EFFECT OF ADRENALIN ON LYSOSOMAL FUNCTION OF THE RAT KIDNEYS DURING LONG-TERM CRUSHING OF SOFT TISSUES

L. N. Kobylyanskii

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Adrenalin, the "emergency hormone," under extremal conditions can bring about the urgent reorganization of various systems of the body with a view to increasing its resistance. This sympathomimetic amine is considered to exert its effect on the plasma membranes of cells and organelles through cyclic AMP, which is synthesized in the cells by adenylate cyclase, activity of which is changed by the action of catecholamines [1, 9].

In the course of development of our research into the study of lysosomal enzyme activity in mechanical trauma, the investigation described below was undertaken in order to determine the effect of adrenalin on lysosomal hydrolase activity in the kidneys of rats during long-term crushing of the soft tissues. No information on this subject could be found in the literature.

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: intact rats and rats receiving an intraperitoneal injection of adrenalin in a dose of 0.1 mg/kg. Parenteral injection of adrenalin was used as a model of excitation of the sympathico-adrenal system. The animals of the remaining 11 groups were killed soon after trauma (fixation of forceps) and at different times thereafter. The total duration of trauma was 5 h. The experimental rats were injured by crushing the soft tissues of the hind limbs with special forceps [2]. To assess the effect of adrenalin on the lysosomes of the rat kidney cells more completely, the forceps were not

TABLE 1. Total Activity of Lysosomal Enzymes (in μmoles substrate/g tissue/min) in Kidneys

Enzymes	Control	Injection of adrenalin	Short-term fixation			
				½	1	1½
Acid DNase	0,358±0,026	0,660±0,015***	0,500±0,016**	0,451±0,023	0,436±0,064	0,451±0,012*
Acid RNase	1,001±0,086	1,210±0,065	1,200±0,027**	1,110±0,012*	1,206±0,011*	1,127±0,015**
AP	0,294±0,023	0,230±0,017*	0,310±0,014***	0,210±0,026	0,340±0,062	0,340±0,025**
Arylsulfatases A and B	0,697±0,064	0,769±0,016*	0,720±0,015**	0,801±0,017**	0,538±0,012**	0,826±0,018***

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

TABLE 2. Nonsedimented Lysosomal Enzyme Activity (in % of total activity) in Kidneys of Rats

Enzymes	Control	Injection of adrenalin	Short-term fixation			
				½	1	1½
Acid DNase	18,3±0,7	25,4±0,6*	24,6±0,4*	21,4±0,8	23,3±0,6*	23,8±0,7*
Acid RNase	12,1±0,5	13,0±0,4	16,0±0,7*	16,1±0,8*	16,0±0,4*	15,8±0,9*
AP	9,8±0,3	18,6±0,9*	17,6±0,9***	17,4±0,7***	18,1±0,6***	18,3±0,4***
Arylsulfatases A and B	3,1±0,1	6,7±0,4	6,4±0,3	6,3±0,4	6,1±0,3	6,0±0,4

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removed until the end of the experiment, thus restricting the generalized distribution of toxic products from the traumatized tissues.

At the end of the period of crushing the soft tissues, the rats were decapitated, the kidneys immediately removed and transferred into cold physiological saline, and a sample of tissue of the necessary weight was homogenized in 0.25M sucrose solution containing 1 mM EDTA [7]. To determine total enzyme activity the kidney homogenate was incubated (3 min) at 0°C in 0.10% Triton X-100. To investigate nonsedimented enzyme activity the kidney homogenate was centrifuged for 30 min at 105,000g on a "Superspeed-65" ultracentrifuge (from MSE, England), after which the translucent cytosol was separated.

Activity of four lysosomal enzymes was determined in the homogenate and cytosol; acid DNase, acid RNase, acid phosphatase (AP), and arylsulfatases A and B. Activity of these enzymes was determined by spectrophotometric micromethods [6, 10], using as substrate 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).

The results were subjected to statistical analysis [11].

EXPERIMENTAL RESULTS

As Table 1 shows, injection of adrenalin into intact animals increased DNase activity by 1.8 times, RNase activity by 1.2 times, activity of arylsulfatases A and B by 1.1 times, but reduced AP activity to 78% of the original value. These results are in agreement with modern views on adrenalin as a powerful inducer of various enzyme systems and, in particular, of lysosomal enzymes [8].

Analysis of the action of adrenalin on lysosomal enzymes of the kidneys of the crushed rats showed that crushing the soft tissues prevented the activating action of adrenalin on DNase. Injection of adrenalin into rats subjected to crushing had no additional stimulating action on acid DNase activity but, on the contrary, reduced it compared with the activity of the enzyme in the kidneys of rats exposed to only brief crushing of soft tissues. RNase activity in the kidney tissue, which was depressed by fixation of the forceps, subsequently increased by 10-21% compared with the intact control between 1.5 and 3 h of crushing. The degree of its activation, consequently, corresponded exactly to the degree of activation of RNase by adrenalin in the intact rats. The inducing effect of adrenalin on RNase was thus exhibited to the full under the conditions of tissue injury to which the animals were subjected.

of Rats with Crushing of Soft Tissues after Injection of Adrenalin (M ± m; n = 10)

Duration of crushing, h						
2	2½	3	3½	4	4½	5
1,501±0,062	0,421±0,013**	0,466±0,018***	0,515±0,014**	0,492±0,015**	0,498±0,012**	0,475±0,013***
1,201±0,019*	1,214±0,018*	1,213±0,012*	1,167±0,011*	1,114±0,011	1,192±0,063	1,231±0,017**
0,210±0,025	0,230±0,022	0,250±0,016*	0,290±0,061	0,320±0,022	0,320±0,022	0,290±0,008*
0,657±0,010*	0,644±0,017*	0,655±0,015*	0,682±0,013*	0,706±0,013***	0,673±0,066	0,671±0,026**

with Crushing of Soft Tissues after Injection of Adrenalin (M ± m; n = 10)

Duration of crushing, h						
2	2½	3	3½	4	4½	5
24,2±0,6*	23,9±0,4*	24,1±0,7*	23,7±0,6*	23,2±0,8*	24,0±0,7*	24,5±0,6**
16,5±0,4**	15,9±0,8*	16,6±0,7**	16,2±0,4*	16,7±0,5**	16,4±0,7*	17,0±0,6**
18,5±0,6***	18,1±0,3***	18,2±0,9***	19,3±0,6***	19,0±0,4***	18,9±0,6***	19,3±0,4***
6,1±0,7	6,3±0,4	6,9±0,6	7,4±0,5	7,3±0,4	7,2±0,6	7,6±0,4

The effect of adrenalin on activity of renal arylsulfatases A and B differed: Whereas in the initial states of crushing of the soft tissues (before 1.5 h) it mainly enhanced activity of the enzyme compared with intact rats, in the later stages of the experiments (from 2 to 5 of crushing), on the contrary, it depressed it.

Adrenalin similarly had different effects on AP activity. During short-term fixation of the forceps and crushing for 1-1.5 h, injection of adrenalin increased AP activity a little compared with the control without trauma. Meanwhile, from 2 to 3.5 h after the beginning of crushing, adrenalin, on the other hand, reduced activity of the enzyme a little compared with that in kidneys of intact rats. The regular peak of increasing AP activity was observed between 1 and 4.5 h of tissue crushing.

The results of investigation of nonsedimented activity of the lysosomal enzymes of the rat kidneys are given in Table 2. They show that injection of adrenalin into intact rats was accompanied by an increase in activity of arylsulfatases A and B by 2.2 times — by 1.9 times compared with the activity of these enzymes in the cytosol from the kidneys of control rats. Activity of acid DNase and acid RNase was increased by a lesser degree — to 139 and 107% of the control respectively.

Crushing the soft tissues after preliminary parenteral injection of adrenalin was accompanied by a significant increase in nonsedimented activity of lysosomal enzymes in the kidneys. In particular, during short-term crushing, activity of arylsulfatases A and B and of AP rose sharply (it was doubled). There was a less marked increase in acid DNase (by 34.4%) and acid RNase (by 32.2%) activity. With an increase in the duration of crushing the level of nonsedimented activity of these enzymes showed little change before the end of the period of observation, namely a very small decrease in acid DNase activity (from 30 to 60 min) and in activity of arylsulfatases A and B (after crushing for 1-2 h), followed by an increase in their activity up to the level at the time of fixation of the forceps, accompanied by a small but progressive increase in AP activity with an increase in the duration of crushing (from 17.6 during short-term crushing to 19.3% in the terminal phase).

Injection of adrenalin thus caused considerable changes in total and nonsedimented activity of the lysosomal enzymes of the kidney both in intact animals and in rats with trauma. In intact animals adrenalin induced general activity of acid nucleases and arylsulfatases and reduced AP activity; the action of adrenalin, moreover, was significantly stronger in respect of acid DNase than of the other enzymes. Nonsedimented activity of hydrolases weakly bound with the lysosomal membranes, namely AP and arylsulfatases A and B, increased considerably in the kidneys of rats exposed to soft tissue crushing after preliminary injection of adrenalin.

These results can be regarded as further experimental confirmation of views on the trophic function of the sympathicoadrenal system, linked with its participation in the control of intracellular metabolism [3-5].

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