ENERGY-DEPENDENT Ca^{++} TRANSPORT AND LIPID PEROXIDATION IN MEMBRANES OF THE SARCOPLASMIC RETICULUM OF RAT MUSCLES DURING HYPOKINESIA

G. V. Chernysheva, V. I. Mel'nik,
and G. G. Amarantova

UDC 612.766.2-08:612.744.16.014.2:576.311

KEY WORDS: hypokinesis; sarcoplasmic reticulum; calcium transport; peroxidation; lipids.

The main factor leading to a change in certain parameters of energy, protein, and lipid metabolism in hypokinesia is hypofunction of the muscles. As a result of atrophy the weight of the muscles decreases, and their content of myofibrillary and sarcoplasmic proteins is reduced [3] through a reduction in their synthesis and increased breakdown [9]; the energy supply for contractile activity of the muscles also is reduced [5], and the parameters of excitability and contractility of the muscles are changed [7]. Disturbance of the ratio between Na^+, K^+, and Ca^{++} ions in the blood and body tissues has been found in hypokinesia [4, 6]. The facts described above suggest a change in the fine mechanisms responsible for contractile function of the muscles.

The object of this investigation was to study the effect of hypokinesia on the rate of accumulation of Ca^{++}, Ca-ATPase activity, and the rate of inflow and outflow of Ca^{++} in the light and heavy fractions of the sarcoplasmic reticulum (SR) of skeletal muscles. It has been shown [10, 15] that mainly longitudinal regions of SR, containing Ca-ATPase, are present in the light fraction of SR. The heavy fraction, corresponding to the terminal cisterns, contains concentrations of Ca^{++}-binding proteins as well as Ca-ATPase.

To characterize the lipid components of the membranes, the intensity of lipid peroxidation (LPO) in the membranes was determined.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar albino rats. Hypokinesia was induced in the animals by keeping them in restraining chambers. The duration of hypokinesia was 30-90 days. Animals kept under standard conditions in the animal house were used as the control. The microsomal fraction was isolated from the rats [14]. Actomysin was extracted with 0.3 M KCl and 25 mM $Na_4P_2O_7$, pH 7.0. The coarse fraction of microsomes thus obtained was fractionated by centrifugation in a sucrose density gradient (20-45% sucrose) at 25,000 rpm (SW-27 rotor, Beckman L-5-65 ultracentrifuge) for 90 min into two fractions: light and heavy, at 29-33 and 37-40% sucrose respectively. The velocity of Ca^{++} transport and of ATP hydrolysis was measured pH-metrically [1]. The rate of inflow and outflow of Ca^{++} from the vesicles was calculated by the equations in [1]. Protein was determined by Lowry's method [12]. The intensity of LPO was judged from the increase in the malonic dialdehyde (MDA) content [2]. The results were subjected to statistical analysis by the Student–Fisher test.

EXPERIMENTAL RESULTS

Ca-ATPase activity and the rate of Ca^{++} accumulation in the light fraction of microsomes isolated from muscles of the control animals were higher than those in the heavy fraction by 33 and 50%, respectively (Table 1). The difference was even more marked when the velocity of inflow and outflow of Ca^{++} from the light and heavy vesicles was compared (49 and 16%, respectively).

The results are in agreement with those of investigations which showed that Ca^{++} is liberated mainly from the terminal cisterns of SR [11, 16].

Hypokinesia for 30 days had no effect on Ca-ATPase activity, the rate of accumulation of Ca^{++}, or the velocity of inflow and outflow of Ca^{++} from the light and heavy SR fractions. The small increase in these parameters in the heavy SR fraction was not statistically significant.

Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 548-549, November, 1981. Original article submitted May 26, 1981.

0007-4888/81/9211-1501$07.50

TABLE 1. Ca^{++} Transport in SR Fractions of Predominantly White Muscles of Rats during Hypokinesia (M ± m)

SR fraction	Parameter	Control (n = 20)	Hypokinesia, 30 days (n = 15)	Control (n = 25)	Hypokinesia, 90 days (n = 10)
Light	V_{ATP}	1,96±0,06	1,99±0,10	1,78±0,18	1,85±0.20
	V_{Ca}	1,99±0,16	2,04±0,17	1,83±0,25	1,98±0,22
	V_{in}	3,98±0,16	4,43±0,21	3,55±0,17	3,96±0,15
	V_{out}	2,02±0,17	1,85±0,18	1,79±0,10	1,75±0,09
	Ca/ATP	1,01±0,08	1,04±0,08	1,03±0,09	1,07±0,07
Heavy	V_{ATP}	1,32±0,29	1,52±0,15	1,18±0,06	1,58±0,10†
	V_{Ca}	0,99±0,05	1,23±0,28	0,86±0,08	1,25±0,21*
	V_{in}	1,98±0,12	2,46±0,08	1,81±0,04	2,55±0,19*
	V_{out}	1,66±0,16	1,80±0,09	1,54±0,01	1,95±0,02
	Ca/ATP	0,78±0,1	0,80±0,03	0,79±0,04	0,73±0,02

Legend. V_{ATP}) Velocity of ATP hydrolysis by SR Ca-ATPase (in μ moles $P_i \cdot mg^{-1} \cdot min^{-1}$), V_{Ca}) rate of Ca^{++} accumulation (in μ moles $Ca^{++} \cdot mg^{-1} \cdot min^{-1}$), V_{in}) velocity of inflow of Ca^{++} into vesicles, V_{out}) velocity of outflow of Ca^{++} from vesicles, Ca/ATP) coefficient of efficiency of SR calcium pump. *$P < 0.01$, †$P < 0.001$; number of experiments in parentheses.

TABLE 2. MDA Concentration (in μ moles/mg protein) in SR of Skeletal Muscles during 30-Day Hypokinesia (M ± m)

Parameter	Light fraction		Heavy fraction	
	control (n = 10)	experiment (n = 10)	control (n = 15)	experiment (n = 15)
Initial level	4,3±0,6	6,0±0,5	5,4±0,7	9,2±0,55
Stimulation by Fe^{2+}:				
5 min	65,1±1,0	59,1±1,8	20,0±2,1	18,4±1,8
10 min	67,6±1,8	63,3±5,8	22,0±3,2	26,6±1,6

Legend. 1-2 mg protein in 1 ml incubation medium (10 mM Tris-HCl, pH 7.4, 52 mM KCl), $12 \cdot 10^{-6}$ M Fe^{++}. Extinction was measured at 535 nm. MDA concentration calculated by using a coefficient of molar extinction of $156 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$; number of animals given in parentheses.

During 90-day hypokinesia the parameters of Ca^{++} transport in the light SR fraction were the same as in the control. In the heavy SR fraction Ca-ATPase activity was increased by 36% and Ca^{++} accumulation by 44%. The Ca/ATP ratio was unchanged, for all the indices increased by the same degree (Table 1).

During hypokinesia for both 30 and 90 days the rate of outlfow of Ca^{++} increased relative to the rate of its inflow into the vesicles, i.e., a decrease in the rate of muscle relaxation was possible.

Determination of LPO in the SR fractions showed that, despite the higher initial level of the MDA concentration in the light and heavy SR fractions during hypokinesia, the degree of intensification of LPO by Fe^{++} ions was lower than in SR of the control animals (Table 2). In the control rats and rats with hypokinesia the intensity of LPO was 3 times higher in the light fraction of microsomes than in the heavy fraction. This was evidently due to differences in the concentration of phospholipids and their composition in the light and heavy microsomes [13].

Hypokinesia for 30 days thus did not affect Ca^{++} transport in SR from predominantly white skeletal muscles. Increasing the duration of hypokinesia to 90 days led to a marked increase in the rate of Ca^{++} accumulation, in Ca-ATPase activity, and in the velocity of inflow and, in particular, of outflow of Ca in the heavy SR fraction, consisting chiefly of terminal cisterns. These parameters were unchanged in the light fraction. The increase in the rate of Ca^{++} accumulation by the heavy SR fraction was evidently connected with the fact that some unatrophied muscle fibers preserved their normal structure and assumed a compensatory function in order to maintain the vital activity of the muscles. This conclusion is supported by the results of electron-microscopic investigations showing a relative increase in the content of SR and dilatation of its lumen [8].

LITERATURE CITED

1. A. A. Boldyrev, in: Transport Adenosine Triphosphatases [in Russian], Moscow (1977), pp. 69-78.
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972), pp. 241-243.
3. M. S. Gaevskaya, L. M. Slez and N. A. Ilyushko, Kosmich. Biol., No. 4, 25 (1970).
4. E. A. Kovalenko, E. S. Mailyan, V. L. Popkov, et al., Usp. Fiziol. Nauk, 6, No. 3, 110 (1975).
5. E. A. Kovalenko, A. A. Kiselev, et al., in: The Pathological Physiology of Extremal States [in Russian], Leningrad (1970), p. 64.
6. V. P. Krotov, Kosmich. Biol., No. 2, 66 (1972).
7. V. S. Oganov and A. N. Potapov, Kosmich, Biol., No. 2, 22 (1973).
8. V. V. Portugalov, E. I. Il'ina-Kakueva, V. I. Starostin, et al., in: Experimental Research into Hypokinesia, Changes in the Gaseous Environment, and Acceleration [in Russian], Moscow (1968), pp. 25-28.
9. I. V. Fedorov, Kosmich. Biol., No. 3, 3 (1980).
10. A. H. Caswell, G. H. Lau, and J. P. Brunschwig, Arch. Biochem., 176, 417 (1976).
11. G. H. Lau, A. H. Caswell, and J. P. Brunschwig, J. Biol. Chem., 252, 5565 (1979).
12. O. H. Lowry, N. J. Rosebrough, et al., J. Biol. Chem., 193, 265 (1951).
13. G. H. Lau, A. H. Caswell, J. P. Brunschwig, et al., J. Biol. Chem., 254, 540 (1979).
14. A. Martonosi, J. Biol. Chem., 243, 71 (1968).
15. G. Meissner, G. E. Connor, and S. Fleischer, Biochim. Biophys. Acta, 389, 51 (1975).
16. S. Winegrad, J. Gen. Physiol., 48, 455 (1965).

INDUCTION OF LIVER MALATE DEHYDROGENASE (DECARBOXYLATING) AND LACTATE DEHYDROGENASE ACTION BY CLOFIBRATE

L. F. Panchenko and V. D. Antonenkov

UDC 612.351.11.014.46:615.272.4

KEY WORDS: clofibrate; lactate dehydrogenase; malate dehydrogenase (decarboxylating).

Numerous investigations have shown that the velocity of ethanol oxidation in liver cells largely depends on the ratio between the concentration of oxidized and reduced forms of pyridine nucleotides (NAD and NADP) in the cytoplasm, which is kept at a certain level through the participation of NAD- and NADP-dependent dehydrogenases. Changes in the activity of these enzymes may have a significant effect on the metabolism of alcohol, especially if it is consumed excessively [2, 10]. The hypolipidemic agent clofibrate can accelerate ethanol oxidation [3, 5] and also modify the steady-state intracellular concentration of various metabolites which are substrates of cytoplasmic dehydrogenases – lactate, pyruvate, glycerol-3-phosphate, etc. [3, 5, 6, 13]. The mechanism of these effects of clofibrate has not been explained. Clofibrate is known to cause marked proliferation of peroxisomes and of the smooth endoplasmic reticulum. Selective induction or repression of the synthesis of catalase, D-amino acid oxidase, and carnitine-acetyltransferase also is observed at the same time [4, 7].

The goal of this research was the discovery of the possible role of certain cytoplasmic dehydrogenases in accelerating the metabolism of ethanol under the action of clofibrate.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. Clofibrate was injected intraperitoneally in a dose of 250 or 800 mg/kg body weight daily. Animals of the control group received injections of physiological saline. The animals were deprived of food for 24 h before sacrifice. Perfusion of the liver, homogenization, preparation of "nuclear-free" homogenate and its differential centrifugation, and also the enzyme control for the composition of the subcellular fractions were carried out as described previously [1, 11]. The effect of clofibrate on cell metabolism was assessed by determining activity of the peroxisomal enzymes catalase, L-α-hydroxy-acid oxidase [1], and carnitine-acetyltransferase [4]. Activity of sorbitol dehydrogenase (SDH) [12], lactate dehydrogenase (LDH), NAD-dependent glycerol-3-phosphate dehydrogenase [1], NAD-dependent malate dehydrogenase (MDH-NAD), and NADP-dependent malate dehydrogenase (decarboxylating; MDH-NADP) [9] was determined. Catalase and carnitine-acetyltransferase activity were determined at 25°C, that of the other enzymes at 37°C, using a Gilford model 250 spectrophotometer. The LDH isozyme spectrum was studied by electrophoresis in

V. P. Serbskii Research Institute of General and Phorensic Psychiatry, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 550-552, November, 1981. Original article submitted March 25, 1981.