GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Parameters of Cell Energy Metabolism during Stress in Laboratory Animals and Energotropic Effect of α_1-Adrenoblocker Doxazosin

E. L. Vishnevskii, A. E. Vishnevskii, E. I. Shabel'nikova*, O. N. Safronova*, A. A. Dreval', G. V. Topchieva**, and V. S. Sukhorukov***

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Cytochemical activity of succinate dehydrogenase and lactate dehydrogenase in blood lymphocytes, activity of hydroperoxides, MDA, and blood plasma antioxidants were studied in experimental animals during immobilization stress after preliminary injection of doxazosin. Preliminary adrenergic receptor blockade prevented changes in cell metabolism characteristic of immobilization stress. The role of adrenergic regulation in the realization of energotropic effects during immobilization stress was examined.

Key Words: *stress; lymphocytes; blood plasma; adrenoblocker; energy metabolic enzymes*

Disturbances in cell energy metabolism in various human diseases are now actively studied. In this context, studies of the key processes in mitochondria are of particular importance. It is established that epinephrine and acetylcholine can directly activate and inhibit mitochondrial functions [4,5]. For example, epinephrine selectively activates succinate oxidation and to some extent inhibits ketoglutarate oxidation, while acetylcholine produces an opposite effects. In the organism, mitochondrial activity is controlled by blood level of these transmitters. After relaxation, mitochondria are activated by epinephrine pulse. It is known that epinephrine activates catabolism of carbohydrates and lipids, *i.e.* increases production of energy substrates and stimulates energy metabolism on the whole. The principal signal triggering inhibition of mitochondria seems to be removal of epinephrine by monoamine oxidase and release of acetylcholine, activated ketoglutarate oxidation and inhibits succinate oxidation.

Measurement of activity of mitochondrial redox enzymes in peripheral blood lymphocytes by quantitative cytochemical methods adequately reflects the level of energy metabolism in the organism [2,3]. In addition to activity of mitochondrial enzymes in lymphocytes, plasma LPO parameters and antioxidant activity (AOA) can be used as laboratory indices of energy metabolism [1].

Our aim was to study polysystemic indices of energy metabolism during immobilization stress in rats under control conditions and after treatment with α-adrenoblocker doxazosin.

MATERIALS AND METHODS

Experiments were carried out on random-bred rats (250 g) divided into 3 groups (n=10 in each group). Group 1 rats were tested 3 h 40 min after intraperitoneal injection of physiological saline (1 ml). Group 2

Department of Urology, Moscow State Medical Dental University; *Research Department of General Pathology, Moscow Research Institute of Pediatrics and Pediatric Surgery, Ministry of Health of the Russian Federation; **Department of Histology, Cytology, and Embryology, Department of Therapy, Russian State Medical University, Moscow. ***Address for correspondence:*** v- sukhorukov@mtu-net.ru. V. S. Sukhorukov

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rats were intraperitoneally injected with physiological saline (1 ml) and after 40-min rest subjected to immobilization stress for 3 h. Group 3 rats intraperitoneally received 1.25 mg α_1-adrenoblocker doxazosin (Cardura) in 1 ml physiological saline (equivalent to average single dose for humans) and after 40 min subjected to immobilization stress. The following parameters were measured: cytochemical activity of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in blood lymphocytes, and levels of hydroperoxides, MDA, and AOA in blood plasma.

Cytochemical activity of mitochondrial enzymes (SDH and LDH) in blood lymphocytes was assessed by the method of R. P. Nartsissov (nitrotetrazolium staining followed by visual morphometry). The assay kits were produced by Khimtekhmash Research-and-Production Complex and IREA State Research Institute. Enzyme activity was expressed in arbitrary units, which corresponded to the mean number of formazan granules.

The intensity of LPO was assessed by the content of hydroperoxides, MDA, and AOA in blood plasma. The concentration of hydroperoxides (primary products of LPO) was determined by measuring UV absorption in lipid extracts from the blood on an SF-26 spectrophotometer (method of V. B. Gavrilov). MDA, a secondary LPO product, was assayed in the sediment of blood plasma β-lipoproteins by the method of I. Minoru. This method is based on the reaction between MDA and 2-TBA, which at high temperature and acid pH yielded stained trimethine complex containing one MDA molecule and two 2-TBA molecules. For evaluation of the total antioxidant potential of the blood plasma we used the method of AOA determination proposed by G. I. Klebanov and based on the ability of blood plasma to inhibit accumulation of TBA-active metabolites in suspension of yolk lipoproteins, which was chosen as the model oxidation system.

RESULTS

Pronounced disturbances in cell energy metabolism were revealed in group 2 rats in comparison with the control group 1 rats (Fig. 1). Stress markedly modified functional activity of mitochondria. The cytochemical indices suggest that these changes result from dramatic activation of energy metabolism in cells. Published data [4,5] suggest that this activation results from direct action of catecholamines on mitochondria.

Activation of tissue respiration increases the content of LPO products (hydroperoxides and MDA) and changes AOA index determined as the ratio of rate constants of hydroperoxides and MDA formation reactions.

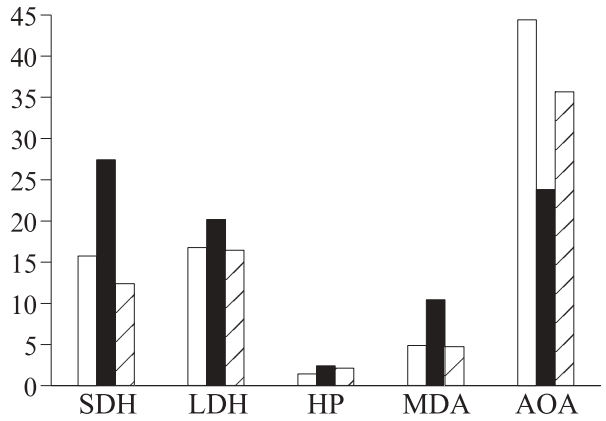

Fig. 1. Effect of immobilization stress alone (solid bars) or in combination with α_1-adrenoblocker (hatched bars) on cell energy metabolism in rat blood. The control data of intact rats are shown by the open bars. SDH (rel. units), LDH (rel. units), HP (hydroperoxides, rel. units/ml), and MDA (nmol/ml), AOA (antioxidant activity, %).

For verification of this hypothesis, we studied the effect of adrenoblocker within the same experimental model. The positive therapeutic effect of α_1-adrenoblockers on cell energy metabolism was established during treatment of the patient with urological diseases [6].

In group 3 rats preliminary injection of α_1-adrenoblocker doxazosin prevented changes in parameters of cell energy metabolism during immobilization stress (Fig. 1).

The data obtained on group 3 rats attest to the involvement of catecholamines in activation of mitochondria during stress. The content of MDA and activity of LDH in groups 1 and 3 were similar, hence preliminary adrenergic receptor blockade completely prevented the stress-induced changes of these indices. Decreased cytochemical activity of SDH in group 3 rats below the control values suggests that under our experimental conditions the energotropic effect is based on adrenergic regulation.

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