# METHODS

# Vibration Model for Hypoxic Type of Cell Metabolism Evaluated on Rabbit Cardiomyocytes

**V. V. Vorobieva and P. D. Shabanov**

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Activity of the energy production system in rabbits myocardium was examined under various conditions of whole-body vibration. The energy-dependent response of native mitochondria from rabbit heart was studied polarographically using a Clark closed membrane electrode. In was established that the energy production system in rabbit myocardium was involved in organism's response to whole-body vibration. Functional changes in myocardial mitochondria were shown to depend on the mode of whole-body vibration (frequency and duration). It manifested in an imbalance between functional activity of FAD- and NAD-dependent components of the respiratory chain. The increase in the frequency and duration of vibration was accompanied by dysfunction of the energy production system in cardiomyocytes. These changes manifested in activation of succinic acid oxidation and inhibition of NAD-dependent components of the mitochondrial respiratory chain. Vibration due to systemic dysregulatory influence can be used as a model for studies of both vibration phenomenon realized at the level of the energy production system in organs and tissues and vibroprotective properties of medicinal products.

**Key Words:** *vibration; mitochondria; myocardial energy metabolism; hypoxia*

The biological effect of vibration is considered as an extreme dysregulating factor inducing neuroendocrine disorders, capillary-trophic insufficiency, and pathological changes in cells and membranes, activating lipid peroxidation, and contributing to the development of hypoxia, deenergization, and dystrophy of organs and tissue [7]. Previous experiments [8-10] and clinical observations [7] showed that the heart is sensitive to vibration. The hypoxic type of cell metabolism forming against the background of vibration and decreased production of macroergic compounds lead to myocardial dystrophy [7]. Taking into account considerable medical and social importance of vibration disease [6], studying of the consequences of whole-body vibration (*i.e.*, function of the myocardial system of energy production) is an important problem.

## MATERIALS AND METHODS

Experiments were performed on 80 chinchilla rabbits weighing 2.5-3 kg and aging 3-4 months. Vertical whole-body vibration (amplitude 0.5 mm) was induced on a UV 70/200 device (Mayak Engineering Company). The animals were daily exposed to whole-body vibration for 60 min (from 9.00 to 11.00, frequency 8 or 44 Hz). The total duration of treatment was 7, 21, and 56 days (without holidays). The study was conducted in the fall-winter period.

Department of Pharmacology, S. M. Kirov Military Medical Academy, St. Petersburg. ***Address for correspondence:*** pdshabanov@mail.ru. P. D. Shabanov

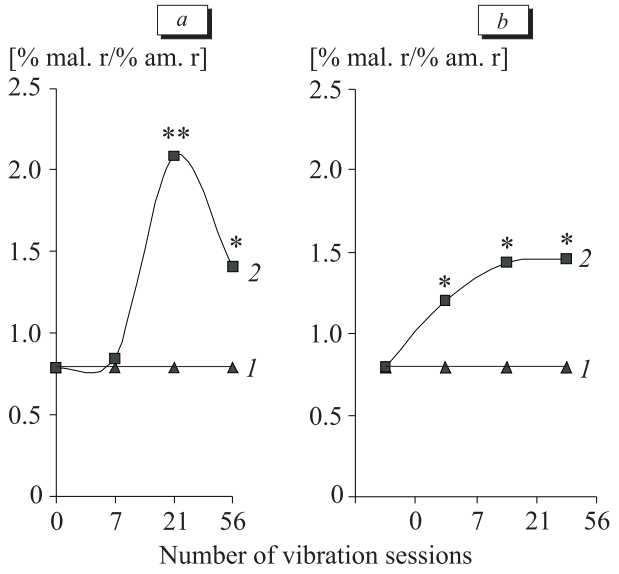


**Fig. 1.** Ratio of partial reactions of endogenous respiration ($V_E$) in rabbit cardiomyocyte mitochondria during vibration with 8 (*a*) and 44 Hz (*b*). Control (intact rabbits, *1*); rate of the increase in malonate sensitivity ($CI_{mal.\ r}$). Here and in Fig. 2: **p*<0.05 and ***p*<0.01 compared to intact rabbits.

Functional activity of native mitochondria from rabbit heart [4] was studied by the polarographic method [3] in a 1-ml well with the incubation medium at 37°C. The mitochondrial respiration rate (V) depended on the added substance in a well. This parameter was expressed in [ng atom O $min^{-1}mg^{-1}$protein]. The metabolic state of mitochondrial "rest" and "activity" was studied *in vitro* upon variation of exogenous energy substrates (before and after addition of 2,4-dinitrophenol to the well) [3,5].

The contribution of NAD- and FAD-dependent substrates to endogenous mitochondrial respiration ($V_E$) was evaluated by the inhibition assay with amytal or malonate. The test compounds were added to the well (concentration 2 mmol) during endogenous respiration [2,5].

The FAD-dependent substrate succinic acid (SA, 1 mmol; $V_{SA}$, substrate respiration rate after administration of SA) or a mixture of NAD-dependent substances glutamic acid and malic acid (Glu+mal, 3 mmol glutamic acid and 3 mmol malic acid; $V_{GLU}$, substrate respiration rate after administration of Glu+mal) was used as the exogenous substrate. Mitochondrial ATPase activity ($V_{DNP}$) was induced by addition of uncoupler 2,4-dinitrophenol (2,4-DNP, 20 μmol) [3,5].

The *in vivo* mitochondrial response to adverse conditions was evaluated from kinetic (V) and estimated parameters. In metabolic states of mitochondria, coefficients of the increase in succinate-dependent respiration (CI) under resting (R) and uncoupling (U) conditions were calculated as follows: $CI_E=[FAD/NAD]_E$=mal. r/am. r, $CI_R=[FAD/NAD]_R=V_{SA}/V_{GLU+MAL}$, and $CI_U=[FAD/NAD]_U=V_{SA\text{-}U}/V_{GLU+MAL\text{-}U}$, where mal. r and am. r are the contribution of malonate-sensitive and amytal-sensitive endogenous respiration, respectively; $V_{SA}$ and $V_{GLU+MAL}$ are rates of oxidation of exogenous succinate and glutamate-malate mixture under "resting" conditions, respectively; and $V_{SA\text{-}U}/V_{GLU+MAL\text{-}U}$ are the substrate oxidation rates in "active" mitochondria under conditions of 2,4-DNP-induced (uncoupler) ATPase load. The regulatory parameters were qualitatively characterized by mitochondrial transition into various states (from the endogenous state to the "resting" state; and from the "resting" state to the "active" state). The coefficients of stimulation (CS) were calculated as follows: $CS_S=V_S/V_E$ and $CU_S=V_{C\text{-}U}/V_S$, where $CS_S$ is stimulation of endogenous respiration by exogenous substrate (S); $V_S$ is the mitochondrial respiration rate after addition of exogenous substrate (succinate or Glu+mal); $CU_S$ is stimulation of substrate respiration with 2,4-DNP; and $V_{C\text{-}U}$ is the oxidation rate of exogenous substrate after addition of 2,4-DNP. $CS_S$ and $CU_S$ were expressed in relative units.

The damaging effect of whole-body vibration on the myocardium was verified histologically. The material for histological study (myocardium from the apex of the left ventricle) was treated routinely with alcohol and paraffin and stained with hematoxylin and eosin. The results were analyzed using Statistica 6.0 software. Intergroup differences were evaluated by the parametric (Student's *t* test) and nonparametric tests (Mann—Whitney *U* test), which depended on the type of data distribution.

## RESULTS

Whole-body vibration had a modulatory effect on functional activity of rabbit heart mitochondria. Changes in the endogenous respiration rate ($V_E$) depended on the frequency and duration of vibration (Table 1, Fig. 1).

**TABLE 1.** Endogenous Mitochondrial Respiration in Rabbit Heart under Various Conditions of Whole-Body Vibration

| Vibration exposure | | Number of animals | $V_E$ |
|---|---|---|---|
| frequency, Hz | number of vibration sessions | | |
| 0* | 0 | 20 | 16.3±4.3 |
| 8 | 7 | 10 | 16.3±3.2 |
| 8 | 21 | 10 | 18.8±5.2 |
| 8 | 56 | 10 | 23.4±5.7 |
| 44 | 7 | 10 | 22.6±5.2 |
| 44 | 21 | 10 | 25.9±6.7 |
| 44 | 56 | 10 | 17.9±5.8 |

**Note.** *Group of intact animals (control).

At 8 and 44 Hz, the sensitivity to malonate increased more rapidly ($CI_{mal.\ r}$) than the sensitivity to amytal (Fig. 2, *a*, *b*), which reflected the prevalence of endogenous SA metabolism in energy supply to adaptive reconstruction of the myocardium. However, the increase in the sensitivity to malonate at 44 Hz was less pronounced than at 8 Hz. The effects of damage to succinate-dependent bioenergetics were probably summarized under conditions of severe vibration.

Independently on the frequency and duration of vibration, the oxidation rate of NAD-dependent substrates in "resting" and "active" mitochondria (Fig. 2, *a*, *c*) was much lower than the rate of succinate oxidation (Fig. 2, *b*, *d*). The observed changes in metabolic stages of mitochondria ("rest" and "activity") reflect preferential increase in succinate-dependent energetics of the myocardium under conditions of long-term vibration. Moreover, the adverse effects of this treatment were summarized.

Stimulation of endogenous respiration in myocardial mitochondria by exogenous substrates also depended on the frequency and duration of vibration. The regulatory effect of NAD-dependent substrates under conditions of "rest" and "activity" was most pronounced during the 7th and 21st sessions of 8-Hz vibration, but decreased by the 56th session. The regulatory role of NAD-dependent substrates was lower under conditions of 44-Hz vibration, which reflects inactivation of this component in the respiratory chain and reduction of its contribution to the myocardial response to vibration. FAD-dependent substrates had a smaller regulatory effect than NAD-dependent substrates during 8-Hz vibration. However, the role of FAD-dependent substrates progressively increased

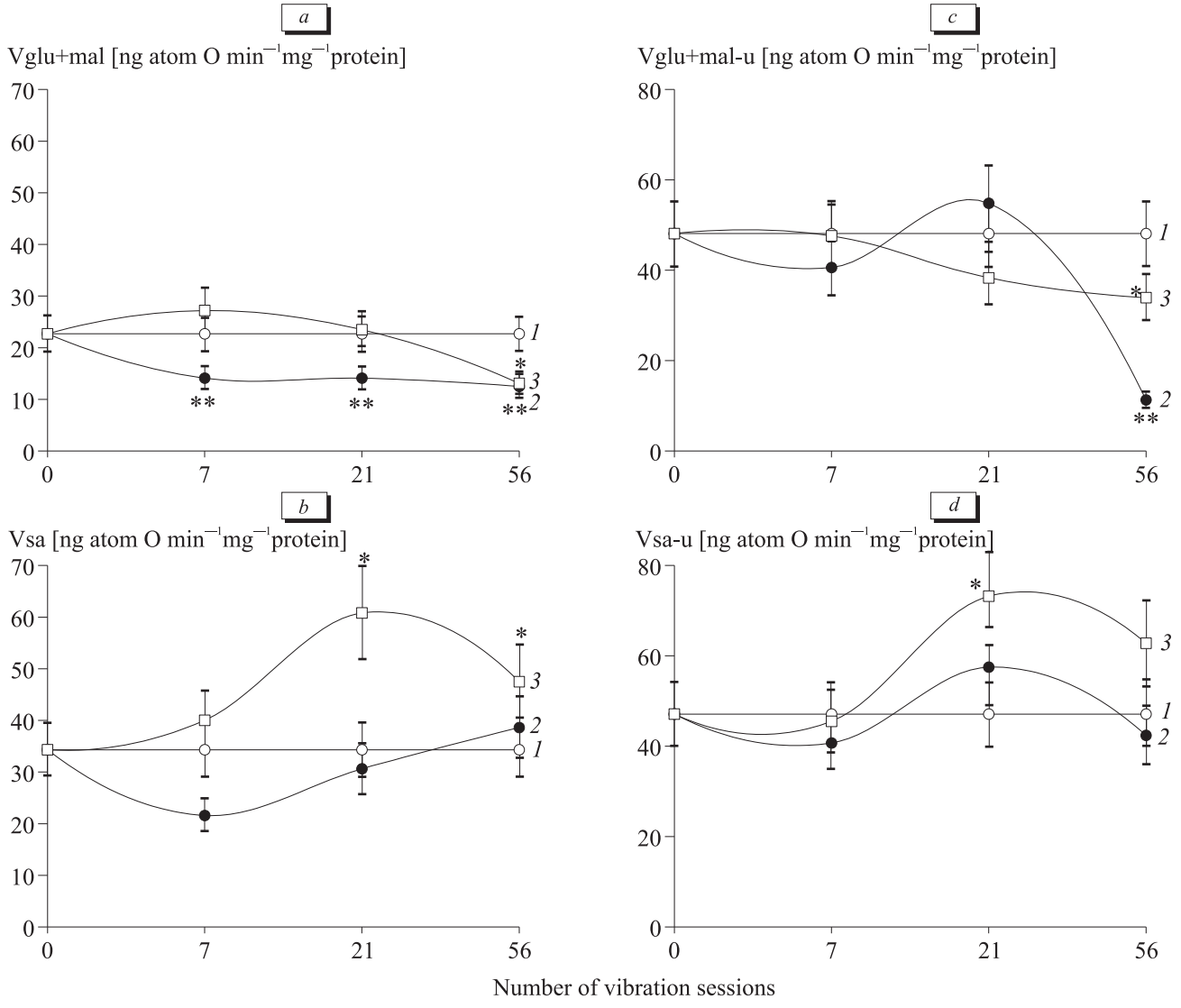


**Fig. 2.** Effect of the frequency and duration of vibration on oxidation of NAD-dependent (*a*, *c*) and FAD-dependent substrates (*b*, *d*) by rabbit cardiomyocyte mitochondria under conditions of "rest" and "activity". Control (intact rabbits, *1*); 8-Hz vibration (*2*); 44-Hz vibration (*3*).

with summation of the effects of 44-Hz vibration. The influence of these substrates was most significant after 56 sessions of vibration under "resting" conditions.

Our results indicate that 21-56 sessions of 44-Hz vibration have the most significant adverse effect on myocardial mitochondria. FAD-dependent components *in vitro* provide a partial response of the mitochondrial respiratory chain with endogenous and exogenous SA. The role of these components was shown to increase under these conditions. Therefore, FAD-dependent components play the major role in the maintenance of respiratory function under adverse conditions [2]. Hyperactivation of the succinate oxidation system was accompanied by suppression and uncoupling, which reflects the low-energy transition [2] of energy supply to the myocardium. This conclusion was confirmed by the results of morphological and histological study.

The adverse effect of vibration was manifested in the following morphological changes: progressive atrophy of cardiomyocytes; reduction of the capillary network; spasm of arterioles; increase and the severity of intercellular edema; and progressive increase in the area of focal hemorrhage and necrosis.

Functional reconstruction of the mitochondrial respiratory chain reflects the adverse effect of exogenous factors on the myocardium. However, vibration has no specific effect on mitochondrial function. The observed changes were similar to typical shift in metabolic pathways caused by exposure to various unfavorable factors. Therefore, "severe" vibration (56 sessions, 44 Hz) serves as a noninvasive model of mitochondrial dysfunction [1]. This model was used for further analysis of the biological phenomenon of vibration, study of the pathogenesis of vibration-induced disorders, and evaluation of the possibility for pharmacological protection of the myocardium from vibration.

We conclude that the energy production system in rabbit myocardium is most sensitive to prolonged exposure to whole-body vibration. Functional changes in myocardial mitochondria depend on the mode of whole-body vibration (frequency and duration). They are manifested in an imbalance between functional activity of FAD- and NAD-dependent components of the respiratory chain. The increase in the frequency and duration of whole-body vibration is accompanied by dysfunction of energy production in cardiomyocytes. The low-energy transition is accompanied by activation of the SA oxidation and inhibition of NAD-dependent components in the mitochondrial respiratory chain.

Our results indicate that vibration can be used as noninvasive experimental model for the hypoxic type of cell metabolism. This model reflects the development of pathophysiological changes in the energy production system of organs and tissues.

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