POSSIBLE ROLE OF CYCLIC AMP IN REGULATION OF MUSCLE TISSUE ACID HYDROLASE ACTIVITY IN AVITAMINOSIS E AND DENERVATION

A. V. Kozlov and B. F. Korovkin

UDC 616.391.041:577.161.3+616.74-009.55-07:616.74-008.931:577.152.3

After denervation and in avitaminosis E the cyclic AMP level falls in rabbit muscle tissue but activity of cyclic AMP phosphodiesterase rises. In experiments in vitro cyclic AMP, in a concentration of 10^{-4} M, inhibits the liberation of acid phosphatase from the lysosome-rich fraction isolated from the muscles of rabbits with avitaminosis E. In muscular dystrophy, the increase in acid hydrolase activity in the skeletal muscle may thus be due to liberation of enzymes from the lysosomes as a result of labilization of their membranes through a fall in cyclic AMP concentration.

KEY WORDS: muscular dystrophy; cyclic AMP; lysosomes; acid phosphatase.

It is now firmly established that acid hydrolase activity in muscle tissue is increased in various forms of muscular dystrophy [2, 3, 8, 9]. This is attributed either to the liberation of enzymes from lysosomes and a subsequent increase in their catalytic activity or to increased biosynthesis of enzymes, or to an "exogenous" increase in the content of acid hydrolases on account of proliferative processes [10].

The uncertainty surrounding this problem and also data on the role of cyclic AMP in the stabilization of lysosomal membranes [6, 7] motivated an investigation of some components of the cyclic AMP system in muscle tissue in avitaminosis E and after denervation. At the same time the relationship between the cyclic AMP level and acid hydrolase activity in muscle tissue was studied.

EXPERIMENTAL METHOD

Three groups of rabbits were used. The animals of group 1 (avitaminosis E) were kept on an artificial diet [4]. In the rabbits of group 2 (denervation) the right sciatic nerve was divided in the upper third of the thigh. The rabbits of group 3 served as the control. The right gastrocnemius muscle was investigated. The cyclic AMP content was determined by a radioimmunological method using a set of ready-made reagents (Radiochemical Centre, Amersham, England) and cyclic AMP phosphodiesterase activity was determined by

TABLE 1. Cyclic AMP Level and Phosphodiesterase Activity in Muscle Tissue in Avitaminosis E and Denervation (M ± m)

Parameter determined	Control	Denervation		Avitaminosis E	
		2 weeks	3 weeks	2 weeks	3 weeks
Cyclic AMP, pmoles/g wet weight (n = 5)	215±12	170±14	165±16	162±17	146±20
p		<0,05	<0,05	<0,05	<0,05
Phosphodiesterase activity, nmoles cyclic AMP/mg protein/min (n = 6)	1,06±0,09	1,89±0,12	2,10±0,13	1,71±0,06	2,86±0,23
p		<0,001	<0,001	<0,05	<0,001

S. M. Kirov Military Medical Academy Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR I. I. Ivanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1068-1069, September, 1976. Original article submitted January 26, 1976.

a modified method of Cheung [5], using the "Ferment-1" instrument [1]. The composition of the incubation medium was: 0.01 M Tris–HCl buffer, pH 8.0, 2.5 ml; 0.1 M $MgSO_4$ solution 0.05 ml; homogenate 0.2 ml. The final concentration of cyclic AMP was $4 \cdot 10^{-4}$ M. Acid phosphatase activity was determined with the kit of ready-made reagents marketed by the firm of Boehringer (West Germany).

EXPERIMENTAL RESULTS AND DISCUSSION

The cyclic AMP level in the muscle tissue fell 2-3 weeks after the beginning of the experiments. This decrease was more marked in avitaminosis E. The fall in the cyclic AMP level was accompanied by a marked rise in activity of the enzyme decomposing it. Since cyclic AMP is a stabilizer of lysosomal membranes, in avitaminosis E and denervation labilization of the lysosomal membranes can be presumed to take place, with liberation of acid hydrolases into the cell cytoplasm. An attempt was made to verify this hypothesis by experiments in vitro. By differential centrifugation a lysosome-rich fraction was isolated from muscle tissue homogenates of rabbits with avitaminosis E, and it was then incubated in 0.01 M Tris–HCl buffer, pH 7.4, at 37°C for 45 min with the addition of either cyclic AMP or 5'-AMP in different concentrations. No nucleotides were added to the control samples. After centrifugation of the samples at 18,000g for 30 min, acid phosphatase activity was determined (in nmoles substrate/mg protein/min) in the supernatant.

In the control its activity was 63 ± 4, and after addition of cyclic AMP in concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M it was 45 ± 5, 51 ± 6, and 57 ± 5 respectively; on the addition of 5'-AMP in the same concentrations its activity was 59 ± 4, 61 ± 6, and 62 ± 6 respectively ($P < 0.05$).

Cyclic AMP, in a concentration of 10^{-4} M, thus inhibits the liberation of acid phosphatase from the lysosome-rich fraction, whereas 5'-AMP had no such action.

It can be concluded from these results that in experimental muscular dystrophy the increase in acid hydrolase activity in muscle tissue is due to liberation of the enzymes from the lysosomes as a result of a fall in the cyclic AMP concentration and labilization of the membranes of these organelles.

LITERATURE CITED

1. V. S. Andreev, A. V. Bashtanov, E. S. Gorshenina, et al., Lab. Delo, No. 7, 492 (1973).
2. B. F. Korovkin and V. V. Budnyakov, Byull. Éksp. Biol. Med., No. 3, 63 (1973).
3. B. F. Korovkin, V. V. Budnyakov, A. V. Kozlov, et al., in: Abstracts of Proceedings of the 3rd All-Union Biochemical Congress [in Russian], Riga (1974), p. 218.
4. D. L. Ferdman, Vopr. Med. Khim., No. 3, 351 (1957).
5. W. I. Cheung, Analyt. Biochem., 28, 191 (1969).
6. L. J. Ignarro, N. Krassikoff, and J. Slywka, J. Pharmacol. Exp. Ther., 186, 86 (1973).
7. L. G. Ignarro and C. Colombo, Science, 180, 1181 (1973).
8. A. A. Iodice, R. Perker, and I. M. Weinstock, in: Muscle Diseases, (ed. by T. N. Walton et al.), Amsterdam (1970), pp. 313-318.
9. I. M. Weinstock and A. A. Iodice, in: Lysosomes in Biology and Pathology, (ed. by J. T. Dingle et al.), Vol. 2, Amsterdam (1969), pp. 450-468.
10. H. Zalkin, A. H. Tappel, K. A. Caldwell, et al., J. Biol. Chem., 237, 2678 (1962).