Systemic Effects of Energy Metabolism Regulator Amber-Antitox during Vibration-Induced Dysregulation

N. K. Mazina and V. A. Khazanov*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 9, pp. 297-303, September, 2006
Original article submitted November 24, 2005

Energy metabolism regulator Amber-antitox had a systemic effect under experimental conditions of vibration-induced dysregulation. Whole-body vibration was accompanied by nonlinear changes in energy metabolism in the heart, liver, kidneys, and lymphocytes and impairment of intrasystemic interorgan relationships between mitochondria. Amber-antitox prevented vibration-induced deenergization of mitochondria and contributed to the preservation of multidimensional relationships of energy metabolism in vital internal organs.

Key Words: *energy metabolism; Amber-antitox; vibration-induced dysregulation; factor analysis*

The introduction of medical methods with energy metabolism regulators (EMR) suggests systemic evaluation [3] of their efficiency by means of multivariate analysis [5,9]. Preclinical studies of EMR are performed on models for the *in vitro* response of the organism to exogenous stimulation. The cycle of mitochondrial function in various tissues (rest—activity—rest) is initiated by addition of substrates and ADP. An incomplete rest—activity cycle is observed after treatment with substrates and uncoupler [4,6,11]. The regulatory role of mitochondria in homeostasis is evaluated by recording of various parameters, which hinders the interpretation of data. Summarization of data involves the analysis of interactions between input factors, whose effects are modified by the mitochondrial response in organs and tissues.

Whole-body vibration is a promising experimental model to study the systemic multilevel effect of EMR. Long-term vibration under experimental conditions or exposure to the adverse industrial or environmental factor over many years is followed by systemic dysregulation of homeostasis, hypoxia, and energy deficiency [2,10]. Systemic approach should be used to develop new schemes for the prevention and therapy of vibration-induced changes (multiple visceropathies and angiopathies [10]) from the positions of bioenergetic pharmacology [12,13].

Here we studied the effect of an EMR Amber-antitox (AA) on vibration-induced dysregulation in energy-producing systems of the myocardium, liver, kidneys, and lymphocytes.

MATERIALS AND METHODS

Experiments were performed on 60 male Chinchilla rabbits weighing 2.5-3.0 kg and obtained from Podgornyi nursery (Kirov region). The study was conducted according to the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Vertical whole-body vibration (amplitude 0.5 mm, frequency 44 Hz) was induced on an UV 70/200 industrial device. The animals were exposed to 60-min vibration in the morning time (9.00-11.00) over 21 and 56 days. Aqueous suspension of AA was administered intragastrically through a tuber 60 min before the start of vibration. Single dose of AA (8.4 mg/kg) was estimated from the

Kirov State Medical Academy; *Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk. ***Address for correspondence:*** vkh@pharm.tsu.ru. V. A. Khazanov

0007-4888/06/1423-0327

coefficient of bioequivalent doses for various mammalian species and humans taking into account the relations between the weight and relative area of the body surface [1]. Control animals received AA, but were not exposed to vibration.

Blood samples were taken from the marginal vein 60 min before vibration. The state of regulatory systems of energy homeostasis was evaluated from the variability of population and cellular characteristics of lymphocyte SDH activity. The cytochemical method was used to estimate the number and distribution of formazan granules in 50 cells [8]. The energy state of lymphocytes was described by mean enzyme activity and coefficients of variation, asymmetry, and excess [7,8]. Oxidative processes in vibration-sensitive organs (heart, liver, and kidneys) [10] were studied *in vitro* by the method of polarography. The rate of mitochondrial respiration in tissue homogenates was estimated on the model of incomplete rest—activity cycle [4]. The metabolic state of mitochondria under rest conditions was modeled by addition of a FAD-dependent substrate (FAD-DS) succinate (1 mM) or a mixture of NAD-dependent substrates (NAD-DS) glutamate and malate (3 mM). ATPase activity was reproduced by oxidation of FAD-DS and NAD-DS with 2,4-dinitrophenol (20 μM). Partial oxidase activity for endogenous substrates was estimated by the sensitivity of endogenous respiration (V_E) to inhibitors of NAD-dependent and FAD-dependent respiration (2 mM amytal and 2 mM malonate, respectively). This index was calculated by the equation: $1\text{-}V_{INH}/V_E$, where V_{INH} is endogenous respiration after addition of amytal (V_A) or malonate (V_M) [6].

The significance of intergroup differences was evaluated by parametric (multiple LSD test) and nonparametric methods (Mann—Whitney *U* test) [9]. Interference of the effects of vibration and AA on mitochondria was estimated by factor analysis and principle component analysis [5]. The data were analyzed by means of ANOVA/MANOVA and Principle components & Classification Analysis (Statistica 6.0 software) [11].

RESULTS

The study of population and cellular characteristics of SDH activity showed that long-term whole-body vibration impairs the energy state of lymphocytes. It was manifested in several stereotypic signs (Table 1). Mean enzyme activity decreased by 20%. According to the variation coefficient, heterogeneity of cells increased by 51% ($p<0.01$). Transition of asymmetry to the region of negative values reflected predominance of cells with decreased activity ($p<0.001$). A negative shift of the excess indicated that lymphocyte pools are unmatched. Low-activity cells dominated under these conditions. Administration of AA before vibration preserved energy status of lymphocytes. SDH activity in treated rabbits was 46% higher than in animals without energy protection ($p<0.001$). Population variability in treated rabbits decreased by 28% ($p<0.001$). According to the decrease in asymmetry, pools of cells with high and low SDH activity were more balanced compared to the control. The negative shift of the excess reflected cell transition from the pool with normal activity to the pool with high activity. Evaluation of the energy state of lymphocytes showed that AA has a consistent and complete effect. Clinical and physiological observations showed that these cytochemical parameters of lymphocyte SDH activity reflect a positive effect of EMR [7,8]. Vibration and energy protection with AA produced phasic changes in mitochondria of the heart, liver, and kidneys, which nonlinearly depended on the duration of exposure. We observed asynchronous changes in several parameters, while other parameters underwent stereotypic changes. Variations in V_E differed in the range and directionality (Fig 1, *a*). V_E decreased in myocardial mitochondria, but returned to the control level in mitochondria of the liver and kidneys. FAD- and NAD-dependent oxidases had similar activity in utilization of endogenous substrates. The slight increase in V_A reflected insignificant acceleration of NAD-dependent reactions in the respiratory chain (Fig. 1, *c*). V_M

TABLE 1. Effect of AA and Long-Term Whole-Body Vibration on Energy State of Rabbit Lymphocytes

Parameter	Control (*n*=12)	Whole-body vibration (*n*=10)	Whole-body vibration+AA (*n*=10)
Mean enzyme activity	10.5 (9.8-11.1)	8.4 (7.5-9.3)*	12.3 (11.1-13.7)$^+$
Variation coefficient	54 (51-58)	82 (75-89)*	59 (55-63)$^+$
Distribution asymmetry coefficient	0.60 (0.56-0.75)	-0.67 (-0.86 - -0.50)*	0.23 (0.08-0.39)$^+$
Excess coefficient	-0.41(-0.67-0.17)	-0.56 (-0.87 - -0.27)	-0.65 (-0.94 - -0.38)

Note. Mean values; 95% confidence intervals are shown in parentheses. $p<0.05$: *compared to the control; $^+$compared to animals of the vibration group.

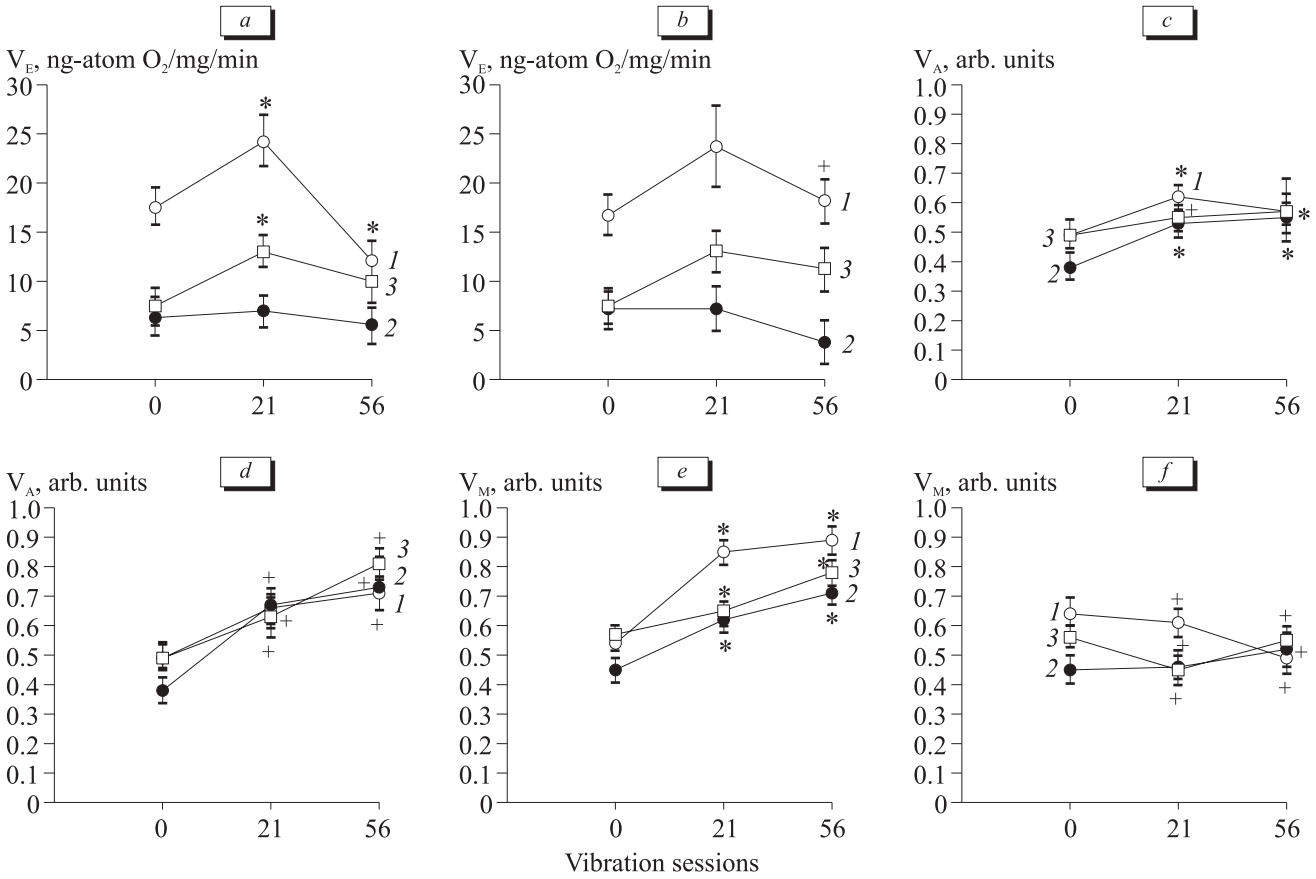

Fig. 1. Effects of whole-body vibration (*a*, *c*, *e*) and AA (*b*, *d*, *f*) on oxidation of endogenous substrates in rabbit myocardium (*1*), liver (*2*), and kidneys (*3*) and sensitivity of V_E to inhibitors of NAD- (*b*) and FAD-dependent fractions of the mitochondrial respiratory chain (*c*). Here and in Fig. 2: $p<0.05$: *compared to pre-vibration parameters; +compared to animals with no pharmacological protection.

underwent a monotonous increase (Fig. 1, *e*) and prevailed over V_A. Therefore, dysregulation is accompanied by synchronous mobilization of the succinate-dependent bioenergetic system in internal organs.

After combined exposure to vibration and AA, myocardial V_E practically did not differ from the control (Fig. 1, *b*). However, this parameter in treated rabbits was higher compared animals of the vibration group. V_E remained nearly unchanged in the kidneys, but decreased in the liver. Inhibitory analysis showed that variations in the contribution of V_A to the monotonous increase of V_E in all organs (Fig. 1, *d*) are in inverse proportion to small changes in V_M (Fig. 1, *f*). These data indicate that AA decreases tissue demands for O_2 and improves mitochondrial function. AA prevents hyperactivation of the FAD-dependent V_E fraction, but increases the contribution of NAD-DS. Oxidation of FAD-DS and NAD-DS in the rest—activity cycle nonlinearly depended on the duration of exposure to input factors (Fig. 2). Oxidation of exogenous succinate corresponded to variations in V_E (Fig. 2, *a*, *e*). However, oxidation of NAD-DS decreased in the myocardium or remained practically unchanged in mitochondria of the liver and kidneys (Fig. 2, *c*, *g*). The regulatory effect of AA was most significant in myocardial mitochondria. The myocardium is one of the organs with high-intensity production and consumption of energy (Fig. 2, *b*, *d*, *f*, *h*). The extremely high rate of respiration decreased, while the respiratory rate corresponding to the range of control values underwent only small changes. Similar results were obtained in experiments with the liver and kidneys. However, the rate of substrate oxidation in these organs was lower. Plasticity of the reactions in rapid metabolic cluster [6,11-13] was higher compared to the NAD-dependent system of energy production (Figs. 1 and 2). The system for production and utilization of succinate probably plays a key role in accumulation of the effects of adverse factor and formation of the response to AA. It is necessary to take into account complex interorgan relationships of mitochondria. They vary under conditions of dysregulation and pharmacological correction, but are unobvious during the data comparison on a plane.

Factor analysis allowed us to decrease the size of information on the interaction of vibration, AA,

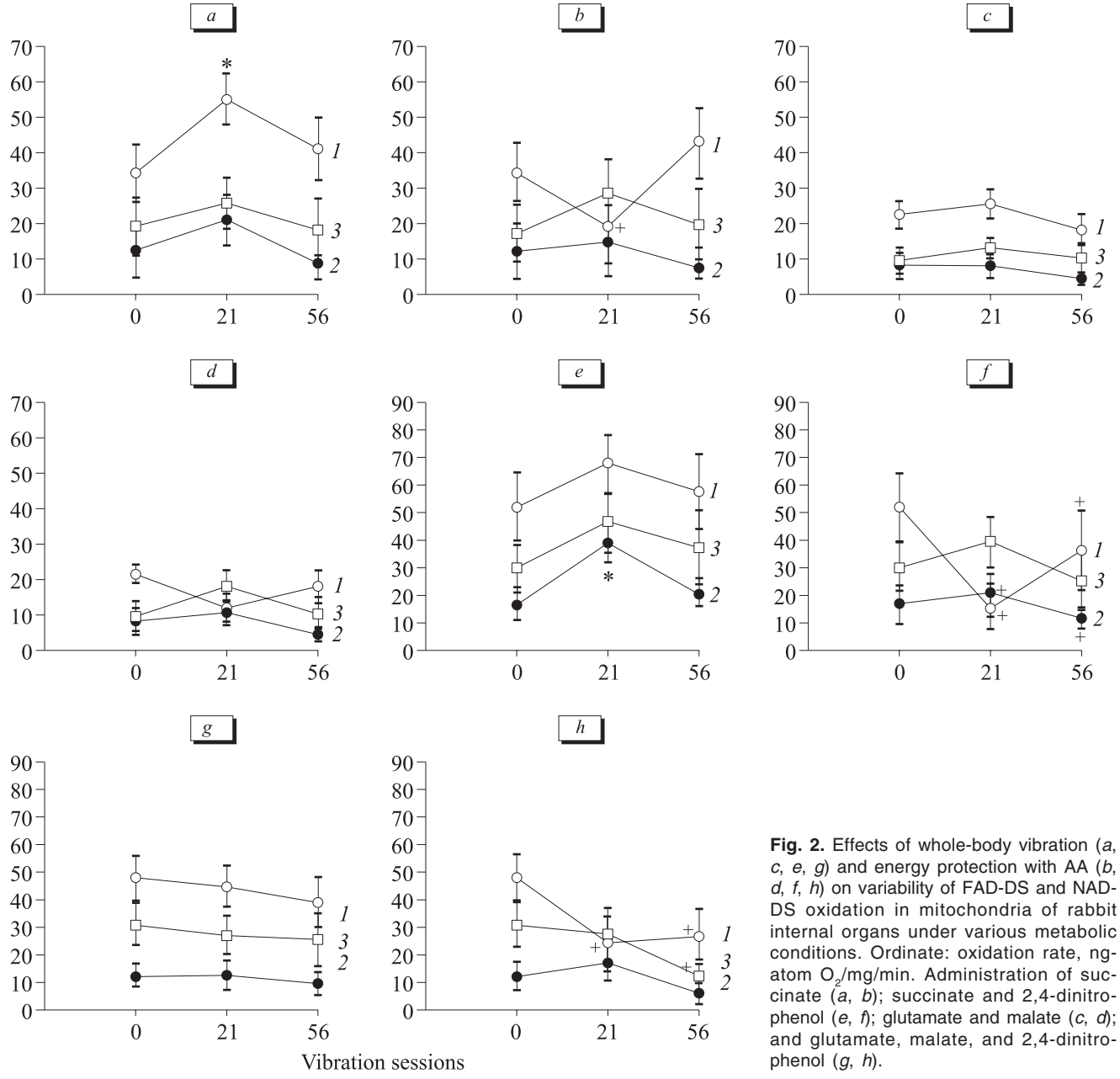

Fig. 2. Effects of whole-body vibration (*a*, *c*, *e*, *g*) and energy protection with AA (*b*, *d*, *f*, *h*) on variability of FAD-DS and NAD-DS oxidation in mitochondria of rabbit internal organs under various metabolic conditions. Ordinate: oxidation rate, ng-atom O_2/mg/min. Administration of succinate (*a*, *b*); succinate and 2,4-dinitrophenol (*e*, *f*); glutamate and malate (*c*, *d*); and glutamate, malate, and 2,4-dinitrophenol (*g*, *h*).

and mitochondria in various organs to 2 independent variables (principle components, Table 2). Vibration and energy production modified the factor structure of the correlation matrix, which reflected the comparability of the strength of their interaction. In animals of the control and vibration groups, the degree of variance absorption by principle components (F_1) was 2.2-2.5 times higher compared to auxiliary components (F_2). F_1 and F_2 were balanced after AA administration. The regulatory effect of EMR counteracted the influence of a dysregulating factor, which contributed to the maintenance of interorgan relationships in mitochondria at the control level. Vibration decreased the ratio of the total variance absorbed by both components (decrease in regulation). The ratio of principle components to the total variance remained unchanged after AA administration (preservation of regulation).

In control animals, strong negative multiple correlations were revealed between kinetic parameters and F_1 (factor loadings >0.8). The contribution of partial reactions was low. However, F_1 mainly depended on V_M. F_2 had a moderate effect only on V_E. The remaining interrelations were insignificant. The factor structure of the matrix significantly changed during dysregulation. Multiple correlations between parameters produced a strong effect on F_1 (factor loadings ≥0.8), but had a posi-

tive sign. Therefore, the regulatory interactions were diminished under these conditions. As distinct from V_A, V_M had little effect on F_1. The value of F_2 absorbing a small part of the total variance distributed the loading on parameters for metabolic activity of mitochondria during administration of succinate, succinate+2,4-dinitrophenol, and glutamate+malate (Table 2). After AA administration, most correlations of test parameters with F_1 had a negative sign. However, significant changes were revealed in absolute parameters of factor weights for individual parameters and their relation to F_2. Factor loadings for V_E, V_M, and administration of glutamate+malate+2,4-dinitrophenol and signs of their influence on F_1 were preserved. Loadings for administration of succinate, succinate+2,4-dinitrophenol, and glutamate+malate on F_2 were redistributed and had a negative sign. These changes show that the system of rapid metabolic cluster is involved in reorganization of tissue bioenergetics. Intersystem regulatory interactions were preserved and partially transformed due to energy production during vibration and accumulation of the effects at the level of mitochondria. Similar tendencies were found in generalized covariations of grouping signs with F_1 and F_2. The influence of vibration and EMR on intersystem interactions of mitochondria (objectively present and implicit regularity) was manifested during formalized presentation of test objects in F_1/F_2 coordinates (Fig. 3). In control animals, the points corresponding to mitochondria of internal organs formed the separate and compact clusters (Fig. 3, *a*). During vibration these clusters became diffuse, superposed, and disoriented and changed the position (Fig. 3, *b*). Administration of AA decreased the degree of dysregulation changes and contributed to the preservation of compactness, separateness, and orientation of point clusters, which did not differ from the control (Fig. 3, *c*).

The strength and directionality of correlations between various parameters reflect the rate and effectiveness of system transition from one state to another state [3]. Our results indicate that EMR not only has a regulatory effect on activity of rapid metabolic cluster in mitochondria of each organ (increase or decrease) [6,11-13], but also optimizes interorgan relationships between parameters of mitochondrial bioenergetics that are impaired under the influence of an adverse factor. This phenomenon can be considered as a sign for systemic integral function of mitochondria [13], which is activated by AA. AA is the exogenous factor that preserves partial energy systems in tissues and organs and maintains stable energy homeostasis during adverse exposure to vibration. Tissue mitochondria summarize the damaging effects, which contributes to the appearance of a material substrate for adaptation stages associated with various levels of energy deficiency [11,12]. The protective effects of EMR are also summarized at the level of tissue mitochon-

TABLE 2. Effect of Long-term Whole-Body Vibration and AA on Factor Structure of Intertissue Interactions of Rabbit Mitochondria

Parameter	Control		Whole-body vibration		Whole-body vibration+AA	
	F_1	F_2	F_1	F_2	F_1	F_2
Ratio of fraction absorbed by each component, %	45.5	17.9	37.4	16.8	35.7	30.0
Dispersion of the amount of basic data, %	63.4	54.2	65.7			
Factor loadings of parameters for mitochondrial function, arb. U						
V_E	-0.84	0.51	0.82	0.26	-0.90	0.26
V_A	-0.40	-0.01	0.10	0.00	0.19	0.00
V_M	-0.55	-0.13	0.32	0.31	-0.52	0.31
after addition of						
succinate	-0.91	0.25	0.79	-0.53	-0.69	-0.53
succinate and 2,4-dinitrophenol	-0.96	0.06	0.89	-0.84	-0.68	-0.84
glutamate and malate	-0.87	0.38	0.87	-0.66	-0.49	-0.66
glutamate, malate, and 2,4-dinitrophenol	-0.95	-0.13	0.91	-0.37	-0.89	-0.37
Covariations of components and grouping signs						
tissue	-0.99	0.12	0.45	0.05	-0.25	-1.85
vibration	0.0	0.0	0.51	-0.21	0.42	1.12

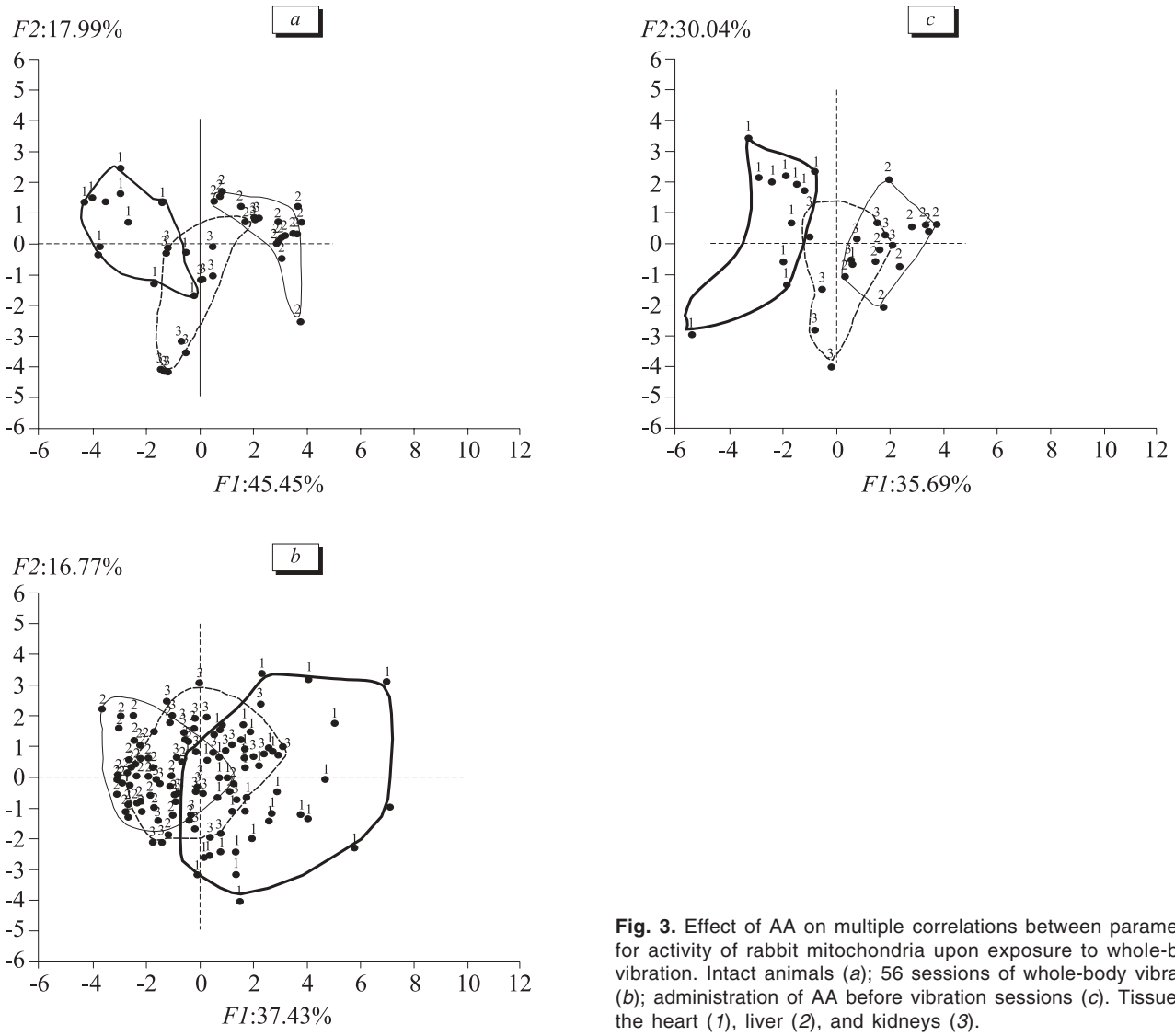

Fig. 3. Effect of AA on multiple correlations between parameters for activity of rabbit mitochondria upon exposure to whole-body vibration. Intact animals (*a*); 56 sessions of whole-body vibration (*b*); administration of AA before vibration sessions (*c*). Tissues of the heart (*1*), liver (*2*), and kidneys (*3*).

dria. EMR contribute to the preservation and stabilization of system interactions between mitochondria in cells and organs upon exposure to an adverse factor. They increase resistance of the organism and have similar pharmacodynamic characteristics under various pathological conditions.

REFERENCES

1. I. A. Volchegorskii, I. I. Dolgushin, O. A. Kolesnikov, and V. E. Tseilikman, *Experimental Modeling and Laboratory Evaluation of Adaptive Reactions in the Organism* [in Russian], Chelyabinsk (2000).
2. O. I. Gogoleva and N. N. Malyutina, *Med. Truda*, No. 4, 20-25 (2000).
3. E. D. Gol'dberg, A. M. Dygai, V. V. Udut, *et al*., *Specific Features of Structural Organization of Life Support Systems under Normal and Pathological Conditions* [in Russian], Tomsk (1996).
4. A. I. Gritsuk, S. M. Sergeenko, and A. N. Koval', *Aviakosm. Ekol. Med*., No. 5, 60-62 (2002).
5. A. M. Dubrov, V. S. Mkhitaryan, and L. I. Troshin, *Multidimensional Statistical Methods* [in Russian], Moscow (2000).
6. M. N. Kondrashova, *Biokhimiya*, **56**, No. 3, 328-405 (1991).
7. L. V. Lukashova, N. B. Smirnova, V. A. Khazanov, *et al*., *Byull. Eksp. Biol. Med.*, Suppl. 1, 86-90 (2005).
8. R. P. Nartsissov, S. V. Petrichuk, Z. N. Dukhova, *et al*., *Succinic Acid in Medicine, Food Industry, and Agriculture* [in Russian], Eds. M. N. Kondrashova *et al*., Pushchino (1997), pp. 155-164.
9. O. Yu. Rebrova, *Statistical Analysis of Medical Data. Use of Statistica Application Package* [in Russian], Moscow (2002).
10. T. M. Sukharevskaya, A. V. Efremov, G. I. Nepomnyashchikh, *et al*., *Microangiopathies and Visceropathies during Vibration Disease* [in Russian], Novosibirsk (2000).
11. V. A. Khazanov and K. Yu. Vasil'ev, *Byull. Eksp. Biol. Med*., Suppl. 1, 54-60 (2005).
12. V. A. Khazanov, *Ibid*., Suppl. 2, 40-44 (2003).
13. V. A. Khazanov, *Energy Metabolism Regulators. Clinical and Pharmacological Aspects* [in Russian], Ed. V. A. Khazanov, Tomsk (2004), pp. 3-7.