ACTIVATION OF RAT BRAIN Na,K-ATPase BY STRESS

T. G. Sazontova, Yu. V. Arkhipenko,
and F. Z. Meerson

UDC 613.863-07:616.831-
008.931:577.152.361

KEY WORDS: stress; sex; brain Na,K-ATPase.

The effect of long-lasting environmental stress situations on the function of different organs differs. For instance, immediately after emotional-painful stress (EPS) a fall in Ca- and Na,K-ATPase activity is observed in heart muscle membranes [1, 4] accompanied by depression of myocardial contractility [5]. Meanwhile in animals exposed to the same stress, the rate of formation and degree of preservation of defensive conditioned reflexes are regularly increased [3]. The molecular mechanisms of this last effect are not clear but it is known [2] that activation of lipid peroxidation (LPO) takes place after EPS both in the brain and in the heart.

The aim of this investigation was to determine the effect of stress on brain Na,K-ATPase activity and the possible role of LPO in this process.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Depending on the experimental conditions the animals were divided into four groups (eight rats in each group): 1) control, 2) stress, 3) receiving the antioxidant ionol (2,6-di-tert-butyl-4-methylphenone), and 4) receiving ionol followed by stress. Ionol was injected intraperitoneally in the form of a suspension in Tween-60, daily for 3 days before the experiment in a dose of 20 mg/kg. EPS was induced in the form of an anxiety neurosis by the usual method [8] in the course of 6 h. The rats were decapitated 2 h after stress. Animals exposed to stress regularly developed gastric ulcers, and these served as the criterion on stress injury.

The gray matter of the brain was homogenized into five fractions with a Teflon-glass homogenizer in medium of the following composition: Tris-HCl 50 mM, EDTA 1 mM, sucrose 0.32 M, ratio of weight of tissue to volume of solution 1:10. The homogenate was centrifuged three times at 1000g for 10 min each time, the supernatant being poured off for keeping at 0°C whereas the residue was resuspended in the same homogenizer. The supernatants were pooled and centrifuged for 20 min at 10,000g. The residue was diluted with 0.32 M sucrose and centrifuged under the same conditions. The resulting residue was resuspended in 6 mM Tris-HCl until the protein concentration was 0.5 mg/ml, and allowed to stand at 0°C after mixing for 2 h. After hypoosmotic shock, the suspension was diluted fourfold with 50 mM Tris-HCl and centrifuged at 105,000g for 1 h. The residue was covered without mixing with keeping medium (50 mM Tris-HCl) and frozen overnight at −20°C. All other operations were conducted at 4°C and the pH of the buffer media was 7.4,

The resulting fraction of unpurified synaptosomes was resuspended, and to determine the protein concentration an aliquot of the suspension was preincubated for 20 min in 1% Na deoxycholate solution, after which protein was determined by the biuret method. Total ATPase activity was determined in medium consisting of: 3 mM $MgCl_2$, 3 mM ATP, 130 mM NaCl, 20 mM KCl, 30 mM imidazole-HCl, pH 7.4, at 37°C. Mg-ATPase activity was determined in the same medium, but with the addition of 1 mM ouabain. The inorganic phosphate concentration was determined by the method in [11]. Na,K-ATPase activity was calculated by subtracting Mg-ATPase activity from total ATPase activity.

LPO was induced *in vitro* with a system of $FeSO_4$ (10 nmoles/mg protein) + ascorbate (0.2 mM). The formation of carbonyl LPO products was recorded by the reaction with 2-thiobarbituric acid [10].

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Gorizontov,) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 97, No. 5, pp. 556-558, May, 1984. Original article submitted April 28, 1983.

 0007-4888/84/9705-0590$08.50

TABLE 1. Effect of Stress on Activity of Brain Na,K- and Mg-ATPases (M ± m)

Experimental conditions	Na,K-ATPase	Mg-ATPase
Control	12,3±0,6 (100)	4,7±0,2 (100)
Stress	17,1±1,7 (139)*	4,9±0,2 (105)
Ionol	11,8±0,4 (104)	4,7±0,2 (100)
Ionol + stress	12,4±0,7 (101)	4,9±0,3 (104)

Legend. *P < 0.05 compared with control. Figures in parentheses are percentages of control.

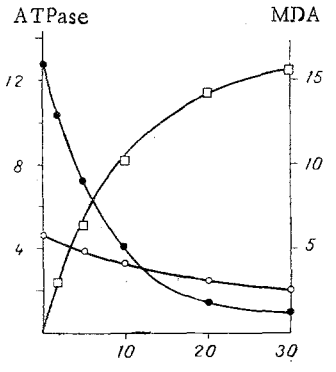

Fig. 1

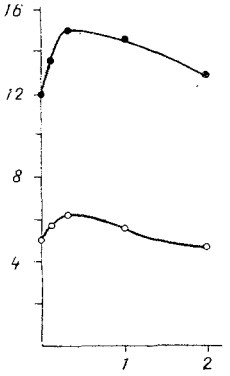

Fig. 2

Fig. 1. Effect of induction of LPO in membrane suspension by system of Fe^{++} + ascorbate on Na,K-ATPase activity (filled circles) and Mg-ATPase activity (empty circles). Abscissa, incubation time (in min); ordinate: on left — enzyme activity (in μmoles P_i/mg protein /h), on right — malonic dialdehyde concentration (in nmoles/mg protein); squares indicate increase in MDA during incubation.

Fig. 2. Effect of detergent Triton X-100 on Na,K-ATPase (1) and Mg-ATPase (2) activity in brain synaptosomes. Abscissa, concentration of Triton X-100 (in mg/mg protein); ordinate, enzyme activity (in μmoles P_i/mg protein/h).

EXPERIMENTAL RESULTS

Stress caused significant activation of Na,K-ATPase of the synaptosomal membranes, but Mg-ATPase activity was virtually unchanged (Table 1). In rats receiving the LPO inhibitor ionol before exposure to stress, activation of Na,K-ATPase did not take place. Activation of LPO in the brain during stress is also known to be prevented by ionol [2]. It can accordingly be concluded that the increase in Na,K-ATPase activity in the brain during stress is caused by LPO.

Meanwhile, modeling the phenomena observed *in vivo* by the use of LPO activation *in vitro* in isolated synaptosomal membranes from the brain of control animals revealed a picture which was not analogous to that *in vivo*. In the course of ascorbate-dependent LPO, Na,K-ATPase was not activated but, on the contrary, it was rapidly inhibited in the presence of only a slight change in Mg-ATPase activity (Fig. 1). This difference between the findings *in vivo* and *in vitro* can explained by several causes.

First, inhibition of Na,K-ATPase during LPO *in vitro* was evidently connected with oxidation of an unidentified group in the active center of the enzyme. In the experiments *in vivo*,

components of the cytoplasm may have a stabilizing action on the enzyme, preserving its reduced form.

Second, the orientation of the enzyme in the membrane must be taken into consideration. *In vivo*, for instance, the active center of Na,K-ATPase is always oriented toward the source of ATP inside the cell, whereas after isolation part of the membrane preserves its original "correct" orientation with the active center inside the vesicles, and part is everted, with the active center of the enzyme facing outward. In that case permeability of the membrane for ATP assumes great importance. If it is increased, the fraction of "correctly" oriented vesicles also is involved in hydrolytic activity; in the absence of permeability for ATP, only the "inside out" vesicles exhibit activity for ATP. A similar effect of demasking of Na,K-ATPase by detergents is well known [7] and was reproduced on the fraction which we isolated with Triton X-100 (Fig. 2). Activation of Na,K-ATPase after stress may thus be apparent, due to an increase in permeability for ATP, whereas true activity of the enzyme may remain unchanged, or it may actually be less than in the control.

Third, activation of Na,K-ATPase may take place through loosening of the lipid microenvironment of the protein on the appearance of polar LPO products (more especially lipid hydroperoxides). The effect of activation of the enzymes during LPO has been demonstrated on several biological membranes [6, 9]; intensification of LPO in the membrane, moreover, is accompanied by a switch from activation to inhibition of the enzyme [1]. This state of affairs must be taken into account when the results of the experiments *in vivo* are analyzed. For instance, activation of LPO in the heart during stress, as was shown previously [1], is expressed to a higher degree than in the brain and, correspondingly, it leads no longer to activation, but to inhibition of Na,K-ATPase.

The principal fact in this account is that Na,K-ATPase plays an important role in maintaining the membrane potential of brain neurons and in realizing their physiological function. There are thus grounds for considering that the increase in the activity of this enzyme revealed by these experiments may play a role in the mechanism responsible for increasing the rate of formation and degree of preservation of conditioned reflexes in animals exposed to stress.

LITERATURE CITED

1. Yu. V. Arkhipenko, V. E. Kagan, and Yu. P. Kozlov, Biokhimiya, No. 3, 423 (1983).
2. F. Z. Meerson, V, E. Kagan, L. L. Prilipko, et al., Byull. Éksp. Biol. Med., No. 12, 661 (1980).
3. F. Z. Meerson, V. I. Pavlova, G. T. Sukhikh, et al., Patol. Fiziol., No. 5, 3 (1982).
4. F. Z. Meerson, T. G. Sazontova, V. E. Kaga, et al., Byull. Éksp. Biol. Med., No. 12, 42 (1983).
5. F. Z. Meerson, N. V. Samosudova, E. V. Glagoleva, et al., Arkh. Anat., No. 2, 43 (1983).
6. A. Baba, E. Lee, A. Ohta, et al., J. Biol. Chem., 256, 3679 (1981).
7. S. P. Banerjee, I. L. Dwosh, V. K. Khanna, et al., Biochim. Biophys. Acta, 211, 345 (1970).
8. O. Desiderato, J. R. MacKinnon, and H. Hissom, J. Comp. Physiol., 87, 208 (1974).
9. H. Heinle, Biochem. Biophys. Res. Commun., 107, 597 (1982).
10. H. J. Kohn and M. Liversedge, J. Pharmacol. Exp. Ther., 82, 292 (1944).
11. W. Rathbun and V. Betlach, Anal. Biochem., 28, 439 (1969).