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.

LITERATURE CITED

1. A. J. Barrett and M. P. Heath, in: Lysosomes [Russian translation], Moscow (1980).
2. P. Syatkin, Vopr. Med. Khim., No. 1, 136 (1981).
3. V. A. Frolov, V. A. Kapustin, and M. Drogova, Patol. Fiziol., No. 4, 136 (1986).
4. V. A. Frolov and B. K. Shkirmante, Byull. Éksp. Biol. Med., No. 5, 434 (1990).
5. C. Ruth, F. F. Kennet, and W. B. Weglicki, J. Molec. Cell. Cardiol., **10**, No. 8, 739 (1978).

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

I. Yu. Malyshev and F. Z. Meerson

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KEY WORDS: adaptation to stress; heart; antibiotics.

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

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

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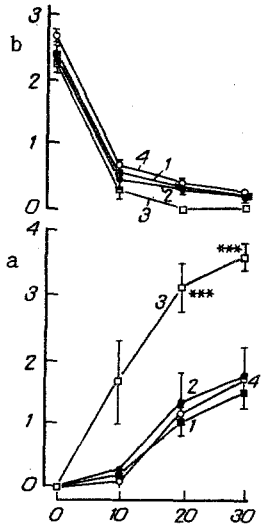

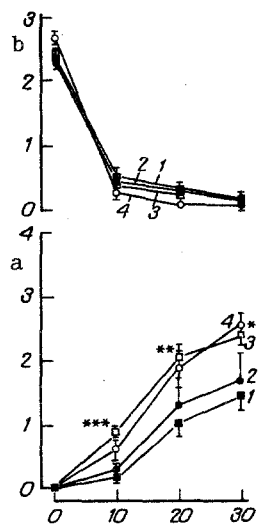

Fig. 1 Fig. 2

Fig. 1. Effect of adaptation of animal to stress on toxic effects of rifampicin, an RNA-polymerase blocker. Here and in Fig 2: abscissa, time after beginning of action of ischemia (in min); ordinate, parameters of mechanical activity of isolated heart (a, b; in mm change of apicobasal length of heart). Significance of differences from control: ***$p < 0.001$ 1) Control (n = 7), 2) adaptation (n = 5), 3) rifampicin (n = 5), 4) adaptation + rifampicin (n = 5).

Fig. 2. Effect of adaptation to stress on toxic effects on polymyxin B, a protein kinase C blocker. Significance of differences from control: *$p < 0.05$, **$p < 0.01$, **$p < 0.001$ 1) Control (n = 7), 2) adaptation (n = 5), 3) polymyxin B (n = 5), 4) adaptation + polymyxin B (n = 5).

The animals were heparinized (2000 U/kg, intraperitoneally) and anesthetized with pentobarbital (50 mg/kg, intraperitoneally). The heart was then quickly removed and perfused on a Langendorff system. A constant coronary flow at the rate of 10 ml/min was created by means of a peristaltic pump, and the heart rate was set at 5 Hz. The remaining conditions of perfusion were described previously [6]. The amplitude of contractions and of contracture was recorded by means of a TD-112S isotonic transducer ("Nihon Kohden"), attached to the apex of the heart [7]. Toxic effects of the antibiotics were reproduced most clearly on the ischemic heart Ischemia was created by Currie's method [6], by lowering the coronary flow rate from 10 to 1 ml/min. Toxic effects of the antibiotics were assessed by measuring the additional reduction of the amplitude of contractions and of contracture. The experimental results are given in the form M ± m. The significance of differences between groups was assessed by Student's test.

EXPERIMENTAL RESULTS

The curves in Fig. 1 show that the amplitude of contractions of the normal, nonischemic hearts in the control, during adaptation, in the rifampicin group, and in the adaptation plus rifampicin group did not differ (see Fig. 1a, at time 0 min). After the creation of ischemia the amplitude of contractions of the control hearts gradually decreased, and toward the 30th minute it was very low, about 0.17 mm. Against the background of the significant reduction of amplitude of the contractions in the hearts of the control animals, marked contracture developed during ischemia, and by the 30th minute it amounted to 1.51 ± 0.29 mm (Fig. 1b).

The isolated hearts of animals receiving rifampicin and of the adapted animals receiving this antibiotic at the same time, did not differ in the time course of ischemic depression of the amplitude of contraction from that in the control group.

The toxic effect of rifampicin and similarly, adaptive protection were assessed in relation to the contracture parameter: on the 30th minute of ischemia contracture of the hearts of animals receiving rifampicin was 2.5 times greater than in the control (Fig. 1b). Adaptation to stress did not affect ischemic contracture of as such, but at the same time, it completely prevented the development of additional contracture in the hearts of the animals receiving rifampicin over a longer period of time.

Adaptation to stress thus effectively protects the heart against the toxic action of rifampicin. This means that an increase in the resistance of the organ to the damaging effect of antibiotics may be an important component of the phenomenon of ASS.

We next assessed the effect of adaptation to stress on resistance of the heart to another antibiotic, namely polymyxin B. The results are given in Fig. 2. Clearly, in the groups compared (control, adaptation, polymyxin B, and adaptation + polymyxin B) ischemia also led to the virtually complete disappearance of the amplitude of contractions (Fig. 2a). Against this background the toxic effect of significant increase of ischemic the protein kinase C inhibitor was realized as contracture compared with the control (Fig. 2b). For instance, in the hearts of the animals receiving polymyxin B, contracture at the 20th and 30th minutes of ischemia was 2.03 ± 0.11 and 2.38 ± 0.16 mm respectively, 1.5-2 times greater than in the control. Adaptation to stress did not lead to restriction of additional contracture, which developed in the ischemic hearts of the animals receiving polymyxin B (Fig. 2b).

The effect of adaptation to stress on the resistance of the heart to the toxic action of the antibiotics is thus differential in character: adaptation limits damage caused by transcription inhibitor rifampicin but is ineffective against the toxic effects of the protein kinase C inhibitor, polymyxin B.

The target for rifampicin is known to be the β-subunit of RNA polymerase [13]. Binding of the antibiotic with this subunit leads to a disturbance of formation of the first phosphodiester bond in the RNA chain and to specific inhibition of the initiation of RNA synthesis. Our experiments showed that in the normal, nonischemic heart, the toxic effects of rifampicin are not manifested, but in the ischemic heart they are completely manifested in the form of additional contracture, so that the consequences of the disturbance of protein synthesis become evident. The protective effect of adaptation against injuries caused by rifampicin may probably be linked with the formation of a mechanism, protecting transcription or, more precisely, preventing inhibition of the β-subunit of RNA polymerase, at the nuclear level. The protective effects of adaptation may perhaps be explained by involvement of heat shock proteins – hsp 70 The writers showed previously that during adaptation to stress the concentration of these proteins in the cytoplasm and nucleus of cardiomyocytes is considerably increased [2, 10]. It has also been shown that hsp 70 can bind with abnormal or damaged proteins of chromatin [15]. These data as a whole suggest that hsp 70 could recognize the rifampicin – RNA polymerase complex as an abnormal protein and bind with it. Binding of hsp 70 with an abnormal structure is known to be accompanied by a change in the conformation of hsp 70, which is transmitted to the substrate protein [11], in this case RNA polymerase. The possibility cannot be ruled out that a change in the conformation of the subunits of the enzyme leads to weakening of the bond of the antibiotic with the β-subunit, or sometimes to dissociation of the inhibitor. The hsp 70 then become detached from the RNA polymerase, utilizing the energy of ATP hydrolase [11]. This hypothesis requires serious experimental confirmation, but one fact is beyond question, namely that RNA polymerase is probably yet another nuclear structure at the level of which the ASS is realized.

The results of these experiments also showed that adaptation to stress did not prevent the toxic effect of polymyxin B. This may be because hsp 70 do not exhibit substrate specificity toward protein kinase C [12].

LITERATURE CITED

1. I. Yu. Malyshev, Byull. Éksp. Biol. Med., No. 4, 411 (1989).
2. I. Yu. Malyshev, A. V. Zamotrinskii, and F. Z. Meerson, Byull. Éksp. Biol. Med. (1991).
3. F. Z. Meerson, Kardiologiya, No. 3, 6 (1990).
4. F. Z. Meerson and I. Yu. Malyshev, Dokl. Akad. Nauk SSSR, **313**, No. 3, 750 (1990).
5. F. Z. Meerson, I. Yu. Malyshev, E. Yu. Varfolomeeva, et al., Byull. Éksp. Biol. Med., No. 5, 460 (1991).
6. R. W. Currie, M. Kamarzyn, M. Kloc, et al., Circulat. Res., **63**, 543 (1988).

7. W. H. Gilst and J. M. Koomen, Basic Res Cardiol., **82**, 233 (1987).
8. F. Z. Meerson, I. Yu. Malyshev, and T. G. Sazontova, Basic Res. Cardiol., **85**, 96 (1990).
9. F. Z. Meerson, Adaptive Protection of the Heart: Protecting Against Stress and Ischemic Damages, CRC Press, Boca Raton, Florida (1991).
10. F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinskii (A. V. Zamotrinsky), Basic Res. Cardiol., **86**, 87 (1991).
11. H. R. B. Pelham, Cell, **46**, 959 (1986).
12. M. J. Schlesinger, J. Biol. Chem., **265**, 12111 (1990).
13. L. Stryer, Biochemistry, San Francisco (1975).
14. W. J. Welch and J. P. Suhan, J. Cell. Biol., **103**, 2035 (1986).

CHANGES IN PARAMETERS OF THE AFTER-DISCHARGE IN THE RAT SENSOMOTOR CORTEX AFTER CESSATION OF CONDITIONING REPETITIVE ELECTRICAL STIMULATION

G. N. Kryzhanovskii, V. K. Lutsenko, N. N. Khlebnikova, and M. Yu. Karganov

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KEY WORDS: rat cerebral cortex; electrical stimulation; increased predisposition to seizures

It was shown previously that repetitive above-threshold electrical stimulation (ES) of the sensomotor cortex, in the form of successive series, induces the formation of generators of pathologically enhanced excitation (GPEE) [1]: primary – in the zone of ES, and secondary – dependent, determined by transcallosal synaptic stimulation – in the homotopic zone of the opposite hemisphere [2]. A characteristic feature of the manifestation of activity of a GPEE in the cerebral cortex, which is an epileptic focus, is the prolonged self-maintained poststimulus after-discharges (AD), consisting of spike-wave complexes, the number, amplitude, and frequency of which increased in the course of repetitive stimulation. These features point to structural transformations and to an increase in predisposition to seizures in the neuron population constituting the GPEE.

It was decided to study whether prolonged ES is necessary in order to preserve the acquired properties of the neuron population of GPEE described above or whether these properties can be continued even after cessation of ES. For this purpose parameters of AD arising in the course of consecutive series of repetitive electrical stimulations (RES), were compared on the 1st day and again 24 h after the last (20th) series of RES.

EXPERIMENTAL METHOD

The operative technique, the parameters of single and repetitive stimulation of the sensomotor cortex, and the arrangement of the electrodes and method of recording the electrocorticogram were all described previously [2]. Threshold values of stimulation to evoke direct (DR) and transcallosal (TCR) responses were determined 20-30 min before the first series of RES and 10 min after the end of AD arising immediately after the last (20th) series of RES. After the end of the experiment, on the 1st day the exposed areas of the sensomotor cortex were flooded with sterile physiological saline and covered with polyethylene film, which was secured to the cranial bones with phosphate cement. The film was removed after

Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 346-347, October, 1991. Original article submitted July 10, 1990.

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