EFFECT OF HYPOTHERMIA ON RAT BRAIN ATPase ACTIVITY

G. ABILOVA*

SUMMARY: The study deals with the activity of Na, K-ATPase and Mg-ATPase in rat sub cellular fractions and cerebral cortex homogenates and its response to 20°C hypothermia during 1 and 3 hours with the intra-abdominal injection of neuropeptide dalargin. The results show that the prolonged hypothermia for 1 and 3 hours intensifies the activity of Na, K-ATPase in all examined fractions with the only exception Mg-ATPase in mitochondrial fraction where 3 hours hypothermia brings the activity down. Intra-abdominal injection of dalargin (200 μg/kg) considerably abates the activity of Na, K-ATPase during 1 hour hypothermia. Under the discussion are the possible causes that advance the activity of Na, K-ATPase with the influence of hypothermia.

Key Words: Hypothermia, Na, K-ATPase, Mg-ATPase.

INTRODUCTION

Now days artificial reduction of temperature is widely used in different spheres of medicine. Hypothermia produces rather positive results is surgery, providing the most reliable conditions for postoperative period (8).

On the other hand, hypothermia is known to produce a harmful effect on biological membrane, that brings about the change in the activity of enzymes connected with them (2).

To get to know the processes in the brain while the animal undergoes the influence of hypothermia demands the search of protective measures against the negative influence of it.

The work was done to study the influence of neuropeptide dalargin (try-d-ala-gly-phe-leu-arg), which is the analog of leu-enkephalin, obtained from Union Cardia Scientific Center, USSR Academy of Medicine which is used in clinic for the treatment of gastric ulcer and as an anti-stressor agent, on the activity of Na, K-ATPase of sub cellular fractions rat brain cellular cortex when they are affected by hypothermia for 1 hour and 3 hours.

MATERIAL AND METHODS

White rats weighing 180–200 g have been investigated. The animals were cooled up to 20°C rectal temperature for 60 mins and this state was prolonged for 1 hour for the animals in the 1st and 2nd groups, and for 3 hours for the animals in the 3rd and in the 4th groups. Physiological salt solution (0.5 ml) intra-abdominal injection was done to the animals of the 1st and 3rd groups 30 minutes before hypothermia, while the 2nd and the 4th groups of animals were subjected to the injection of dalargin (200 μg/kg weight of animal). Control group animals were immobilized in hypothermic cameras 40–60 mins without cooling.

Sub cellular fractionation was conducted according to Gray *et al.* (5).

Total ATPase activity was assayed in an incubation medium containing (in mol; 1^{-1}): tris-HCL buffer, pH 7.4 (50), NaCl (120), KCl (20), $MgCl_2$ (3) and Na_2ATP in a total volume 1.5 ml. Mg-ATPase activity was measured in a similar reaction mixture, but containing ouabain.

Inorganic phosphate was estimated by the method of Lowry and Lopez (7). The protein content was measured by the method of Lowry *et al.* (6) using bovine serum albumin as standard. The enzyme activity was expressed as μmol Pi h^{-1} mg $protein^{-1}$. The Na, K-ATPase activity was calculated by subtracting ouabain resistent, Mg-ATPase activity from the total ATPase activity.

The statistical significance of differences was evaluated by Student's t-test.

RESULTS AND DISCUSSION

Results of hypothermia 20°C effect on the activity of Na, K-ATPase and Mg-ATPase during 1 hour and 3 hours are given in the Table 1. The figures show that 1 hour hypothermia of body increases Na, K-ATPase activity

*From Problem Neurochemistry Laboratory, Daghestan State University, Makhachkala, USSR.

Table 1: The effect of 20°C hypothermia for 1 hour and 3 hours on ATPase activity of homogenate and sub cellular fractions rat brain cerebral cortex (M^+m).

Fractions	Animal control group		Prolonged 1 hour hypotermia		Prolonged 3 hours hypotermia	
	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na, K-ATPase
H	9.91±0.45 (19)	6.51±0.28 (19)	13.99±1.41 (6) $0.001<p_1<0.01$*	12.24±1.41 (6) $p_1<0.001$*	11.23±0.49 (11) $0.001<p_1<0.01$*	8.28±0.66 (11) $0.001<p_1<0.01$*
S	13.48±0.26 (6)	11.61±0.80 (6)	16.15±0.61 (10) $0.001<p_1<0.01$*	15.18±0.90 (10) $p_1<0.01$*	13.99±1.33 (5) $p_1>0.2$	12.92±0.48 (5) $0.2<p_1<0.1$
M	25.38±2.18 (6)	2.36±0.65 (6)	24.63±1.68 (10) $p_1>0.2$	7.91±0.63 (10) $p_1<0.001$*	17.24±0.68 (5) $0.001<p_1<0.01$*	4.11±1.19 (5) $0.02<p_1<0.05$*

Notes to Tables 1 and 2:
in brackets are given number of animals under investigation; stars denote true differences ($p<0.05$);
P_1: in comparison with control; P_2: in comparison with applied hypothermia.

consequently 88.0%, 30.8% and 235.2% in homogenate (H), synaptosomal (S) and mitochondrial (M) fractions. Mg-ATPase activity surely increases in H, S, does not change in M, acquiring tendency to abate.

Three hours prolonged hypothermia varies the enzyme activity as well to a much lesser degree. Therefore, Na, K-ATPase activity for certain growth 27.2% and 74.2% in H and M, consequently. Mg-ATPase activity arises 13.3% in H and decreases 32.1% in M.

There may be several causes of changes in Na, K-ATPase activity in sub cellular fractions of rat cerebral cortex when they are affected by hypothermia.

First: The fall of the body temperature brings about the rise of lipid peroxidation and this in turns changes chemical and physical properties of membrane (9). The synaptosomes with sedimental properties that differ from those in control animal group appear in brain tissue of hypothermic animals derived by homogenization. These synapto-

Table 2: The effect of dalargin on ATPase activity of homogenate and sub cellular rat brain cortex fractions hypothermic animals (M^+m).

Fractions	Animal control group		Injection of peptide during 1 hour hypotermia		Injection of peptide during 3 hours hypothermia	
	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na, K-ATPase
H	9.91±0.45 (19)	6.51±0.28 (19)	13.57±0.41 (6) $p_1<0.001$* $p_2>0.2$	9.74±0.69 (6) $p_1<0.001$* $0.1<p_2<0.2$	11.21±0.38 (10) $0.001<p_1<0.01$* $p_2>0.2$	8.01±0.32 (10) $p_1<0.001$* $p_2>0.2$
S	13.48±0.26 (6)	11.61±0.80 (6)	16.95±0.63 (15) $p_1<0.001$* $p_2>0.2$	13.22±0.86 (15) $0.1<p_1<0.2$ $0.1<p_2<0.2$	15.96±0.37 (6) $p_1<0.001$* $0.1<p_2<0.2$	12.25±1.58 (6) $p_1>0.2$ $p_2>0.2$
M	25.38±2.18 (6)	2.36±0.65 (6)	18.98±1.42 (9) $0.02<p_1<0.05$* $0.02<p_1<0.01$*	6.21±0.53 (10) $p_1<0.001$* $0.05<p_2<0.1$	23.53±1.22 (7) $p_1<0.2$ $p_2>0.001$*	4.01±0.73 (7) $0.1<p_1<0.2$ $p_2>0.2$

somes are characterized by the greater permeability of membranes and under ultra centrifugation get into mitochondrial fraction. That means that the extension of Na, K-ATPase activity in M derived by prolonged hypothermia for 1 hour and 3 hours may be accounted for by redistribution of the activity between S and M fractions.

Second: The sharp increase of Na, K-ATPase activity in fraction S during 1 hour (77.0%) may be caused by activity of the enzyme itself. The number of organs both in man and animal including brain contain NADH-vanadate-oxidoreductase that converts vanadate (VO_3–intovanadyl (VO^{2+}) thus lowering the presence of vanadium in its +5 oxidation state is a strong inhibitor of Na, K-ATPase in cells (3, 4). Under the lower temperature the regaining processes prevail over oxidizing events providing the increase of NADH, which in turn increases the activity of NADH-vanadate-oxidoreductase and the latter decreases the quantity of vanadate - the inhibitor of Na, K-ATPase.

Third: The fact that 1 hour hypothermia increases Na, K-ATPase activity to a much higher degree than 3 hours makes us think that a shorter time period hypothermia may serve a great stressor factor for an organism. As the result of this there occur a series of processes that together with CNS and humoral system increase the activity of the enzymes and Na, K-ATPase activity as well. The obtained changes in the enzyme activity with 3 hours prolonged hypothermia evidently have nothing to do with the stress reaction, but being equilibrators of body temperature.

To clear out this assumption the influence of neuropeptide dalargin on rat brain sub-fractions Na, K-ATPase activity has been thoroughly studied. In research papers dalargin is known as anti-stressor drug (1). The data obtained shows that intra-abdominal injection of dalargin with 1 hour hypothermia decreases Na, K-ATPase activity 20.4%, 12.9% and 21.5% respectively in H, S and M in comparison with 1 hour hypothermia (Table 2). The changes of Mg-ATPase under these circumstances are insignificant, with the exception of M where the enzyme activity falls by 22.9%. With 3 hours hypothermia dalargin does not cause considerable changes in Na, K-ATPase and Mg-ATPase activity, except M, where the latter rises a 36.5%.

Therefore, the results of experimental research indicate that dalargin can correct the changes caused by hypothermia in animal body without anesthesia.

REFERENCES

1. Aleklinskaya LA, Vasilyev YaS, Savenkova GM : The influence of dalargin on the content of biogenous amines in the plasma of blood in patients with cardio vessel disease. In Neuropeptides: their role in physiology and pathology. Tamsk, pp 158-159, 1985.

2. Belous AM, Bondarenko VA : Structural changes of biomembrane with hypothermia. Science Dumka, Kiev, p 255, 1982.

3. Cantley LC, Josepson L, Warnes R, Yanagisawa M, Lechene C, Guidotti G : Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. J Biol Chem, 252:7421-7423, 1977.

4. Erdmann E, Werdan K, Kravietz W, Lebuhn M, Christl S : Significance of NADH-vanadate-oxidoreductase of cardiac and erythrocyte cell membrane. Basic Res Cardiol, 75:460-465, 1985.

5. Gray EG, Whittaker VP : The isolation of nerve endings from brain: An electron microscopic study of cell fragments derived by homogenization. J Anat, 96:79-87, 1962.

6. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ : Protein measurement with the Folin phenol reagent. J Biol Chem, 193:265-275, 1951.

7. Lowry OH, Lopes JJ : The determination of inorganic phosphate in the presence of labile phosphate esters. J Biol Chem, 162:421-242, 1946.

8. Petrow IV, Gubler EV : Artificial hypothermia: Med, p 149, 1961.

9. Vladimirov Yu A, Archakov AJ : Peroxide oxidation of lipids in biological membranes. Science, Moscow, p 252, 1972.

Correspondence:
G. ABILOVA
Problem Neurochemistry Laboratory,
Daghestan State University,
Makhachkala, USSR.