Adaptation to Multiple Electrostimulation Limits Hemorrhages in the Brain of Rats with Audiogenic Epilepsy

F. Z. Meerson, V. G. Pinelis, V. B. Koshelev, T. V. Ryasina, T. P. Storozhevykh, T. B. Mareeva, E. N. Arsen'eva, E. Ya. Vorontsova, and O. E. Fadyukov

UDC 616.853-06:616.831-005.1-02:615.844]-092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 2, pp. 127-129, February, 1994
Original article submitted July 19, 1993

Exposure of Krushinsky-Molodkina rats (a strain genetically predisposed to audiogenic epilepsy) to multiple electrostimulation (a course consisting of 10 sessions) prolongs the latency of epileptic seizures, lowers blood levels of stress hormones, lessens the severity of seizures, and reduces by half the area occupied by subdural hemorrhages. It is concluded that the major role in the mechanism of these protective effects is played by adaptation to stress, accompanied by the accumulation in the brain of heat-shock proteins that stabilize cellular structures.

Key Words: *electrostimulation; hemorrhage; audiogenic epilepsy*

Adaptation to stress has been shown to increase the resistance of animals not only to stress-induced injuries but also to cardiac fibrillation, acute ischemia, and myocardial infarction and to sharply reduce mortality among animals with sublethal hypoxia [6,9].

Similar results have been obtained with "mild" electrostimulation (ES), which adapts the animals to stress [7,8]. The influence of preadaptation to multiple ES on the severity of audiogenic epilepsy and of the brain hemorrhages associated with it [3,10], has not been investigated. However, a loud noise, which induces convulsive seizures in Krushinsky-Molodkina (KM) rats (a strain genetically predisposed to audiogenic epilepsy), is certainly an agent of stress, and adaptation of these rats to short-term stressors such as multiple ES may therefore be expected to make them more resistant to audiogenic epilepsy.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow; Institute of Pediatrics, Russian Academy of Medical Sciences, Moscow; Moscow State University

The purpose of this work on KM rats was to evaluate how adaptation to short-term stress agents (multiple ES) might influence the severity of epileptic seizures, the extent of hemorrhages, ATP levels in the brain, and the magnitude of the stress reaction accompanying convulsions in these rats.

MATERIALS AND METHODS

A total of 39 male KM rats genetically prone to audiogenic epilepsy were used, divided into 4 groups: group 1 comprised 8 intact rats and group 2, 11 rats exposed to sound in order to elicit convulsions. Groups 3 and 4 included 20 rats that were subjected to transauricular ES daily for 10 days, after which audiogenic epilepsy was induced in 12 of these rats.

For ES, standard needles for facial acupuncture were introduced into a particular point on the auricular concha of each ear. The needles were then connected to a Lasper CS-504 electrostimulator (Japan). A course of ES consisted of 10 sessions each lasting 10 min on the first day and 20 min on the following nine days. ES was carried

0007-4888/94/0002-0125$12.50

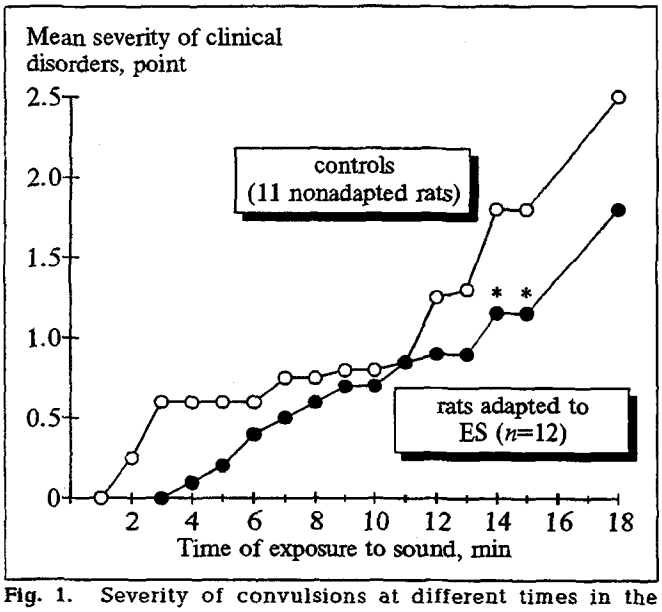

Fig. 1. Severity of convulsions at different times in the course of acoustic stimulation. Asterisks indicate significant differences from nonadapted rats.

out with solitary sawtooth pulses of 1.5-2 mA in amplitude and 1.5 sec in duration.

Rats were exposed to electric bell sounds in a box as follows: a sound of 115 dB for 1.5 min followed by alternating loud (115 dB) and softer (85 dB) sounds of 10 sec each at 10 sec-intervals for a total of 15 min, followed by a 3-min interval after which the animals were again exposed to a loud sound for 1 min. In the first 1.5 min of exposure, the latency before the onset of a convulsive seizure and the intensity of the latter were recorded. During the subsequent 15 min of intermittent exposure, the rats developed behavioral abnormalities whose severity was rated on a three-point scale.

Immediately after the termination of exposures to sound, the rats were decapitated and their blood was collected into cooled test tubes for biochemical analysis, the cerebellum was rapidly removed for biochemical study, and the rest of the brain was fixed in 10% formalin for subsequent assessment of the frequency and severity of intracranial hemorrhages. The hemorrhages were rated macroscopically as either severe (extensive confluent hemorrhages - 3 points), moderate (pronounced hemorrhages but limited in area - 2 points), or mild (occasional hemorrhages on the brain surface - 1 point). Rats without hemorrhages were assigned 0 points. The area of subdural hemorrhages was calculated in mm^2 on photographs using a Pericolor 1000 system (France).

The method used to determine ATP was essentially similar to that described previously [12], with some modifications. The cerebellar cortex (100-120 mg) was homogenized in the cold in 2 ml of 0.01 M EDTA (pH 7.6), and ATP was extracted from the homogenate with 0.6 N $HClO_4$. The supernatant was neutralized with 1 M K_2CO_3 and the sediment, after removal of salts, was kept frozen at -40°C until assay. ATP concentration was determined on an LKB bioluminometer (Sweden) using the luciferin-luciferase test [19]. Cortisol in the peripheral blood was determined by radioimmunoassay.

The results were treated statistically using Student's t test or Wilcoxon-Mann-Whitney's U test.

RESULTS

The adaptation to multiple ES significantly prolonged the latency of convulsions and also decreased their severity as assessed at minute 15 after their onset (Table 1). The severity of convulsions at different times of acoustic stimulation is shown in Fig. 1. It can be seen that the curve for adapted rats has all points but one lying below the corresponding points on the curve for nonadapted animals, and that the anticonvulsive effect of adaptation was significant at minutes 3, 4, 13, and 15. The decreases in the severity of the convulsive syndrome in the adapted rats were paralleled by decreases in ACTH and cortisol levels in these rats - from 32.4±2.6 to 21.8±1.3 pg/ml and from 43.9±3.8 to 23.9±3.5 pg/ml, respectively ($p<0.05$). This latter factor may also have played

TABLE 1. Effect of Multiple Electrostimulation (ES) on Selected Parameters Characterizing Resistance of KM Rats to Audiogenic Epilepsy

Group	Latency of epileptic seizure, sec	Severity of seisures (points)	ATP level in cerebellar tissue, ng/mg protein
Intact rats ($n=8$)	–	–	5.1±0.9
After multiple ES ($n=8$)	–	–	4.9±0.7
Audiogenic epilepsy ($n=11$)	3.8±0.6	2.3±0.1	3.2±0.3*
Multiple ES + audiogenic epilepsy ($n=12$)	4.8±0.3	1.3±0.2*	5.1±0.5**

Note. Asterisks indicate significance of differences ($p<0.05$): *from rats not exposed to multiple ES; **from intact rats.

TABLE 2. Effect of Adaptation to Multiple Electrostimulation (ES) on the Severity of Cerebral Hemorrhage (in %) and the Mean Hemorrhagic Area in KM Rats

Group	Degree of hemorrhage				Mean hemorrhagic area, mm²
	severe	moderate	mild	none	
Audiogenic epilepsy ($n=16$)	37.5 (6)	43.5 (7)	12.5 (2)	6.3 (1)	54.3±9.4
ES + audiogenic epilepsy ($n=12$)	8.3* (1)	33.2 (4)	41.5* (5)	16.6* (2)	27.1±7.9*

Note. Asterisk indicates a significant difference ($p<0.05$) from the audiogenic epilepsy group. Figures in parentheses are the numbers of rats.

a role in limiting the manifestations of audiogenic epilepsy.

The decreases in cerebellar ATP during audiogenic convulsions were considerable, but they were almost completely prevented by the adaptation (Table 1).

Thus, the adaptation to multiple ES, like that to other short-term stressors, prolonged the latency of seizures and limited their severity, and it also prevented the fall of cerebellar ATP, which is an important indicator of impaired energy metabolism in the brain.

The data in Table 2 make it possible to assess the effect of adaptation to multiple ES on the incidence of severe, moderate, and mild subdural hemorrhages arising during an attack of audiogenic epilepsy and on the mean area occupied by the hemorrhages. Thus, the adaptation reduced more than fourfold the number of rats with severe hemorrhages and also decreased the number of those with moderate hemorrhages, while more than doubling the number of those with mild hemorrhages. In the ES-adapted group, the mean hemorrhagic area was only half that in the nonadapted group. Multiple ES thus proved to be an important factor of protection against epileptogenic subdural hemorrhages.

Taken as a whole, the results of this study indicate that adaptation to stress in the form of ES was effective in limiting the manifestations of audiogenic epileptic seizures and even more so in protecting the brain from their consequences, such as subdural hemorrhages.

Circulation in the brain of KM rats is impaired because of the rupture of its veins [1-3,10], which is preceded by an increase in cerebral blood flow [12,13] and a considerable elevation of arterial blood pressure [3,18]. It appears that rupture of venous walls may result not only from their direct damage by stress but also from the stress-induced rise of blood pressure in the veins. This possibility is supported by the clinical observation that the incidence of stroke in stressful situations is high and by evidence of impaired reactivity of large veins in stress [4]; stress has been found to have a greater damaging effect on the venous than on the arterial wall [14,17].

The foregoing suggests that, apart from the well-known ischemic disease of the brain caused by atherosclerosis of its vessels [19], an important factor is likely to be stress-induced cerebral circulatory disorders which may form the basis of another nosologic entity.

In evaluating the mechanism of the protective effects of adaptation to stress, an important consideration is the development, during this adaptation, of adaptive stabilization of structures - a phenomenon which underlies the above-mentioned protective cross-effects from such adaptation. One manifestation of this phenomenon of adaptive stabilization of structures (PASS) is the high resistance of isolated hearts from adapted animals to ischemia and reperfusion, toxic doses of catecholamines, high calcium concentrations, and heat shock [9]. Organelles extracted from the cardiomyocytes of such hearts have proved to be highly resistant to autolysis [7,15,16]. Also, functions of the portal vein in stressed animals are grossly impaired, but isolated portal veins from animals were found to be well protected if the animals had been adapted to repeated short-term immobilization stress [17]. It has been shown that at the basis of PASS and of all of its diverse manifestations may be the accumulation in the brain and effector organs of so-called heat-shock proteins having molecular weights of 70-72 kD [15,16]. These proteins are able to disperse denatured proteins and thus prevent or limit damage to cellular structures. The observed adaptive protection from subdural hemorrhages afforded by a course of ES was probably associated with the development of PASS at the level of venous walls in the brain.

The finding that a course of ES can offer effective adaptive protection may well be of practical relevance, for the electrostimulation in this study was carried out with a stimulator used in clinical practice and did not elicit defensive reactions because it was painless.Mean severity of clinical disorders (points).

REFERENCES

1. S. G. Vlasov, I. V. Storozhenko, A. M. Khak, *et al.*, *Fiziol. Zh. im. I. M. Sechenova*, **77**, № 4, 52-58 (1991).
2. L. V. Krushinskii and L. N. Molodkina, *Dokl. Akad. Nauk SSSR*, **66**, № 2, 289-292 (1949).
3. L. V. Krushinskii, T. I. Ryasina, V. B. Koshelev, *et al.*, *Fiziol. Zh. im. I. M. Sechenova*, **75**, № 11, 1576-1584 (1989).
4. E. B. Manukhina, *Usp. Fiziol. Nauk*, **19**, № 3 (1988).
5. F. Z. Meerson and M. G. Pshennikova, *Adaptation to Stress and to Physical Loads* [in Russian], Moscow (1988).
6. F. Z. Meerson, V. P. Pozharov, *et al.*, *Byull. Eksp. Biol. Med.*, **115**, № 4, 339-342 (1993).
7. F. Z. Meerson and I. Yu. Malyshev, *The Phenomenon of Adaptive Stabilization of Structures, and Protection of the Heart* [in Russian], Moscow (1993).
8. F. Z. Meerson, M. G. Pshennikova, B. A. Kuznetsova, *et al.*, *Byull. Eksp. Biol. Med.*, **117**, № 1, 16-18 (1994).
9. F. Z. Meerson, *Kardiologiya*, № 3, 3-12 (1990).
10. S. V. Nichkov and G. N. Krivitskaya, *Acoustic Stress and Cerebrovisceral Disorders* [in Russian], Moscow (1969).
11. H. K. Beutler and G. Michal, in: *Methods of Enzymatic Analysis*, Vol. 4, Acad. Press, New York-London (1974), pp. 1708-1713.
12. A. C. Ludolph, M. Seelig, A. G. Ludolph, *et al.*, *Ann. New York Acad. Sci.*, **648**, 300-302 (1992).
13. W. G. Mayman and D. D. Heisted, *Circulat. Res.*, **59**, № 1, 216-220 (1986).
14. F. Z. Meerson, *Adaptive Protection of the Heart: Protecting against Stress and Ischemic Damage*, CRC Press, Boca Raton (1991).
15. F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinsky, *Basic Res. Cardiol.*, **86**, 87-98 (1991).
16. F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinsky, *Canad. J. Cardiol.*, **8**, № 9 (1992).
17. F. Z. Meerson and E. B. Manukina, *Basic Res. Cardiol.*, **80**, 407-416 (1985).
18. B. S. Meldrum and B. Nilson, *Brain*, **99**,№3, 523-542 (1976).
19. B. K. Siesijo, *Brain Energetic Metabolism*, New York (1978).

Ca-Transporting System of the Left Ventricular Sarcoplasmic Reticulum in the Rat Heart and Damage to Its Membrane During Ischemia and Reperfusion

T. G. Sazontova, L. M. Belkina, Fu Syan Zun, and F. Z. Meerson

UDC 616.127-005.4-07:616.124.2]-092-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 2, pp. 130-135, February, 1994
Original article submitted November 15, 1993

Ischemia and reperfusion of various duration are shown to result in a nonlinear increase in the level of free Ca in myocardial homogenates. A striking dissociation has been observed in the effect of ischemia and reperfusion on the rate of Ca transport in the sarcoplasmic reticulum, on the one hand, and the permeability of its membranes on the other.

Key Words: *sarcoplasmic reticulum; ischemia; reperfusion*

A considerable increase in the concentration of free Ca in ischemia is one of the most important mechanisms by which ischemia and reperfusion exert their damaging effects, because excess Ca can not only induce the development of contracture but also lead to the activation of destructive cellular enzymes, namely proteases [13,26], lipases, and phospholipases [1,3,5,26]. This aggravates the damage to intracellular, including membrane, structures. Ca accumulation in cardiomyocytes in ischemia and especially during reperfusion is the overall result of several processes, the prime ones being damage to the sarcolemma and the entry of Ca

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/94/0002-0128$12.50