PREVENTION OF STRESS-INDUCED DISTURBANCE OF MYOCARDIAL Na,K-ATPase ACTIVITY BY ADAPTATION TO SHORT-TERM STRESSES

F. Z. Meerson and T. G. Sazontova

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Disturbances of electrical stability of the heart arising after long-term stress, namely lowering of the electrical threshold of its fibrillation, can be prevented by preliminary adaptation of animals to short-term stresses [4]. The Na,K-ATPase of the myocardial sarcolemma plays a definite role in maintenance of the membrane potential and electrical stability of the heart, and its activity also is disturbed during stress [7, 8]. It can accordingly be postulated that preliminary adaptation to short-term stresses prevents the stress-induced disturbance of Na,K-ATPase activity.

To test this hypothesis it is not sufficient to assess the influence of stress and of adaptation to stressors only on the rate of ATP hydrolysis by Na,K-ATPase as has been done in previous investigations [8]. It is necessary to study the effect of these factors on the conformational stability of the enzyme, which is determined both by intramolecular bonds and by lipid-protein interactions of the membrane-bound protein. An accepted method of assessing the conformational stability of such proteins is an analysis of their thermal denaturation kinetics.

The aim of this investigation was, first, to study the effect of long-term stress on Na,K-ATPase activity and on the kinetics of its thermal denaturation and, second, to study whether the disturbances found under these conditions can be prevented by preliminary adaptation to short-term stresses.

EXPERIMENTAL METHOD

Male rats weighing 180-200 g were used. The animals were divided into four groups: 1) control; 2) stress, in the form of an anxiety neurosis, produced by the method in [10], for 6 h; 3) adaptation to short-term stresses (seven sessions of stress, each lasting 1 h, on alternate days); and 4) adaptation to short-term stresses followed by exposure to stress for 6 h, 24 h after the end of adaptation. The animals were decapitated 2 h after long-term stress or 24 h after the end of the course of adaptation.

The hearts were removed, washed, and the heavy sarcolemma fraction was isolated by the method described by us previously [8]. ATPase activity was determined by measuring accumulation of inorganic phosphorus (P_i) by the method in [13] after incubation of the preparation in the presence and absence of ouabain. Protein was determined by the method in [12]. Thermal denaturation was carried out within the 50-60°C range, the temperature being maintained constant with an accuracy of ±0.1°C; the thermodynamic parameters were calculated as described in [11]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

After stress the rate of Na,K-dependent ATP hydrolysis in the sarcolemma preparation isolated from the myocardium was reduced by 20% (Fig. 1). It is unlikely that the effect of stress would be limited to this small change in Na,K-ATPase activity, for it is well known that the contractile function of the heart is significantly altered in stress [2]. To detect fine conformational changes in structure of the enzyme, capable of impairing operation of the sodium pump *in vivo*, we analyzed the kinetics of thermal denaturation of Na,K-ATPase. The kinetics of inhibition of the enzyme during thermal denaturation at 53°C is shown in Fig. 1: As a result of stress the rate of thermal inactivation of the enzyme was increased.

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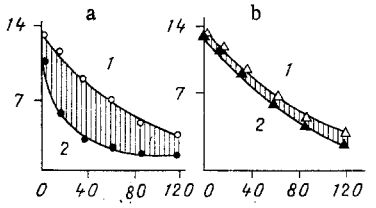

Fig. 1

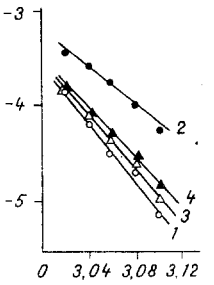

Fig. 2

Fig. 1. Thermal inactivation of Na,K-ATPase of the myocardial sarcolemma (a) at 52°C in control (1) and after stress (2), and effects of preliminary adaptation to short periods of stress (b) on thermal inactivation kinetics of Na,K-ATPase. Abscissa, time of thermal denaturation (in min); ordinate, Na,K-ATPase activity (in μmoles P_i/ml protein/h). 1) Adaptation to short periods of stress; 2) stress in rats adapted to short periods of stress.

Fig. 2. Temperature dependence of velocity constant of thermal denaturation (K_{td}) of Na,K-ATPase of the myocardial sarcolemma in control (1), after stress (2), during adaptation to short periods of stress (3), and after stress in rats adapted to short periods of stress (4). Abscissa, $10^3/T$ (T denotes absolute temperature); ordinate, log K_{td}.

Depending on temperature, sarcolemma Na,K-ATPase of rats exposed to stress was inhibited 2-3 times faster than in control animals. By carrying out thermal denaturation of Na,K-ATPase at different temperatures within the 50-60°C range, the thermodynamic parameters of the process could be determined. Dependence of log K_{td} on 1/T, when K_{td} is the velocity constant of thermal denaturation of the enzyme and T the absolute temperature, is shown in Fig. 2. The slope of the straight lines between these coordinates is proportional to the activation energy (E_a) of the process. Values of the change in enthalpy (ΔH^*), entropy (ΔS^*), and free energy (ΔF^*) of thermal denaturation of the enzyme were then calculated, and these are shown in Table 1. The data show that as a result of stress values of E_a, ΔH^*, and ΔS^* were significantly reduced. This is evidence that as a result of stress the conformational stability of Na,K-ATPase is disturbed, and transformation of the enzyme molecule into the denaturated state during heating is thus facilitated.

The data given in Figs. 1 and 2 and Table 1 reflect the main results of the investigation, namely the effects of preliminary adaptation to short periods of stress on the harmful effects of long-term stress. Adaptation itself affected neither the hydrolytic activity of Na,K-ATPase nor the kinetics of its thermal denaturation, but at the same time it completely prevented both the decrease in activity of this enzyme and its more rapid thermal denaturation discovered in animals exposed to stress.

To understand this protective effect of adaptation, it must be recalled that stress injury to the heart and, in particular, to the cardiomyocyte plasma membrane, where Na,K-ATPase is located, is produced as a result of a powerful and prolonged adrenergic effect, which activates lipases, phospholipases, and lipid peroxidation (LPO) [2]. Under these circumstances, as the writers have shown, the role of this last process is very important [8].

We can accordingly propose at least two mechanisms by which adaptation to short periods of stress prevents disturbances in the system of the Na pump. First, such adaptation leads to an increase in the functional capacity of central stress-limiting systems [3] and, in particular, to the accumulation of opioid peptides [5] and gamma-hydroxybutyric acid [2] in the brain, resulting in reduction of the stress reaction itself and of the injuries caused by it in target organs [9]. Second, we know that the isolated heart of animals adapted to stress is less reactive to adrenalin [6] and has increased resistance to induction of LPO, which is accompanied by increased activity of the antioxidant enzyme, catalase [1].

The prophylactic effect of adaptation to short periods of stress may thus come about as a result of activation of both central and peripheral stress-limiting systems. In the context

TABLE 1. Thermodynamic Parameters of Thermal Denaturation of N,K-ATPase of Myocardial Sarcolemma in Control, after Stress, and after Adaptation to Short Periods of Stress (M ± m)

Parameter	Control	Stress	Adaptation	Adaptation + stress
E_a, kcal/mole	75,6±2,3	60,7±2,0	73,5±2,8	71,6±3,1
ΔH^*, kcal/mole	75,0±2,3	60,1±2,0	72,9±2,8	71,0±3,1
ΔS^* cal/mole·deg·C	156,8±0,6	111,3±3,6	148,1±3,8	142,4±4,0
ΔF^*, kcal/mole	24,4±1,5	23,7±1,9	24,1±1,7	23,9±2,3

of this description, what is important is that this adaptation simultaneously prevents stress-induced disturbances of the electrical stability of the heart and the state of the Na,K-ATPase, which is in harmony with the view that this enzyme plays a key role in maintenance of the electrical stability of the heart and that disturbance of Na,K-ATPase activity plays an important part in the genesis of arrhythmias.

LITERATURE CITED

1. L. S. Katkova, "Prevention of disturbances of myocardial contractility during long-term stress by preliminary adaptation to short periods of stress," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1985).
2. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
3. F. Z. Meerson, Byull. Vses. Kardiol. Nauchn. Tsent., No. 1, 34 (1985).
4. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., Kardiologiya, No. 10, 36 (1985).
5. F. Z. Meerson, A. D. Dmitriev, and V. I. Zayats, Kardiologiya, No. 9, 81 (1984).
6. F. Z. Meerson, L. S. Katkova, Yu. P. Kozlov, et al., Byull. Éksp. Biol. Med., No. 12, 25 (1983).
7. F. Z. Meerson, L. N. Medvedev, L. Yu. Golubeva, et al., Byull. Éksp. Biol. Med., No. 2, 61 (1982).
8. F. Z. Meerson, T. G. Sazontova, V. E. Kagan, et al., Byull. Éksp. Biol. Med., No. 12, 42 (1983).
9. F. Z. Meerson, G. T. Sukhikh, L. S. Katkova, et al., Dokl. Akad. Nauk SSSR, 274, No. 1, 241 (1984).
10. O. Desiderato and M. Testa, Physiol. Behav., 16, 67 (1976).
11. F. H. Johnson and H. Eyring, The Theory of Rate Processes in Biology and Medicine, New York (1974).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. W. B. Rathbun and M. W. Betlach, Anal. Biochem., 28, 436 (1969).