LITERATURE CITED

1. M. G. Voronkov and V. M. D'yakov, Silatranes, Novosibirsk (1978).
2. M. G. Voronkov, G. I. Zelchan, and É. Ya. Lukevits, Silicon and Life [in Russian], Riga (1978).
3. I. G. Kuznetsov, A. T. Platonova, S. K. Suslova, et al., in: Biologically Active Compounds of Group IVB Elements [in Russian], Irkutsk (1975), p. 34.
4. I. G. Kuznetsov, M. S. Sorokin, and M. G. Voronkov, Khim.-farm. Zh., No. 2, 70 (1980).
5. L. A. Mansurova, A. T. Platonova, I. G. Kuznetsov, et al., in: Biologically Active Compounds of Group IVB Elements [in Russian], Irkutsk (1975), p. 28.
6. L. A. Mansurova, M. G. Voronkov, L. I. Slutskii, et al., Dokl. Akad. Nauk SSSR, 262, No. 6, 1505 (1982).
7. L. I. Slutskii, Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969).
8. L. I. Slutskii, Izv. Akad. Nauk Laty. SSR, 408, No. 7, 94 (1981).
9. R. B. Dean and W. I. Dixon, Anal. Chem., 23, 636 (1951).
10. T. Sekino, K. Murata, Y. Saito, et al., Digestion, 16, 28 (1977).
11. K. Takagi, S. Okabe, and R. Sazaki, Jpn. J. Pharmacol., 19, 418 (1969).
12. M. G. Voronkov (M. G. Voroncov), in: Biochemistry of Silicon and Related Problems, New York (1978), p. 395.
13. M. G. Voronkov (M. G. Voroncov), M. S. Sorokin, and I. G. Kuznetsov, in: Fifth International Symposium of Organosilicon Chemistry, Karlsruhe (1978), p. 152.
14. M. G. Voronkov (M. G. Voroncov), Top. Curr. Chem., 84, 77 (1979).

ADRENERGIC STRUCTURES AND MONOAMINE OXIDASE ACTIVITY IN DYSTROPHIC SKELETAL MUSCLES

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UDC 616.892-009.54-092:
616.74-008.931:
577.152.143

KEY WORDS: muscular dystrophies; adrenergic structures; catecholamines; mitochondria; monoamine oxidase.

The study of hereditary neuromuscular diseases is becoming increasingly urgent in connection with the relative increase in the contribution of genetically determined diseases to the general structure of human pathology. Important advances in clinical neurogenetics have been achieved as a result of close collaboration with the basic natural sciences: molecular biology, biochemistry, etc. However, the absence of adequate animal models makes the study of such a large group of diseases as the progressive muscular dystrophies much more difficult.

In recent years, skeletal muscles from human patients taken at biopsy have been used on an increasing scale for study. Histochemical investigations [4] have revealed accumulation of catecholamines (CA) in the skeletal muscles of patients with Duchenne's progressive muscular dystrophy. More recent investigations [2, 6, 7] have demonstrated the important role of biogenic amines in the determination of the muscular dystrophic process. However, the causes of pathological accumulation of CA in the skeletal muscles of such patients have not yet been explained.

The aim of this investigation was to study the characteristics of adrenergic structures and monoamine oxidase (MAO) activity in skeletal muscles of patients with Duchenne's muscular dystrophy and with Charcot–

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 660-662, June, 1984. Original article submitted November 16, 1983.

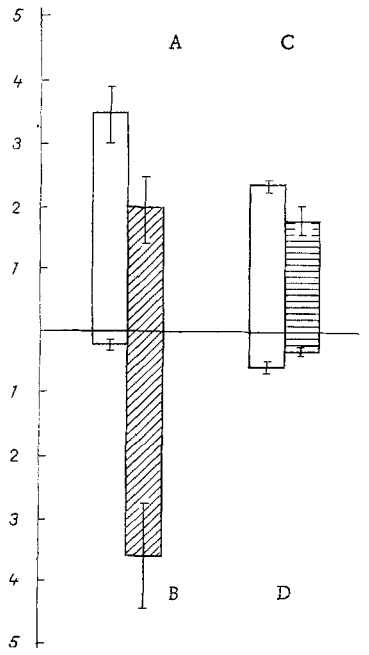

Fig. 1. MAO activity (in μmoles ammonia/g tissue) in skeletal muscle. Substrate – dopamine. A, C) Fraction No. 1; B, D) fraction No. 2. Unshaded columns – control; obliquely shaded columns – Duchenne's progressive muscular atrophy; horizontally shaded column – Charcot–Marie peroneal muscular atrophy.

Marie peroneal muscular atrophy.

METHODS

We studied 17 male patients (14 patients with Duchenne's muscular dystrophy and 3 with Charcot–Marie peroneal muscular atrophy). All patients were in stage II of the disease [2]. The control group consisted of 11 patients with no neuromuscular pathology.

Biopsy was performed on the deltoid muscle of the patients with Duchenne's muscular dystrophy and the peroneal muscle of patients with Charcot–Marie peroneal muscular atrophy, under local anesthesia with 0.25% procaine solution.

CA were detected in adrenergic nerve formations by the luminescence-histochemical method [13], using glyoxylic acid. CA were identified by their emerald green luminescence.

To determine MAO activity dopamine hydrochloride (Ferak, West Germany) was used as the substrate. A 10% homogenate was prepared from the biopsy material in 0.25 M sucrose in 0.01 M potassium-phosphate buffer, pH 7.3. By centrifugation of the homogenate for 10 min at 200g the residue of nuclei and cell fragments was separated, washed three times with 0.25 M sucrose in 0.01 M potassium-phosphate buffer, and recentrifuged each time for 10 min, twice at 110g and once at 17,000g. The supernatants were pooled after four centrifugations and mitochondria were sedimented from the resulting suspension by centrifugation for 30 min at 17,000g. The resulting supernatant was described as fraction 2, and the residue of mitochondria was washed with 0.01 M potassium-phosphate buffer and centrifuged for 30 min at 17,000g. The residue of "washed" mitochondria was then suspended in 0.1 M potassium-phosphate buffer and the mitochondrial fraction was obtained [1]. Protein was determined in both fractions by the method in [9].

Samples 1.8 ml in volume contained: enzyme, i.e., one of the fractions, taken in a quantity of about 1.4 mg protein in each; 0.1 ml of potassium-phosphate buffer, pH 7.3; and substrate, namely dopamine, in saturating concentration (10 μ moles per sample). The samples were incubated for 30 min at 37°C in an atmosphere of oxygen. The reaction was stopped by the addition of 0.2 ml of 50% TCA (final concentration 5%). MAO activity in protein-free filtrate was determined by measuring the quantity of ammonia liberated by a modified method [11] with Nessler's reagent.

EXPERIMENTAL RESULTS

Individual long branching fluorescent fibers and short terminal fibrils were found in biopsy material from muscles of the control subjects. Small varicose expansions containing CA were present in them. The intensity of fluorescence of the adrenergic formations was moderate. MAO activity with respect to dopamine deamination was detected in the mitochondria and absent in the supernatant.

Unlike in the control, adrenergic structures in skeletal muscles of patients with Duchenne's muscular dystrophy were represented by highly branched plexuses. The fibers were thickened and equipped with numerous large varicose expansions. Characteristically they showed bright luminescence, indicating considerable accumulation of CA.

The results of investigations of MAO activity in the skeletal muscles of patients with Duchenne's muscular dystrophy and Charcot–Marie peroneal muscular atrophy are given in Fig. 1.

It will be clear from Fig. 1 that the intensity of deamination of dopamine in the mitochondria (Fig. 1A) in patients with Duchenne's muscular dystrophy was reduced by 1.7 times, but in the supernatant it was increased by 19 times (Fig. 1B) compared with the control. Single sympathetic efferent endings were revealed in the skeletal muscles of patients with Charcot–Marie peroneal muscular atrophy. Single fibers had the form of separate luminescent fragments or long, extremely thin, twisted structures. The intensity of luminescence of adrenergic formations was much lower than in the control. The intensity of dopamine deamination in the mitochondrial fraction (Fig. 1C) and in the supernatant (Fig. 1D) in the skeletal muscles of patients with Charcot–Marie peroneal muscular atrophy was virtually indistinguishable from the control.

The increase in the CA concentration in the skeletal muscles during progressive muscular dystrophy may be associated with an increase in their circulation [7], disturbance of interaction with adenylate cyclase [10], or changes in the activity of enzymes responsible for CA utilization [1, 3, 5].

Data in the literature on MAO activity in dystrophic skeletal muscles are scanty and contradictory [8, 14]. Meanwhile experimental studies using the MAO inhibitor pargyline have demonstrated that an induced myopathy histochemically similar to Duchenne's muscular dystrophy can develop [15].

The results of the present investigation showed that excessive accumulation of CA in skeletal muscle combined with depression of mitochondrial MAO activity, detectable by a histochemical method, is present in children with Duchenne's muscular dystrophy. Differences in the degree of dopamine deamination in the mitochondrial fraction and in the supernatant fraction suggest that "leakage" of the enzyme takes place on account of disturbance of permeability of the mitochondrial membranes.

The decrease in mitochondrial MAO activity is probably an important cause of CA accumulation in skeletal muscle in Duchenne's muscular dystrophy.

The absence of any similar changes in adrenergic formations and in MAO activity in Charcot–Marie peroneal muscular atrophy indicates the possible specificity of disturbances of the mitochondrial membranes in Duchenne's progressive muscular dystrophy.

LITERATURE CITED

1. O. M. Avakyan, Substances Acting on the Sympathoadrenal System [in Russian], Moscow (1980).
2. L. O. Badalyan, Hereditary Diseases of the Neuromuscular System [in Russian], Moscow (1974).
3. T. D. Bol'shakova, Zh. Vses. Khim. Ova. im Mendeleeva, 21, 196 (1976).
4. T. L. Wright et al., Neurology (Minneapolis), 23, 510 (1973).
5. V. Z. Gorkin, Zh. Vses. Khim. Obshch. im Mendeleeva, 21, 181 (1976).
6. G. N. Dunaevskaya and T. G. Mosunova, in: Problems in Clinical Neurogenetics [in Russian], Moscow (1973), p. 255.
7. J. Dalmaz et al., J. Neurol. Transmiss., 46, 17 (1979).
8. N. C. Kar and C. M. Pearson, Clin. Chim. Acta, 50, No. 1-3, 431 (1974).
9. O. H. Lowry, N. J. Rosebrough, et al., J. Biol. Chem., 193, 2 (1951).
10. S. Mawatary et al., Neurology (Minneapolis), 26, 1021 (1976).
11. I. S. Severina, Biokhimiya, 44, 195 (1979).
12. A. M. Utevskii and V. O. Osinskaya, Vopr. Biokhim. Mozga, No. 13, 260 (1978).
13. V. N. Svalev and N. N. Zuchkova, Arkh. Anat., No. 6, 114 (1979).
14. H. R. Scholte and H. F. M. Busch, J. Neurol. Sci., 45, No. 2-3, 217 (1980).
15. M. K. Yu et al., Neurology (Minneapolis), 24, 237 (1974).