EFFECT OF PRELIMINARY ADAPTATION ON ACCUMULATION OF ^{99m}Tc-PYROPHOSPHATE AND DEVELOPMENT OF STRUCTURAL CHANGES IN HEART MUSCLE DURING STRESS

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Previous investigations showed that preliminary adaptation of animals to physical exertion and to short exposures to stress prevents the overexcitation of the adrenergic and pituitary-adrenal systems, disturbances of energy metabolism, and depression of cardiac contractility, characteristic of prolonged emotional stress [2, 3, 7, 8].

Meanwhile the problem of ability to prevent stress-induced structural disturbances of the myocardium by preliminary adaptation, and the use of radionuclide methods to assess the protective effect of adaptation, whereby the degree of both ischemic [11] and stress-induced [13] damage to the heart can be quantitatively determined, remains largely unsolved.

This paper compares the results of the effect of preliminary adaptation of animals to physical exertion and to short periods of stress on the severity of stress-induced changes in structure of the cardiomyocytes and accumulation of ^{99m}Tc-pyrophosphate by the heart muscle during long-term emotional stress.

EXPERIMENTAL METHODS

Experiments were carried out on 108 male albino rats weighing 180-220 in the fall and winter. The animals were divided into six groups: 1) control, 2) animals subjected to prolonged emotional stress, 3 and 4) animals adapted to physical exertion, and animals so adapted and then exposed to stress 1 day later, respectively, 5 and 6) animals adapted to short periods of stress and animals so adapted and later subjected to long-term stress. Emotional stress for 6 h was produced in the form of an anxiety neurosis by the method described previously [5]. The animals were adapted to physical exertion for 45 days (swimming at 32°C

TABLE 1. Effect of Preliminary Adaptation on ^{99m}Tc-Pyrophosphate Accumulation by the Heart during Stress (M ± m)

Experimental conditions	Index of ^{99m}Tc-pyrophosphate accumulation by the heart
Control	0.06 ± 0.05
Stress	0.27 ± 0.015†
Adaptation to physical exertion	0.058 ± 0.004
Adaptation to physical exertion + stress	0.086 ± 0.01*
Adaptation to short periods of stress	0.056 ± 0.006
Adaptation to short periods of stress + long-term stress	0.075 ± 0.009

Legend. Nine determinations in each experiment. *P < 0.05, †P < 0.001 compared with control.

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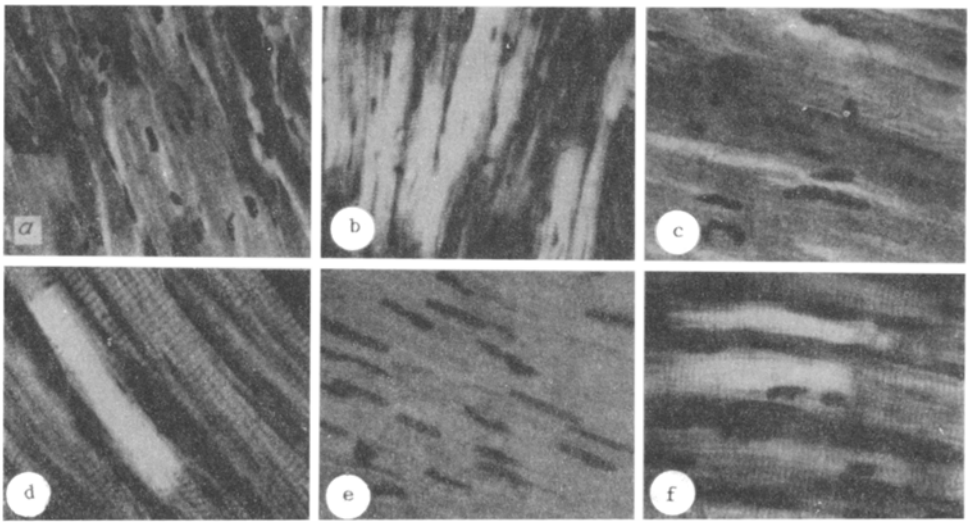

Fig. 1. Effect of preliminary adaptation on morphological changes in rat myocardium during stress: a) Stress. Perls' reaction. 126×; b) The same. Polarization microscopy. 126×; c) Adaptation to physical exertion + stress. Perls' reaction. 197×; d) The same. Polarization microscopy. 197×; e) Adaptation to short periods of stress followed by long exposure to stress. Perls' reaction. 197×; f) The same. Polarization microscopy. 197×. Explanation in text.

for 1 h daily 6 days a week). The animals were adapted to short periods of stress by 12 sessions of exposure for 40 min to emotional stress, with an interval of 2 days.

The myocardial structure and accumulation of ^{99m}Tc-pyrophosphate by heart muscle were studied in animals of groups 3 and 5, 24 h after the end of adaptation, and in animals of groups 2, 4, and 6, 45 h after exposure to stress for 6 h, for it was shown previously that it is at this time that the severest structural disturbances of cardiomyocytes are observed and that maximal accumulation of ^{99m}Tc-pyrophosphate in the heart is found [2, 4].

For morphological investigation the animals were decapitated and the heart removed, fixed in 10% neutral formalin, and embedded in paraffin wax. Serial topographic sections 5-7 μ thick were stained by Van Gieson's and Selye's methods, and by Perls' reaction with counterstaining with hematoxylin and eosin. The PAS reaction and Brachet's reaction were carried out. To study the contractile system of the heart muscle cells and the character of changes in the myofibrils, the method of polarization microscopy was used. Histologic sections were examined under the MBI-15U microscope. For morphometric analysis the number of contractures of muscle fibers in 20 visual fields was determined in sections through the heart under a magnification of 112×, and this was followed by statistical analysis of the results for all the experimental animals in each series per field of vision. The degree of the contractual changes also was taken into consideration.

To assess the degree of heart damage based on accumulation of ^{99m}Tc-pyrophosphate, the Pirfotekh-99m reagent, to which eluate from ^{99m}Tc generator from the "Medradiopreparat" Factory was added, was used. The resulting complex of ^{99m}Tc-pyrophosphate, in a volume of 0.3 ml with activity of between $11.1 \cdot 10^3$ and $29.6 \cdot 10^3$ MBq (the dose was monitored on a "Radioisotope Calibrator CRC-k5" from Nuclear Chicago, USA) was injected into the caudal vein of the rats. Accuracy and completeness of injection of the isotope into the blood stream was monitored visually on an LFOV gamma-camera with Scintiw computer (Searle, The Netherlands). Thoracotomy was performed under pentobarbital anesthesia (8 mg/100 g) 100 min after injection of the radionuclide, and the heart was extracted and washed to remove radioactivity not bound with the myocardium. Radiometry of the heart was carried out on an NK-150 scintillation well counter (Hungary). Accumulation of ^{99m}Tc-pyrophosphate by the heart muscle was expressed as an index of radionuclide by the heart, equal to the ratio of the quantity of ^{99m}Tc-pyrophosphate taken up by the heart as a percentage of the injected dose, to the weight of the heart as a percentage of the body weight of the rat [13].

EXPERIMENTAL RESULTS

Emotional stress produced in the course of 6 h in the form of an anxiety neurosis caused marked focal necrotic and contractural lesions of the myocardium (Fig. 1a, b). A sharp increase in the intensity of Perls' reaction, fuchsinophilia, and the positive PAS reaction, with disappearance of cross-striation, continuous or partial homogenization, and fragmentation of muscle fibers were observed in the affected muscle fibers. Among the many injured cardiomyocytes (18.2 ± 4.1 contractures per field of vision) most were cells with profound changes (12.2 ± 3.2 3rd-degree contractures), and also cells in a state of necrosis. The nuclei of these cardiomyocytes showed lytic or pycnotic changes, or they were completely absent. The contractural and necrotic lesions found in the myocardium after prolonged exposure to emotional stress reflect the realization of one of the last links of the complex pathogenic chain of stres-induced heart damage [5, 6]. No structural changes in the myocardium were discovered in adapted animals not exposed to stress.

The effect of preliminary adaptation to physical exertion and short exposure to stress on the morphological changes in the myocardium of rats exposed to prolonged stress is illustrated in Fig. 1. The results show that both types of adaptation largely prevents the development of profound contractural and necrotic changes in the heart muscle. Only regions of myocardial fibers with weakening of cross-striation and of the positive Perls' reaction were observed. Some increase in anisotropy and single first-degree contractures of myofibrils were observed (in the case of preliminary adaptation of the animals to physical exertion before stress 1.9 ± 0.5 first-degree contractures were observed per field of vision, compared with 1.2 ± 0.4 in the case of adaptation to short periods of stress).

It will be clear from Table 1 that the ability of the myocardium of adapted animals not exposed to stress to accumulate ^{99m}Tc-pyroposphate was the same as in the control. Meanwhile, during long-term emotional stress the ability of the myocardium to accumulate ^{99m}Tc-pyrophosphate was increased by 4.5 times. Preliminary adaptation of the animals to short-term stress prevented the increase in ^{99m}Tc-pyrophosphate accumulation in the myocardium characteristic of stress virtually completely, but preliminary adaptation to physical exertion before stress reduced this effect more than threefold.

When the results are assessed it must be recalled that several hypotheses have now been put forward to explain the mechanism of ^{99m}Tc-pyrophosphate accumulation in damaged heart tissue. According to the two main points of view, the radionuclide accumulates inside the cell by binding with Ca^{2+} ions and being deposited in the form of hydroxyapatites [1], or it is assimilated by cardiomyocytes on account of the increased permeability of the cell membrane, due to inhibition of processes of energy metabolism [12].

In connection with the description of the results of this investigation it is important to note that disturbances of Ca^{2+} transport in the mitochondria [9], accumulation of this ion in the sarcoplasm [10] and, as was shown above, necrotic and contractural changes in the cardiomyocytes, forming the main stages in the pathogenesis of stress-induced cardiac damage, discovered in previous investigations, taken together determined the increased ability of the heart muscle to accumulate ^{99m}Tc-pyrophosphate during long-term emotional stress.

Prevention of accumulation of the radionuclide in the heart muscle by preliminary adaptation of the animals thus correpsonds to a significant reduction in the severity of the structural changes in the myocardium. Consequently, accumulation of ^{99m}Tc-pyrophosphate by the heart is an objective quantitative criterion of the antistressor effect of preliminary adaptation of the organism to short-term stress and physical exertion.

LITERATURE CITED

1. A. A. Kramer (ed.), Radionuclide Methods of Investigation of the Myocardium [in Russian], Moscow (1982).
2. V. V. Malyshev, Human Adaptation in Different Climatic, Geographic, and Occupational Conditions [in Russian], Vol. 2, Novosibirsk (1981), p. 73.
3. V. V. Malyshev, Injury and Regulatory Processes of the Organism [in Russian], Moscow (1982), p. 140.
4. V. V. Malyshev, V. I. Lifant'ev, V. A. Petrova, et al., Theoretical and Clinical Aspects of Pathology of the Circulation [in Russian], Irkutsk (1981), p. 30.
5. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981).

6. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Lesions [in Russian], Moscow (1984).
7. F. Z. Meerson, L. S. Katkova, Yu. P. Kozlov, et al., Byull. Éksp. Biol. Med., No. 12, 23 (1983).
8. F. Z. Meerson, L. S. Katkova, and V. V. Malyshev, Kardiologiya, No. 2, 74 (1985).
9. F. Z. Meerson, V. I. Lifant'ev, and V. V. Malyshev, Vopr. Med. Khimii, No. 1, 119 (1984).
10. F. Z. Meerson, N. V. Samosudova, E. V. Glagoleva, et al., Arkh. Anat., No. 2, 43 (1983).
11. E. I. Chazov, A. A. Kramer, and A. Z. Zhitov, Ter. Arkh., No. 6, 12 (1977).
12. I. I. Shepeleva, D. B. Saprygin, V. A. Saks, et al., Med. Radiol., No. 5, 16 (1979).
13. D. G. Miller and S. Mallow, Pharm. Biochem. Behav., 7, 139 (1977).

MECHANISMS OF VALINOMYCIN-INDUCED CHANGES IN OSMOTIC RESISTANCE OF ERYTHROCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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In a previous study of the kinetics of ^{45}Ca accumulation by rat erythrocytes loaded with a calcium chelating agent it was found that valinomycin significantly reduces the sensitivity of the cells to osmotic hemolysis, as reflected in hemoglobin release [6].

The data on the mechanism of this phenomenon and differences found in spontaneously hypertensive rats are given in this paper.

EXPERIMENTAL METHODS

Blood from male spontaneously hypertensive Kyoto-Wistar rats (SHR) aged 4-5 months, with a blood pressure of 183 ± 10 mm Hg, and from normotensive Kyoto-Wistar rats (NR), of the same age and sex (control, blood pressure 108 ± 7 mm Hg), and also from normal healthy blood donors was used in the experiments. The procedure of taking blood and obtaining erythrocytes was described previously [3]. The blood samples were kept on ice for not more than 2 h before the experiment. To assess the osmotic resistance of the erythrocytes, one volume of cells was treated with four volumes of medium containing 140 mM NaCl, 5 mM Kcl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 10 mM glucose, and 10 mM HEPES-Tris-buffer, pH 7.4 (37°C). In some cases the medium contained 2.5 μM valinomycin and 0.2 μCi/ml of $^{86}RbCl$. After definite incubation times 150 μl of suspension was transferred into 1 ml of cold medium containing 150 mM NaCl, 0.1 mM EDTA, and 5 mM sodium phosphate (pH 7.4), after which the cells were sedimented and hemolyzed in 0.5 ml water for 20 sec with constant shaking. After recentrifugation the supernatant was diluted 100 times with water and the optical density determined at 407 nm (FP-9 instrument, Finland). Before determination of the K^+, Na^+, and ^{86}Rb concentrations in the erythrocytes the cells were washed twice in the cold with an iso-isomotic solution of choline chloride, with the addition of 10 mM Tris-HCl, pH 7.4. For determination of the total potassium and sodium concentrations on an atomic absorption spectrophotometer (Nippon Jarrel, Japan), the erythrocytes were diluted with water $3 \cdot 10^5$ and 10^3 times respectively. Radioactivity of ^{86}Rb was determined by a liquid scintillation spectrometer (Intertechnique, France) after treatment of the erythrocytes with 5% TCA.

EXPERIMENTAL RESULTS

In the presence of valinomycin the quantity of hemoglobin released from rat erythrocytes on the addition of water was sharply reduced (Fig. 1). The maximal effect recorded after

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