The hypothesis that blocking of serotonin extrusion from Ec cells plays a beneficial role is confirmed by investigations [5, 8] which demonstrated a connection between the fall in the serotonin level in the gastric mucosa and the frequency of gastric ulcers.

Considering data in the literature on the inhibitory effect of serotonin on HCl secretion and the stimulation of secretion of alkaline components of the mucus, it can be postulated that the increase in the number of detectable Ec cells and elevation of the serotonin level in the gastric mucosa of hyperthyroid rats, receiving injections of hydrocortisone together with dextran, is compensatory and adaptive in nature.

Intravenous injection of dextran with hydrocortisone, against the background of experimental hyperthroidism, thus reduces the percentage of lesions in the gastric mucosa by restricting serotonin release.

It is evident that an adequate concentration of serotonin in the stomach and, according to recent data [6], of melatonin also, is responsible for maintaining its structural integrity, and it increases the resistance of the gastric muscosa to the ulcerogenic action of glucocorticoids.

LITERATURE CITED

1. S. N. Fedchenko, Arkh. Anat., No. 12, 76 (1977).
2. S. N. Fedchenko, D. A. Maslakov, and A. V. Miron, Zdravookhr. Belorussii, No. 3, 41 (1980).
3. T. C. McAlpin, T. Moore, A. Cerlas, et al., Arch. Surg., 109, 65 (1974).
4. W. Rees, J. Rhodes, and M. Wheeler, Gastroenterology, 72, 67 (1977).
5. N. Sadao and S. Takeshi, Jpn., J. Pharmacol., 24, 241 (1974).
6. J. A. Smith, in: Melatonin: Current Status and Perspectives, Oxford (1981), pp. 113-118.
7. S. Snyder, J. Axelrod, and M. Zweig, Biochem. Pharmacol., 14, 831 (1965).
8. T. Tobe and C. Tanaka, Am. J. Gastroenterol., 58, 133 (1972).

EFFECT OF ACETYLCHOLINE ON LYSOSOMAL FUNCTION IN THE LIVER AND KIDNEYS OF RATS WITH SEVERE MECHANICAL TRAUMA

L. N. Kobylyanskii

UDC 617-001.32-036.17-092:[616.36+616.61]

KEY WORDS: acetylcholine; lysosomes of liver and kidneys; enzyme activity; mechanical trauma.

An important role in the pathogenesis of severe trauma, caused by crushing of soft tissues, is played by changes in the cholinergic system expressed primarily as general depression of its physiological activity and a disturbance of acetylcholine metabolism [1, 2]. As a clinical transmitter of nervous excitation in M- and N-cholinergic structures, acetylcholine affects the functional state of organs and tissues by regulating active transport of materials through cell membranes [10, 11]. Acetylcholine accumulation in synapses enchances the readiness of the organism for seizure activity and lowers its resistance to traumatic factors, whereas stimulation of acetylcholinesterase activity or exogenous preparations of this enzyme increase the resistance of animals to trauma [3, 4]. Consequently, direct correlation between the state of function of the cholinergic system and the resistance of the organism to traumatic factors can be postulated.

Department of Pathological Physiology, Kishinev Medical Institute.

[Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh (deceased).] Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 27-30, April, 1983. Original article submitted September 24, 1982.

0007-4888/83/9504-0434$07.50

TABLE 1. Changes in Total (in μmoles substrate/g tissue/min) and Nonsedimented (in % of total) Liver Lysosomal Enzyme Activity in Rats with Crushing of Soft Tissues after Preliminary Injection of Acetylcholine (M ± m, n = 6).

Enzyme	Control (intact animals)	After injection of acetylcholine	Short-term fixation	Duration of crushing, h									
				½	1	1½	2	2½	3	3½	4	4½	5
				Total activity									
Acid DNase	0,217±0,013	0,213±0,018	0,320±0,021*	0,274±0,021	0,276±0,023	0,305±0,021	0,346±0,027	0,326±0,021	0,445±0,032	0,398±0,028	0,430±0,031	0,450±0,032	0,519±0,037**
Acid RNase	0,717±0,040	0,691±0,029	0,700±0,036	0,736±0,031	0,803±0,039*	0,814±0,035	0,696±0,030	0,726±0,031	0,885±0,053	0,823±0,047	0,868±0,039	0,923±0,058	0,957±0,064**
Acid phosphatase	0,380±0,024	0,307±0,012*	0,340±0,019	0,334±0,021	0,327±0,020	0,310±0,021	0,318±0,017	0,304±0,019*	0,285±0,021	0,322±0,023	0,321±0,027	0,375±0,029	0,409±0,033
Arylsulfatases A and B	0,474±0,023	0,317±0,021*	0,330±0,029**	0,339±0,025*	0,340±0,031*	0,348±0,030*	0,256±0,019**	0,274±0,023**	0,422±0,030*	0,319±0,022**	0,328±0,027**	0,355±0,031*	0,462±0,032*
				Nonsedimented activity									
Acid DNase	14,1±0,7	14,3±0,7	17,4±1,0	14,4±0,6	14,1±0,5	14,2±0,7	14,2±0,6	14,1±0,4	14,3±0,5	14,2±0,6	14,1±0,7	14,3±0,4	14,9±0,6
Acid RNase	9,5±0,8	1,8±0,6*	11,4±0,7	10,3±0,5	10,3±0,4	10,2±0,6	10,4±0,5	10,3±0,4	10,2±0,6	10,4±0,7	10,6±0,3	10,5±0,8	10,7±0,4
Acid phosphatase	6,7±0,2	8,2±0,4*	9,1±0,3	9,0±0,5	9,0±0,4	9,4±0,6*	10,2±0,7**	10,7±0,6*	10,9±0,4**	10,9±0,7**	11,9±0,6*	13,4±0,7*	13,6±0,5
Arylsulfatases A and B	1,5±0,1	2,1±0,1***	3,2±0,2*	3,5±0,1	3,3±0,2*	3,7±0,3**	4,1±0,1**	4,4±0,2**	4,4±0,3***	4,8±0,4**	5,6±0,7*	6,2±0,4	6,7±0,3

Legend. Here and in Table 2: *P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 2. Changes in Total (in μmoles substrate/g tissue/min) and Nonsedimented (in % of total) Kidney Lysosomal Enzyme Activity in Rats with Crushing of Soft Tissues after Preliminary Injection of Acetylcholine (M ± m, n = 6)

Enzyme	Control (intact animals)	After injection of acetylcholine	Short-term fixation	Duration of crushing, h									
				½	1	1½	2	2½	3	3½	4	4½	5
				Total activity									
Acid DNase	0,358±0,026	0,347±0,021	0,400±0,028	0,336±0,018**	0,292±0,011**	0,293±0,015**	0,429±0,026	0,371±0,015	0,248±0,012**	0,339±0,019**	0,328±0,020**	0,338±0,021**	0,213±0,011*
Acid RNase	1,001±0,086	0,787±0,041*	0,890±0,061*	0,853±0,049	0,870±0,051	0,884±0,047	0,764±0,031	0,635±0,021*	0,923±0,037	0,670±0,028*	0,713±0,032*	0,776±0,030	1,038±0,058*
Acid phosphatase	0,394±0,023	0,204±0,011*	0,260±0,019***	0,220±0,011	0,223±0,010*	0,240±0,012	0,209±0,011	0,195±0,010*	0,271±0,017*	0,219±0,011*	0,233±0,015*	0,249±0,013*	0,345±0,021
Arylsulfatases A and B	0,697±0,064	0,510±0,021*	0,430±0,031	0,469±0,030*	0,465±0,034**	0,456±0,021	0,438±0,017	0,432±0,028*	0,390±0,016**	0,472±0,037**	0,479±0,029	0,476±0,031*	0,331±0,014**
				Nonsedimented activity									
Acid DNase	18,3±0,7	19,3±0,9	20,2±1,0	20,8±0,8	20,1±0,9	20,7±1,1	21,0±1,1	20,9±0,9	21,2±1,2	21,5±1,1	20,0±0,8	20,8±0,7	21,6±0,9
Acid RNase	12,1±0,5	14,0±0,4*	15,2±0,4	15,2±0,4	15,0±0,6	14,9±0,8	15,1±0,9	15,0±1,1	15,3±0,9	15,4±0,4	16,0±0,9	16,2±1,2	16,4±1,0
Acid phosphatase	9,8±0,3	12,0±1,3*	15,3±1,1	15,5±1,0	15,2±0,8	15,9±1,1	16,2±0,9	16,3±1,0	16,6±1,3	17,2±0,7	17,7±0,9	18,2±1,4	18,6±1,0
Arylsulfatases A and B	3,1±0,1	4,2±0,1**	5,0±0,2	5,2±0,3	5,2±0,4	5,1±0,2	5,4±0,3	5,3±0,2	5,5±0,4	5,7±0,7*	6,0±0,5	6,7±0,6	6,6±0,4

The aim of this investigation was to study lysosomal function in liver and kidney cells of rats with severe mechanical trauma caused by crushing of soft tissues against the background of acetylcholinesterase — a key component of the cholinergic system.

EXPERIMENTAL METHOD

Experiments were carried out on 78 male Wistar rats divided into 13 groups (six animals in each group). Of these 13 groups two served as controls: intact rats and rats receiving acetylcholine intraperitoneally in a dose of 0.001 mg/100 g body weight, decapitated 10 min later. Acetylcholinesterase activity was induced in the experimental rats by four injections of acetylcholine. The soft tissue of the rats' hind limbs was crushed for 2-3 min (short-term fixation), for 30 min, and thereafter the duration of traumatization was increased by 1.5 h in each subsequent series. The whole period of traumatization was 5 h. The soft tissues were crushed by means of special forceps [4]. To assess the effect of acetylcholine on the lysosomal system of the cells of the above-mentioned organs more completely, a modified model of long-term soft-tissue crushing was chosen — the forceps were not removed until the end of the experiment, thereby restricting generalization of toxic products.

At the end of the time of compression of the soft tissues the rats were decapitated, the liver and kidneys were removed and washed in cold physiological saline, and weighed samples were homogenized in a Potter—Elvehjem glass homogenizer with Teflon pestle in 0.25 M sucrose with 0.001 M EDTA [6]. To determine total activity of lysosomal hydrolases a homogenate of liver and kidneys was incubated for 3 min at 0°C in Triton X-100 in a final concentration of 0.1%. To investigate nonsedimented enzyme activity, the liver and kidneys homogenate was centrifuged for 30 min at 105,000g on a "Superspeed-65" ultracentrifuge (MSE, England), after which the translucent supernatant was separated.

Activity of four lysosomal hydrolases — acid DNase, acid RNase, acid phosphatase, and arylsulfatases A and B — was determined in the homogenate and supernatant obtained from rat liver and kidneys. Activity of these enzymes was determined [5, 7] by spectrophotometric micromethods, using as substrates high-polymer DNA (Koch-Light, England), high-polymer RNA (Sigma, USA), sodium β-glycerophosphate (Merck, West Germany), and p-nitrocatechol sulfate (Sigma, USA).

The results were subjected to statistical analysis [9].

EXPERIMENTAL RESULTS

The results of determination of total enzyme activity of the liver and kidneys of rats at different times of soft tissue trauma are given in Tables 1 and 2. A single injection of acetylcholine into the animals did not affect total activity of liver acid DNase and RNase, whereas activity of acid phosphatase and arylsulfatases A and B fell by 21-31% compared with their activity in the liver of intact (control) animals. Total activity of acid RNase, acid phosphatase, and arylsulfatases A and B in the kidneys fell significantly compared with that in intact animals. The opposite character of changes in enzyme activity in the liver and kidneys during short-term soft-tissue crushing is noteworthy. For instance, a significant rise in activity of acid RNase, acid phosphatase, and arylsulfatases A and B was observed in the kidneys, whereas in the liver, on the other hand, there was some decrease in total activity of acid DNase and RNase and of arylsulfatases A and B. The total activity of all the enzymes studied in the liver and kidneys 30 min after the beginning of traumatization differed by not more than 10-25% from that in animals not receiving acetylcholine. With an increase in the duration of traumatization, the changes in total lysosomal hydrolase activity in the liver and kidneys began to assume a distinctive appearance under the influence of acetylcholine. The most characteristic feature was a regular fall in total activity of all enzymes in the kidneys in the interval from 30 min to 4.5 h and of most liver hydrolases with the exception of acid DNase activity, the level of which was increased a little after the 3rd hour of trauma. By the end of the period of crushing a sharp increase (by 54 and 44%, respectively, in acid RNase activity was observed in both the liver and kidneys, with a smaller increase (by 33%) in acid DNase activity in the liver.

Two facts connected with the effect of acetylcholine on total lysosomal enzyme activity in animals with severe mechanical trauma were thus established: first, changes in opposite directions in activity of nearly all hydrolases during short-term crushing, and second, a regular fall in enzyme activity at subsequent stages of the experiment. The fall in enzyme activity after injection of acetylcholine into the animals is evidence of increased resistance

of cellular homeostasis as a whole and of the lysosomes in particular to the action of trauma. With the development of the traumatic process the effect of acetylcholine in ensuring stable function of the cholinergic system through induction of acetylcholinesterase also diminished. This was probably reflected in a selective increase in total lysosomal enzyme activity toward the 5th hour of crushing of the soft tissues. In turn, activation of enzymes in the kidneys during short-term crushing was evidently the result of an adaptive reaction mainly of the hypothalamic—hypophyseal—adrenal system to the extremal situation.

Investigation of nonsedimented lysosomal hydrolase activity in the liver and kidneys after injection of acetylcholine (Tables 1 and 2) revealed ill-defined but, in our view, important and similar changes. For instance, injection of acetylcholine led to a very small increase in nonsedimented activity of acid nucleases (by 6-16%) and a slightly greater increase in nonsedimented activity of acid phosphatases (by 22-40%). In the subsequent stages of trauma changes in nonsedimented acid phosphoesterase activity were found to depend on the duration of trauma: It fell progressively until the 3rd hour by 29-38%, after which the activity of these enzymes in the supernatants increased just as progressively, and after 5 h it differed from the control level by only 16%.

Analysis of the results leads to the following conclusions. Since evidence has been obtained that the harmful action of acetylcholine on the lysosomal membrane of choline creates a temporary excess of the compound, the interval of 10 min before sacrifice of the animals was not long eneough for complete induction of acetylcholinesterase. With an increase in the time after injection of acetylcholine, normalization of cholinergic and pathophysiological processes took place. This evidently explains the tendency of the lysosomal membranes to be more resistant if trauma to the soft tissues occurred after the triggering action of exogenous acetylcholine. With an increase in severity of the traumatic state the effect of activation of the cholinergic system was reduced, nonhydrolyzed acetylcholine accumulated, and ultimately this was reflected, in particular, in its active harmful action on the lysosomal membranes. Changes in nonsedimented acid phosphatase and arylsulfatase A and B activity reflect sufficiently clearly the general pattern of the action of acetylcholine as an inducer of the cholinergic system. So far as the differences in the degree of changes in nonsedimented lysosomal hydrolase activity are concerned, the main factor responsible can be considered to be the structural features of the lysosomes [8].

LITERATURE CITED

1. D. E. Al'pern, Cholinergic Processes in Pathology [in Russian], Moscow (1963).
2. G. Ya. Bazarevich, A. L. Kirillov, and G. N. Romanenko, Patol. Fiziol., No. 3, 12 (1976).
3. A. M. Ermakov, "The role of disturbances of cholinergic processes in the pathogenesis of traumatic shock," Author's Abstract of Candidate's Dissertation, Leningrad (1970).
4. V. K. Kulagin, The Pathological Physiology of Trauma and Shock [in Russian] Leningrad (1978).
5. A. A. Pokrovskii, in: Chemical Basis of Vital Activity [in Russian], Moscow (1962), pp. 311-331.
6. A. A. Pokrovskii and V. A. Tutel'yan, Biokhimiya, No. 4, 809 (1968).
7. A. A. Pokrovskii and A. I. Archakov, in: Modern Methods in Biochemistry, ed. V. N. Orekhovich [in Russian], Vol. 2, Moscow (1968), pp. 5-59.
8. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
9. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).
10. D. J. Cash and G. P. Hess, Anal. Biochem., 112, 39 (1981).
11. J. D. Fernstrom, Diabetologia, 20, Suppl., 281 (1981).
12. L. J. Ignarro, J. Slywka, and N. Krassikov, Life Sci., 10, 1309 (1971).
13. L. J. Ignarro, Nature New Biol., 336, 191 (1973).