokhimiya, 33, 809 (1968).
9. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
10. V. I. Ovsyannikov, Kardiologiya, No. 1, 53 (1970).
11. L. F. Semenov, Experimental Prevention of Acute Radiation Sickness [in Russian], Leningrad (1967).
12. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).
13. Zh. A. Chotoev, "Effect of serotonin on oxidative and carbohydrate-phosphorus metabolism of heart muscle," Candidate's Dissertation, Moscow (1964).

EFFECT OF HETEROLOGOUS PROTEINS ON THE INITIAL STAGES OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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Human γ-globulin is widely used in medical practice for preventitive and therapeutic practice, especially in children. Injection of large doses of heterologous proteins (250 mg/kg body weight or more) into animals has been shown to increase the permeability of the endothelium of arteries and to accelerate the development of atherosclerosis in animals fed with cholesterol [6, 12, 13, 15]. Bovine serum albumin or whole blood serum was used as antigens in these investigations, and human γ-globulin, which is used in medical practice in the form of parenteral injections, was never once used for immunization. Yet there are indications in the literature that experimental atherosclerosis is not accelerated if animals are given injections of smaller doses of heterologous proteins [9, 14].

It was therefore decided to study how immunization with various doses of human γ-globulin (HGG) and also of bovine serum albumin (BSA), most frequently used in investigations of this kind, affects the development of experimental atherosclerosis in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 57 male rabbits weighing 2.5 kg. Hypercholesteremia was induced by daily administration of 500 mg cholesterol, dissolved in sunflower oil, via gastric tubes [3].

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