Protective Effect of Energy Metabolism Regulators in Alteration of Gravitation Load under Experimental Conditions

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 10, pp. 423-429, October, 2006
Original article submitted January 19, 2006

Mitochondrial substrate-based preparations corrected disorders, caused by long-term exposure to abnormal gravitation vector in head-down tilt (hanging) test in rats. The preparations produced systemic and polyorgan protective effects consisting in correction of the blood prooxidant/antioxidant balance, energy metabolism in musculus soleus, and minimization of morphological changes in the liver and kidneys.

Key Words: *amber-cardio; amber-energovit; head-down tilt status; systemic effect; factor analysis*

Long-term space missions cause total systems disorders impeding the recovery after the mission despite high requirements to the health status of team members [3,4]. Dependence of many functions and their metabolic support on gravitation formed in the phylogenesis. Low gravitation or its abnormal vector disorients functional systems and causes moderate stress, drawing off energy resources for the maintenance of homeostasis at a different level [3, 4,7]. Minimization of systemic manifestations of gravitation dysregulation is an important problem of medical provision of mission safety [3]; the solution of this problem requires modern pharmacological technologies used from the total-systems standpoint [2]. A new trend in bioenergetic pharmacology is based on the leading system-forming role of the mitochondria in energy homeostasis [6,8]. The use of drugs containing mitochondrial substrates for the treatment and prevention helps to maintain homeostasis through regulation of organelle functions, to prevent diseases caused by energy deficiency, and to stimulate recovery after exercise or disease [6,8,9]. It is assumed that mitochondrial substrates preserve evolution-fixed gravitation-dependent functions and provide their metabolic support under conditions of microgravitation.

The aim of this study was total systemic analysis of the effects of drugs, regulators of energy metabolism (REM) [6] created on the basis of mitochondrial substrates, on a complex of energy metabolism parameters of the skeletal muscle, liver and kidney morphology, LPO and antioxidant defense status of the blood under conditions of altered gravitation factor.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=48) weighing 180-220 g. The protocol of experiment including all manipulations on animals was approved by Biomedical Ethics Committee of Institute of Biomedical Problems. Control rats (groups 1-3) were

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0007-4888/06/1424-0441

kept in a vivarium, experimental animals (groups 4-6) were exposed to head-down tilt (hanging). Under these conditions, *m. soleus* (performing antigravitation function) was exposed to hypodynamia, the kidney to hemodynamic devastation, and the liver to hemodynamic overload because of redistribution of body fluids towards the cranial direction [4,7,10]. Animals of groups 1 and 4 received vivarium rations; in groups 2 and 5 these rations were supplemented with amber-cardio REM (AC; succinic and malic acids, 50 mg each), in groups 3 and 6 with amber-energovit REM (AE; 50 mg succinic acid, 70 mg glutamic acid, 2 mg vitamin B_2, and 2 mg vitamin B_6) in doses of 50 mg succinic acid/kg. After experiment the animals were decapitated.

Isolated *m. soleus* was frozen in liquid nitrogen and serial sections were made for cytochemical detection of SDH and α-glycerophosphate dehydrogenase (GPDH). Activities of enzymes were measured in the slow and fast fibers using a Leica image analyzer. The fibers were identified immunohistochemically with detection of slow and fast isoforms of myosin heavy chains, using immunofluorescent methods. Antibodies to slow (MHCs) and fast (MHCf) myosin chains were used (clones (NCL-MHCf (a+b) and NCL-MHCs, Novocastra Laboratories) were used. The morphology of the liver and kidneys was evaluated by light microscopy of the parenchymal and stromal cells and the microcirculatory bed. The intensity of LPO and the level of antioxidant defense were evaluated by the content of MDA [1] and catalase activity in the plasma and erythrocytes.

The data were analyzed in ANOVA/MANOVA moduli of Statistica 6.0 software. The statistical

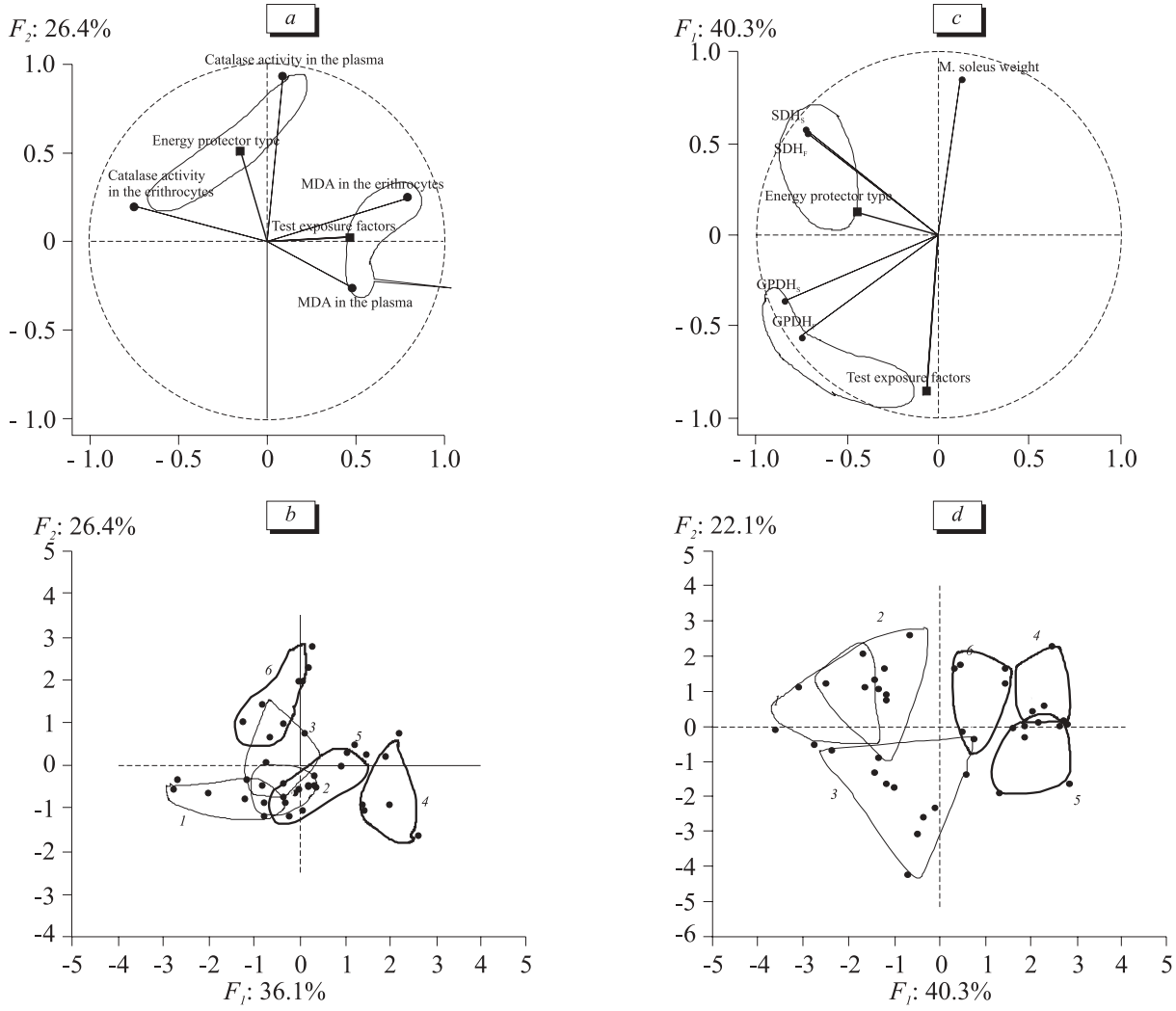

Fig. 1. Relationship between REM effects, test exposure factors, and energy protector type, presented in coordinates of the main components F_1 and F_2 by the sum of parameters of the blood prooxidant/antioxidant status (*a*, *b*) and energy metabolism in m. soleus (*c*, *d*). Figures show the groups of control (*1-3*) and experimental animals (*4-6*); no energy protector (*1*, *4*), treatment with AC (*2*, *5*) or AE (*3*, *6*). S: slow; F: fast fibers.

significance of differences between the groups were evaluated by parametric (multiple LSD test) or nonparametric (Mann—Whitney *U* test) tests. The relationship between the test exposure, energy protection with REM, and the energetic and oxidative processes was studied by the main components method [5].

RESULTS

The parameters of the blood oxidant/antioxidant status, energy metabolism in *m. soleus* during REM treatment and test exposure were in reciprocal relationships, formalized as informative clusters reflecting the axes of stimulating relationships: "energy protection" with catalase activity (increase in antioxidant defense) and SDH (mitochondrial energization); head-down tilt with MDA content (LPO initiation), GPDH activity, and *m. soleus* weight as a result of hypodynamia (Fig. 1, *a*, *c*). The effects of REM and head-down tilt were comparable (Fig. 1, *a*, *c*), because they unambiguously grouped the objects of observation in the coordinates of multidimensional parameters F_1 and F_2. In case of reduction of the mass data to F_1 (head-down tilt) and F_2 (energy protection), the control and experimental groups were differentiated as dots characterizing each animal by a complex of oxidant/antioxidant status parameters (Fig. 1, *b*). Intact controls (group 1) or animals treated with REM (groups 2 and 3) fused into homogenous communities, indicating similarity of their states.

In head-down tilt test the objects were differentiated by the REM type: group 4 (no energy protection) was concentrated along F_1 axis with a shift to the positive area, while groups 5 and 6 joined intact animals. Summation of REM protective effects manifested by the similarity of grouping: animals treated with AE (groups 3 and 6) united along axis F_2 and their projections shifted to the zero and negative F_1 values. The location of the groups trea-

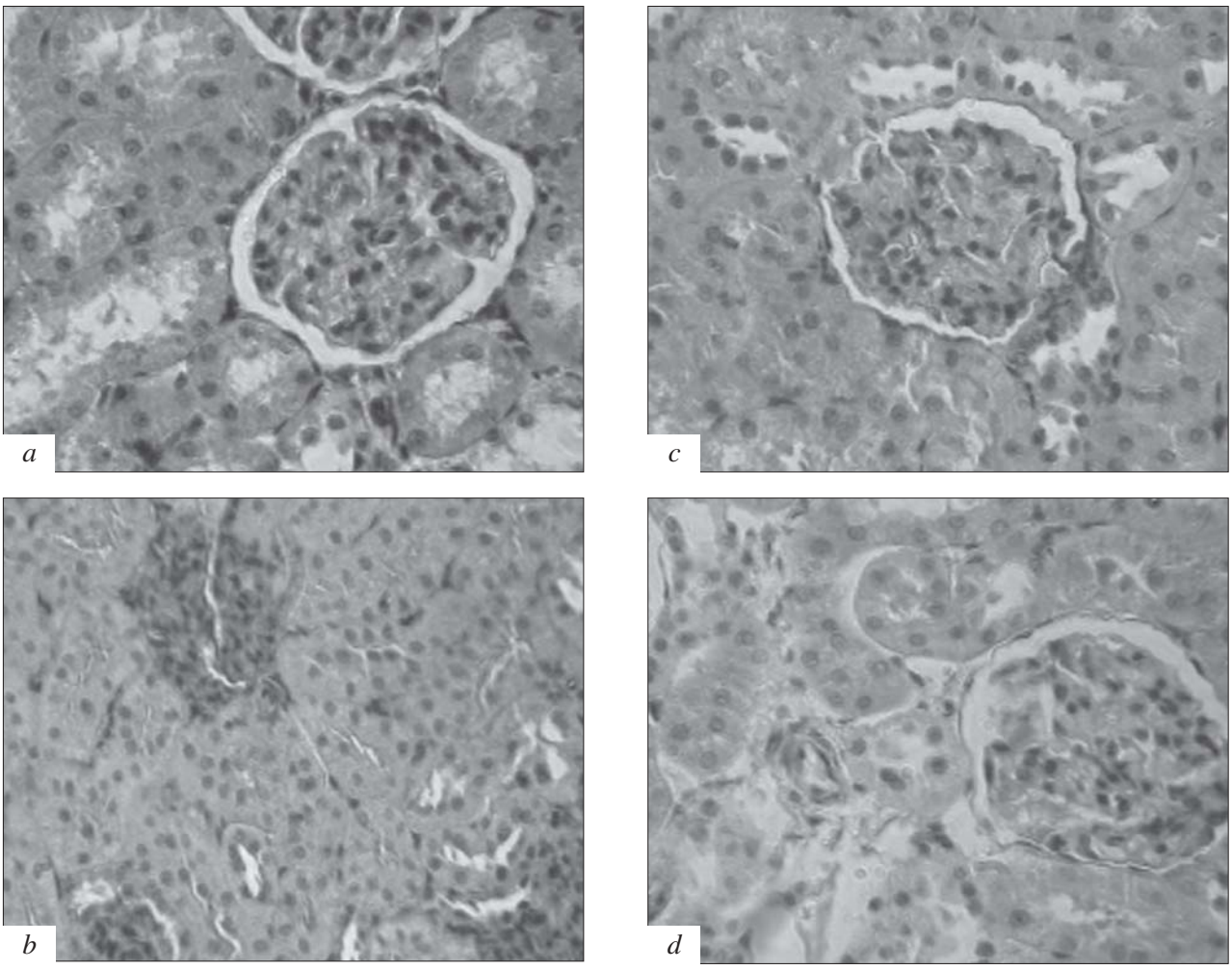

Fig. 2. Effects of head-down tilt (hanging) (*b*) and energy protection with AC (*c*) and AE (*d*) on the morphology of rat renal cortical layer in comparison with the control (*a*). Here and in Fig. 3: hematoxylin and eosin staining, ×300.

ted with AC indicated better protective effects of AE in comparison with AC. The specific pharmacodynamics of the preparations was demonstrated by multidimensional analysis of energy metabolism in m. soleus (Fig. 1, *d*). Animals in control group and after head-down tilt grouped as dots concentrated predominantly along axis F_1 (test exposure) and were differentiated by the type of REM. The dots representing intact and AC-treated rats were united, while the AC group was separated. In head-down tilt the position of all objects was determined by REM: rats treated with AC (group 5) tended to the group 4 (head-down tilt without energy protection), while AE (group 6) approached the control in the center of coordinates, reflecting a more potent effect of this drug.

There were in fact no morphological changes in the kidneys of control rats, treated with REM (Fig. 2, *a*). Hemodynamic devastation during head-down tilt was associated with compression of vascular glomeruli in the cortical layer (Fig. 2, *b*). Capillary loops of the glomeruli tightly stuck to each other, forming a compact cell conglomeration. Multiple hemostasis with solitary thromboses was seen in the peritubular vessels; hemorrhagic foci were seen in the interstitial tissue between the tubules. Release of vacuoles into the tubular lumen was observed in the nephron tubules, particularly in the epithelium of the proximal twisted compartments. The epithelium of Henle's fine loop tubules and collection tubes in the medulla and renal papilla was unchanged. Renal bodies with normal morphology of capillary loops predominated in the group "REM+head-down tilt", with just few compressed glomeruli.

Treatment with AC was associated with negligible plethora, hemostasis in peritubular vessels, and slight degenerative changes (Fig. 2, *c*), while AE minimized the changes in the renal parenchyma (Fig. 2, *d*).

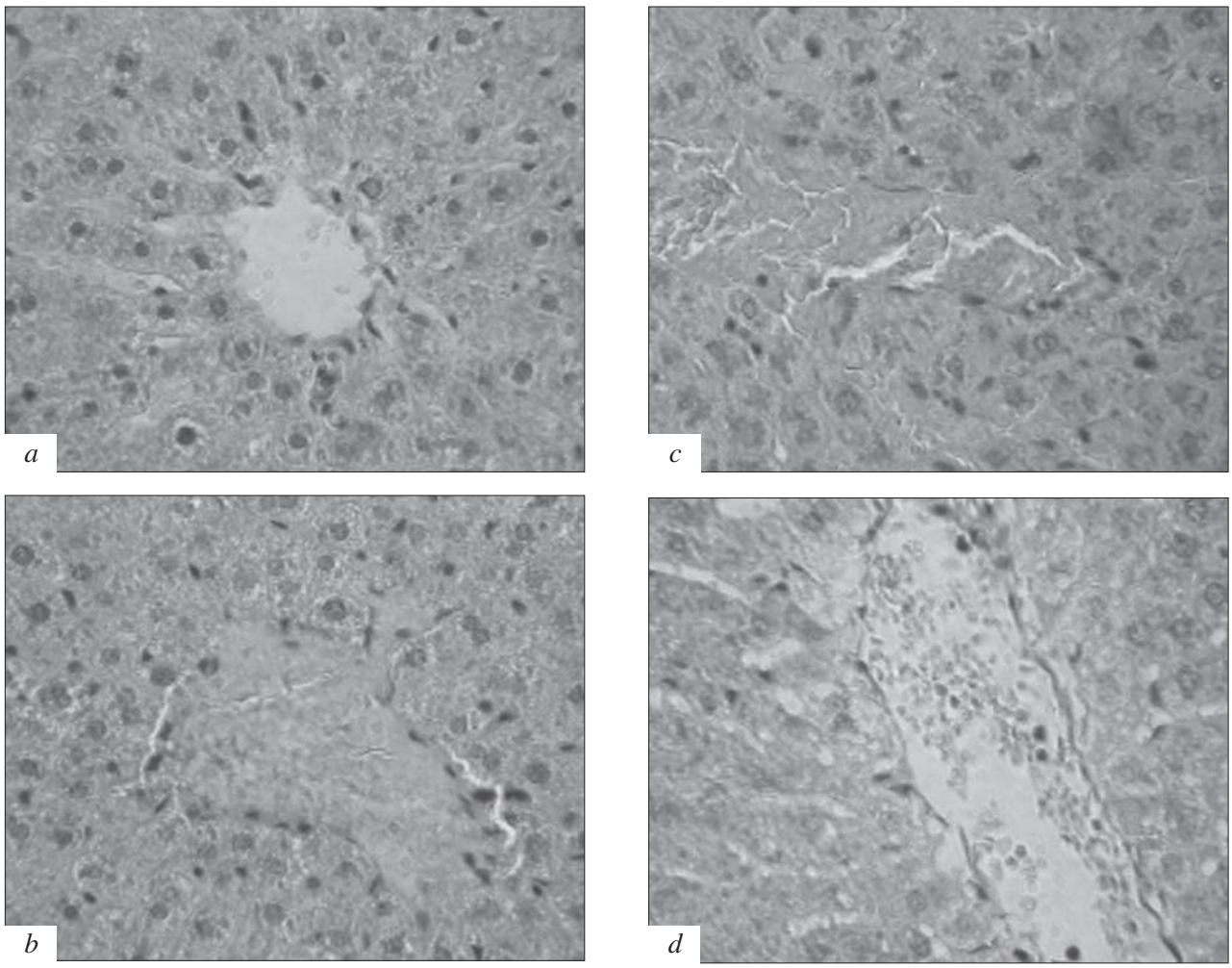

Fig. 3. Effects of test exposure (head-down tilt by hanging) (*b*) and energy protection by AC (*c*) and AE (*d*) on liver morphology in the central vein area in comparison with the control (*a*).

In the control, hepatocytes had normal structure, the sinusoidal cells being retained, irrespective of drug treatment. The hepatic lobule in the central vein area was characterized by bar structure and open sinusoids (Fig. 3, *a*). Signs of sharply congestive plethora in the central vein manifested under conditions of hemodynamic overload (Fig. 3, *b*), with deformation of the vein, shrinkage of the sinusoidal spaces, "blurring" of the bar structure because of hepatocyte swelling caused by the central vein overload. Treatment with AC (Fig. 3, *c*) led to a decrease in the central vein plethora and deformation, the bar structure was preserved, there were no pronounced destructive changes in the cells. Treatment with AE minimized the shifts (Fig. 3, *d*): plethora decreased, the bar structure approximated the control and empty sinusoids were retained. Hence, REM exhibited a protective polyorgan effect in animals exposed to head-down tilt: hepatoprotective (in hemodynamic load of the liver) and nephroprotective (in hemodynamic devastation of the kidneys). Analysis of morphological changes indicates a more pronounced effect of AE than AC in this model. Quantitative evaluation of differences between the groups showed that erythrocyte catalase activity of controls did not change under the effect of REM (Table 1). Head-down tilt caused depression of the enzyme, but energy protection "saved" its activity at the level of control. Treatment of control rats with REM promoted an increase in the plasma activity of the enzyme, this effect being more pronounced in AC treatment (Table 1). Plasma catalase activity did not change under conditions of experimental exposure, while treatment with REM promoted an increase in this parameter, the effect being significantly higher after AE treatment.

Erythrocyte MDA content slightly decreased under the effect of REM in the control (Table 1). Head-down tilt initiated LPO and led to accumulation of MDA in erythrocytes and plasma. Treatment with AC during this period did not modify the level of MDA, while AE inhibited MDA accumulation in erythrocytes and plasma (Fig. 1, *c*, *d*). The content of MDA increased under the effect of REM in the plasma of controls, which can be explained by total pre-activation of oxidative processes in the tissues through the fast metabolic cluster and a certain intensification of free radical processes because of synchronous activation of mitochondria (systemic cell integrators [6,8]) in involved organs and tissues. Head-down tilt led to intensification of LPO and suppression of antioxidant defense, while treatment with REM, particularly with AE, inhibited LPO and activated antioxidant defense. Treatment of controls with AE (in contrast to AC) increased SDH activity in *m. soleus* slow and fast fibers (Table 2). SDH activity in slow fibers (predominating in this muscle) decreased significantly during unloading and deficiency of the tonic component of movements, while fast muscle SDH remained unchanged, this being in line with published data [7]. Under these conditions energy protection was associated with increase in SDH activity in slow fibers but did not manifest in fast ones. Slow fibers as the main functional elements of *m. soleus* were more sensitive to hypodynamia, and the protective effects of EMR towards these muscles were more pronounced. Activity of GPDH (glycolytic marker) in slow and fast fibers of *m. soleus* of controls virtually did not change under the effect of AC and increased under the effect of AE (Table 2). In hypodynamia activity of GPDH increased only in fast fibers, reflecting activation of glycolysis as a catabolic

TABLE 1. Effects of REM on Blood LPO and Antioxidant Defense System of Rats during Alteration of the Gravitation Vector in Head-Down Tilt (Hanging) Test (*M*±*m*)

Parameter	Group	No REM	AC	AE
Catalase activity, kg-O_2 atom $min^{-1}mg^{-1}$				
in erythrocytes	Control	2086±409	2060±409	1964±383
	Test	1277±408**	1563±442	1811±484
in blood plasma	Control	139±77	348±77	262±72
	Test	182±77	243±83	394±91*
MDA content, μmol/ml				
in erythrocytes	Control	47±5	42±5	42±5
	Test	59±5**	56±5**	49±6
in blood plasma	Control	3.7±0.4	4.3±0.3	4.1±0.3
	Test	4.5±0.3**	4.4±0.3	3.4±0.3**

Note. Here and in Table 2: *$p<0.05$, *$p<0.01$ compared to control.

TABLE 2. Effects of REM and Hypodynamia on SDH and GPDH Activities in Fibers of Different Types in *m. soleus* and Its Weight (*M*±*m*)

Parameter	Group	No REM	AC	AE
SDH, opt. dens. units				
in slow fibers	Control	0.49±0.11	0.43±0.12	0.64±0.11
	Test	0.33±0.11**	0.42±0.12	0.46±0.12*
in fast fibers	Control	0.71±0.13	0.61±0.14	1.04±0.13
	Test	0.62±0.14	0.66±0.15	0.64±0.16**
GPDH, opt. dens. units				
in slow fibers	Control	0.18±0.05	0.18±0.04	0.31±0.04
	Test	0.24±0.04	0.34±0.04*	0.23±0.06*
in fast fibers	Control	0.25±0.07	0.30±0.08	0.47±0.07
	Test	0.45±0.07*	0.56±0.08*	0.38±0.09*
M. soleus weight, mg	Control	123±9	126±9	114±9
	Test	47±9	52±9	53±10

process under conditions of passive hind paws of animals during head-down tilt. Treatment with AC stimulated GPDH in fibers of both types. By contrast, injection of AE inhibited activation of glycolysis. The detected differences in the protective effects of AC and AE can be explained by the known effects of components of these preparations on activity of mitochondrial fast metabolic cluster with consideration for the reaction of organelles on the "weak" and "strong" activators during low-energy shift of different intensity [6,8,9]. The weight of *m. soleus* decreased in hypodynamia, which was due to intensification of catabolism and protein degradation processes. Injection of AC to control animals did not change the weight of *m. soleus*, while AE slightly decreased it (Table 2). A trend to reduction in *m. soleus* weight loss was observed during treatment with AE (in contrast to AC), indicating opposite effects of REM on the oxidative and glycolytic processes in fast and slow fibers during normal functioning and hypodynamia.

Formalized systemic analysis of the initial mass data formed by multidimensional correlations between the exposure factor and responses of the blood oxidant/antioxidant system and *m. soleus* metabolism and tissue morphology not only helped detect the "prograviation" effects of the preparations, but also differentiated them by the intensity of these effects. The results indicate that REM are characterized by systemic polyorgan protective effects, manifesting simultaneously in different tissues and metabolic processes, which was due to multiplicity of targets located in the mitochondria of cells in the organs with gravitation-dependent functions. Hence, REM preparations based on substrates and compounds essential for activity of the mitochondrial fast metabolic cluster are perspective means for pharmacological protection from disorders caused by alteration or decrease of the gravitation vector.

The study was supported by the Ministry of Industry, Science, and Technologies of the Russian Federation (contract No. 43.600.11.1043).

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