GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Opposite Effects of Adaptation to Short-Term Stress and Adaptation to Periodic Hypoxia on Na,K-ATPase Activity in Liver Plasma Membranes

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Na,K-ATPase activity is shown to be lowered more than twice 2 hours after emotional pain stress in comparison with the initial level, remaining practically unchanged during the subsequent 24 hours. Adaptation to repeated stress results in a 50% activation of Na,K-ATPase. A protective effect is demonstrated in long-term stress against the background of preadaptation. Adaptation to periodic hypoxia inhibits liver Na,K-ATPase to the same extent as does acute stress. Against the background of preadaptation to periodic hypoxia, stress does not aggravate the drop of Na,K-ATPase activity. Adaptation to stress inhibits accumulation of products of *in vitro*-induced lipid peroxidation in the heart 1.4-fold and does not affect it in the liver, whereas adaptation to hypoxia sharply accelerates the accumulation of oxidized products in both organs, which probably explains the activation of liver Na,K-ATPase in adaptation to stress and its inhibition in adaptation to hypoxia.

Key Words: *heart; liver; Na,K-ATPase; lipid peroxidation; stress; adaptation to stress; adaptation to hypoxia*

Adaptation to short-term stress and adaptation to hypoxia are known to reduce the intensity of the stress-induced reaction of the organism and to prevent stress-induced damage to the myocardium, including disturbances in the functioning of membrane-bound enzymes [6]. Among them Na,K-ATPase of the sarcolemma and Ca-ATPase of the sarcoplasmic reticulum are of primary importance since these enzymes are responsible for maintaining the transmembrane potential and calcium homeostasis in cardiomyocytes. The efficiency of both these transport systems of the myocardium and their resistance to endogenous adverse factors are impaired in stress [7,11], while adaptation to stress protects these transport enzymes and, if the activity of Na,K-ATPase just returns to the initial level, the function of the Ca pump is considerably improved [5,11]. Adaptation to hypoxia only partially protects the myocardial ion-transport systems from stress-induced damage; moreover, some parameters, such as the resistance of the Ca-transport system of the sarcoplasmic reticulum to endogenous damaging factors, are even impaired [2]. Furthermore, the two types of adaptation differ fundamentally with respect to other important parameters. The isozyme spectrum of heat shock proteins synthesized in the heart, liver, and brain in adaptation to stress is found to

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exceed that in adaptation to hypoxia. Heart shock proteins are known to exhibit protective and reparative effects on cell structures and to characterize the general improvement of the adaptive reserves of the organism [6]. Moreover, adaptation to hypobaric hypoxia, but not adaptation to stress, reduces the content of natural antioxidants in the liver despite activation of the antioxidant enzymes [6].

Thus, there is a fundamental difference between the two types of adaptation — to stress and to hypoxia — which is probably determined to a great extent by the difference in the intensity of oxidative processes and resistance of the ion-transport systems to lipid peroxidation (LPO). In light of the above we studied the effect of adaptation to hypoxia and to short-term stress on the intensity of LPO and the activity of Na,K-ATPase in the liver plasma membrane, an extremely LPO-sensitive enzyme in the stress-sensitive organ, and attempted to prevent stress-induced damage of this enzyme by means of adaptation to various environmental factors.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 180-200 g. The animals were divided into the following 6 groups: group 1 were controls; group 2 was subjected to 6-hour acute emotional pain stress (EPS) as described previously [10]; group 3 was adapted to short-term stress (eight 1-hour EPS sessions every other day); group 4 was adapted to hypoxia (pressure chamber, 6000 m, 6 hours daily during 20 days); group 5 was adapted to stress+long-term stress (6 hours); and group 6 was adapted to hypoxia+long-term stress (6 hours).

The liver was removed the day after adaptation to stress or hypoxia, 2 or 24 hours after stress against the background of or without preadaptation to hypoxia, and 2 hours after stress against the background of adaptation to stress, washed in physiological saline, and frozen in liquid nitrogen. The liver was homogenized in a glass-Teflon homogenizer at a 1:10 ratio of tissue to medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 0.32 M sucrose. The homogenate was washed and centrifuged at 1000 g (10 min) three times and the resultant supernatant was centrifuged at 10,000 g for 20 min and washed in 0.32 M sucrose under the same conditions. The pellet was subjected to hypoosmotic shock (6 mM Tris-HCl, 0.5 mg protein/ml) at 0°C for 2 hours under constant stirring. The suspension was then diluted 4-fold with 50 mM Tris-HCl and centrifuged at 105,000 g for 1 hour. The pellet in 50 mM Tris-HCl was stored at -20°C prior to the experiments. Na,K-ATPase activity was evaluated from the accumulation of inorganic phosphate (P_i) in the incubation medium in the presence and absence of ouabain measured on a Hitachi-557 spectrophotometer (λ=660 nm) [14]. LPO in liver and heart homogenates was induced by the Fe^{2+}-ascorbate system (10 μM+0.5 mM). Thiobarbituric acid (TBA)-reactive products were measured as described previously [12]. Protein concentration was determined from the fourth derivative of the absorption spectrum (320-240 nm) using an earlier-described approach [13].

RESULTS

Data on the effect of stress on Na,K-ATPase activity are presented in Fig. 1. Long-term stress is seen to sharply reduce Na,K-ATPase activity more than 2-fold after just 2 hours, and this parameter did not return to the control values even after 24 hours. There are no reliable differences between Na,K-ATPase activity in the liver plasma membranes 2 and 24 hours after stress, which implies early and maximally expressed disturbances in the functioning of this enzyme. The twofold drop in Na,K-ATPase activity attests to marked stress-induced damage of the enzyme, which may result in a shift of the ionic equilibrium in hepatocytes.

Adaptation to repeated short-term stress not only does not reduce Na,K-ATPase activity but even drastically activates the enzyme (by 50%, Fig. 2). Repeated exposure to one-hour acute stress, the damaging effect of which even exceeds that of long-term stress [9], may trigger the development of

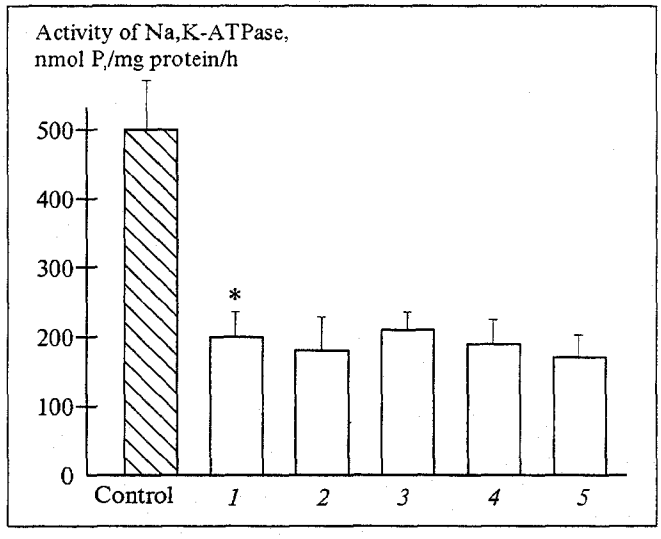

Fig. 1. Effect of emotional pain stress and adaptation to periodic hypoxia on Na,K-ATPase activity in the liver. *1*) 2 hours after stress; *2*) 24 hours after stress; *3*) adaptation to hypoxia; *4*) stress against the background of adaptation to hypoxia 2 hours after the cessation of stress; *5*) stress against the background of adaptation to hypoxia 24 hours after the cessation of stress. *$p<0.001$ between the control and stress.

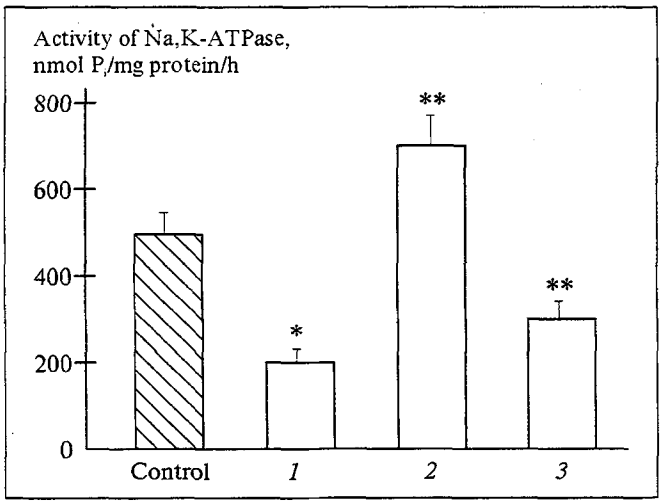

Fig. 2. Effect of adaptation to short-term stress on Na,K-ATPase activity in the liver. *1*) 2 hours after exposure to stress; *2*) adaptation to stress; *3*) long-term stress (measurement 2 hours after stress) against the background of preadaptation to stress. *$p<0.001$, **$p<0.02$ in comparison with the control.

some protective mechanisms to compensate for the effect of LPO, which is known to be activated in stress [4]. Previously we found that adaptation to stress activates the antioxidant defense enzyme system in the heart [8] accompanied by a lower intensity of LPO and, on the whole, may be responsible for the absence of any inhibition of Na,K-ATPase in the myocardium in adaptation to repeated short-term stress [5] and for the maintenance of the transmembrane potential on the control level.

The absence of inhibition of Na,K-ATPase in the liver is probably due to analogous processes. However, in contrast to the situation in the heart, we observe a considerable activation of the enzyme in the liver, which probably results from its higher sensitivity to free-radical processes [1,3,4,6] and a more pronounced activation of LPO in this organ during each exposure to short-term acute stress, leading to the formation of more substantial protection in adaptation to repeated short-term stress. It is important that this substantial protection of Na,K-ATPase highly susceptible to free-radical oxidation is able to a great extent to prevent also a drop in the activity Na,K-ATPase induced by each subsequent stress. Whereas long-term stress reduces the activity of Na,K-ATPase to 42% of its initial level, after adaptation to stress 71% of its activity is preserved.

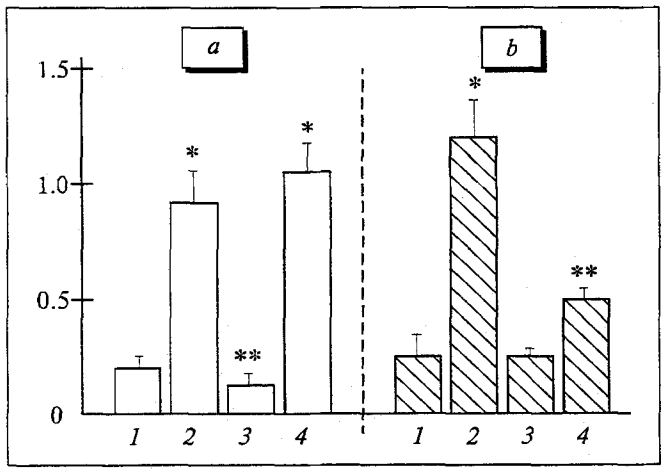

Fig. 3. Effect of stress, adaptation to short-term stress influences, and adaptation to hypoxia on the initial rate of accumulation of TBA–reactive products in the heart (*a*) and liver (*b*). *1*) control; *2*) emotional pain stress; *3*) adaptation to stress; *4*) adaptation to hypoxia. Ordinate: initial rate of accumulation of LPO products, A_{532} optical density units/min/mg protein. *$p<0.001$, **$p<0.05$ in comparison with the control.

Adaptation to hypoxia not only does not activate Na,K-ATPase but even inhibits it virtually to the same extent as does a 6-hour single stress (Fig. 1). In this case one should take into account the exceptional sensitivity of Na,K-ATPase to various oxidants. Therefore, repeated "hyperoxia" arising when the hypobaric hypoxia gives way to normoxia at the normal pressure may lead to a considerable noncompensated LPO activation and oxidative damage to Na,K-ATPase. To verify this assumption we assessed the degree of LPO activation in the liver and heart in different types of adaptation (Fig. 3). Long-term stress is seen to markedly intensify *in vitro*-induced LPO in the heart and liver, 3.6- and 4.3-fold, respectively. Repeated exposure to stress factors during adaptation leads to the formation of a protective mechanism which prevents the activation of LPO, so that its intensity in the liver remains at the control level and drops 1.4-fold in the heart. Unlike adaptation to stress, adaptation to hypoxia carried out in a rather severe regime (6000 m, 6 hours) did not inhibit but even induced LPO, the accumulation of LPO products being accelerated 1.7- and 4.4-fold in the liver and heart, respectively, in comparison with the control, i.e., not less than after long-term stress.

Six-hour exposure to the stress factor against the background of adaptation to hypoxia could have been anticipated to aggravate the damage to Na,K-ATPase. However, no reliable inhibition of the enzyme was found 2 and 24 hours after stress.

At least two conclusions may be drawn from these observations. First, unlike adaptation to hypoxia, which in the given severe regime is itself a damaging factor leading to the accelerated accumulation of LPO products in tissues and inhibition of Na,K-ATPase, an enzyme highly sensitive to oxidation, adaptation to stress results in the formation of a protective mechanism which not only activates Na,K-ATPase but also protects the enzyme against stress-induced damage.

Second, adaptation to hypoxia, despite the pronounced damaging effect, better protects liver Na, K-ATPase against the single long-term stress influence. However, the entire damaging effect of the stress against the background of preadaptation is more pronounced in the case of adaptation to hypoxia. Adaptation to short-term stress followed by long-term stress is characterized by a higher absolute Na,K-ATPase activity in the liver.

Thus, the absence of activation and direct inhibition of LPO caused by adaptation to stress considerably improved the function of Na,K-ATPase but did not completely protect its activity from the following stress. Adaptation to hypobaric hypoxia sharply activating LPO impaired the function of Na,K-ATPase but completely protected the enzyme from the subsequent stress. This probably means that adaptation to stress should be combined with adaptation to hypoxia of lower intensity. It should be noted that adaptation to hypoxia carried out in a milder regime does not damage Ca-ATPase of the sarcoplasmic reticulum, which is less sensitive to LPO, and even slightly activates the enzyme. A more effective approach may be a combination of adaptation to stress with adaptation to hypoxia at the normal pressure, which has been shown by us recently to be unaccompanied by activation of free radical processes in the liver [15].

REFERENCES

1. Yu. V. Arkhipenko, V. V. Didenko, T. G. Sazontova, and F. Z. Meerson, *Dokl. Akad. Nauk SSSR*, **304**, No. 6, 1500-1503 (1989).
2. Yu. V. Arkhipenko, T. G. Sazontova, I. I. Rozhitskaya, and F. Z. Meerson, *Kardiologiya*, **32,** No. 6, 57-61 (1992).
3. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
4. M. M. Melkonyan, E. A. Ararityan, V. G. Mikaelyan, and V. G. Mkhitaryan, *Zh. Eksp. Klin. Med.*, **18**, No. 4, 25-30 (1978).
5. F. Z. Meerson and T. G. Sazontova, *Byull. Eksp. Biol. Med.*, **102**, No. 8, 153-155 (1986).
6. F. Z. Meerson, *Adaptation Medicine: Mechanisms and Protective Effects of Adaptation* [in Russian], Moscow (1993).
7. T. G. Sazontova, *Byull. Eksp. Biol. Med.,* **102**, No. 12, 685-687 (1986).
8. T. G. Sazontova, Yu. V. Arkhipenko, and F. Z. Meerson, *Ibid.*, **104**, No. 10, 411-413 (1987).
9. T. G. Sazontova, V. I. Vovk, and F. Z. Meerson, *Ibid.*, **109**, No. 2, 122-124 (1990).
10. O. Desiderato and M. Testa, *Physiol. Behav.*, **16**, No. 1, 67-73 (1976).
11. F. Z. Meerson, T. G. Sazontova, and Yu. V. Arkhipenko, *Biomed. Sci.*, **1**, 73-78 (1990).
12. H. Okhawa, N. Ohishi, and K. Yagi, *Anal. Biochem.*, **95**, No. 2, 351-358 (1979).
13. E. Padros, M. Dunach, A. Morros, *et al.*, *Trends Biochem. Sci.*, **9**, No. 12, 508-510 (1984).
14. W. B. Rathbun and M. V. Betlach, *Anal. Biochem.*, **28**, No. 1, 436-446 (1969).
15. T. G. Sazontova, E. N. Tkachuk, S. N. Kolmycova, *et al.*, *Hypoxia Med. J.*, No. 4, 4-7 (1994).