PHOSPHOLIPID COMPOSITION OF THE SARCOPLASMIC RETICULUM OF NORMAL AND DENERVATED RABBIT MUSCLE TISSUE

O. V. Dobrynina, V. A. Severina, and S. Z. Khaitina

UDC 612.744.15:547.953

The content of total phospholipids and phospholipid fractions and the acetylcholinesterase (ACE) and Mg^{++}-activated ATPase activity were studied in the sarcoplasmic reticulum of denervated rabbit muscle tissue. The considerable increase in the content of all the phospholipid fractions in the membranes of the sarcoplasmic reticulum of the denervated muscles was accompanied by a sharp increase in ACE and Mg^{++}-ATPase activity.

Besides proteins, lipids and, in particular, phospholipids play an important role in the structural organization of biological membranes. Injury to muscle tissue accompanying atrophy induced by denervation is accompanied by marked changes in the phospholipid components of the membranes of the sarcoplasmic reticulum [11].

This paper describes the results of an investigation of quantitative changes in the phospholipid fractions and also of the activities of certain enzymes in the sarcoplasmic reticulum of denervated rabbit skeletal muscles at various stages of the pathological process caused by division of the sciatic nerve.

EXPERIMENTAL METHOD

Male rabbits weighing 2-3 kg were used. The animals were denervated under ether anesthesia. The sciatic nerve was divided in the left hind limb at the level of the upper third of the thigh. The leg muscles (excluding the soleus, a red muscle) of the left denervated and right (contralateral) control limbs of the animal were used in the experiments. To obtain native preparations of sarcoplasmic reticulum (SR) the muscles of the left leg of healthy rabbits were used. The muscle tissue was passed through a hand mincer, and 5-6 g of the mince was homogenized for 75 sec in 4 volumes of 0.1 M KCl solution, pH 7.4, in a Potter's homogenizer. SR was obtained by differential ultracentrifugation as the fraction sedimented between 13,000 g and 105,000 g. The purity of the SR preparations was verified by electron microscopy and by the enzyme activity. Protein was determined by Lowry's method [6]. Acetylcholinesterase (ACE) activity of the SR was determined by Hestrin's method [5] with certain modifications [2]. Activity of Mg^{++}- dependent ATPase was determined from the increase in inorganic phosphate in the incubation medium (10 min, 25°C) in a volume of of 2 ml containing 0.1 M KCl, 5 mmole $MgCl_2$, 5 mmole ATP, and 5 mmole histidine buffer, pH 7.4; 100-200 μg protein. Inorganic phosphate (Pi) in TCA-filtrates was determined by Rathbun's method [9]. Phospholipids were extracted from the freshly obtained SR residue with a mixture of chloroform and methanol (2:1) with homogenization. The phospholipids were fractionated by two-dimensional thin-layer chromatography in a strengthened layer of mark KSK silica gel in the following systems: 1) chloroform–methanol–water (65:25:4) and 2) chloroform–methanol–25% ammonia (14:6:1). To detect phospholipids a solution of ninhydrin in butanol and 50% H_2SO_4 solution was used [1]. The content of identified phospholipids was measured as the phosphate content [3].

Department of Biochemistry, Medical Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 11, pp. 57-59, November, 1973. Original article submitted March 21, 1973.

TABLE 1. Phospholipid Composition of Membranes of Sarcoplasmic Reticulum in Normal Muscle and after Denervation (M ± m)

SR	Phospholipid component (in mg Pi/g SR protein)				
	phosphatidylcholine	phosphatidylethanolamine	phosphatidylinositol and phosphatidylserine	sphingomyelin	cardiolipin and phosphatidic acid
Native	5±0,2	1,3±0,1	1,1±0,2	0,5±0,1	0,4±0,1
Control	5±0,4	1,73±0,3	1,1±0,1	0,7±0,1	0,5±0,1
Denervated	7,6±0,1	3±0,3	2,75±0,25	1,5±0,1	0,45±0,05

EXPERIMENTAL RESULTS

Preparations of SR obtained from denervated muscle tissue contained much less protein (calculated per gram wet weight of tissue) than SR preparations isolated from the control muscles. One week after the operation the yield of the SR fraction, calculated as the ratio

$$\frac{\text{yield of SR (in mg protein/g tissue) of denervated limb}}{\text{yield of SR (in mg protein/g tissue) of control limb,}}$$

was reduced to 0.85 ± 0.01, and 3 weeks after the operation it was reduced to 0.057 ± 0.03 from the control level of 1.07 ± 0.07).

This decrease in the yield of the SR fraction (by almost half) was accompanied by a considerable increase in the content of total lipid phosphorus in the SR membranes. SR preparations isolated from the muscles of healthy rabbits contained 8-9 μg lipid phosphorus per mg protein of the fraction (or 0.26-0.29μ mole Pi/mg protein). Changes in the muscle after denervation led to an increase in the content of phospholipid phosphorus to 15-16 μg/mg protein of the fraction (or 0.48-0.52 μmole Pi/mg protein). The distribution of the phospholipid composition of the reticulum of the denervated muscle tissue with that of SR obtained from both control and native muscles showed the following features:1) the quantitative distribution of the phospholipid fractions in SR of the normal and control muscles was reflected in similar values; 2) the range of phospholipid components of SR obtained from the denervated muscle tissue was qualitatively indistinguishable from that of the native and control preparations; 3) the phospholipid components of the SR of the control and denervated muscles differed chiefly in the quantitative proportions of the phospholipid components. For instance, after denervation atrophy the content of phosphatidylcholine was increased by 52% and that of phosphatidylethanolamine by 76% compared with the control. Meanwhile the content of sphingomyelin and of the combined fraction of phosphatidylserine and phosphatidylinositol was increased by several times. The fraction of acid phospholipids (phosphatidic acid and cardiolipin) showed no appreciable changes as a result of denervation atrophy.

The change in the relative proportions of the phospholipid components in the SR membranes during denervation atrophy was accompanied by a sharp change in the activities of ACE and Mg^{++}-ATPase. For instance, 3 weeks after denervation the ACE activity of the SR membranes was increased by 3 times (from 1.1 ± 0.2 to 3.4 ± 0.4 moles acetylcholine/ mg SR protein/ h. Similar changes took place in the Mg^{++}-ATPase activity (from the control level of 0.07 mole Pi/ mg SR protein/ min to 0.41 after denervation).

These differences in the phospholipid composition and enzyme activities indicate damage to the membranous structures of SR in this particular pathological condition.

This conclusion is supported by data in the literature showing changes in the functional and morphological properties of the SR membranes of denervated muscles, reflected as an increase in the Ca-absorptive power [10], changes in the lactate dehydrogenase isoenzyme spectrum in the SR matrix [7], and changes in the electron-microscopic picture of this subcellular fraction [8].

LITERATURE CITED

1. É. V. Dyatlovitskaya, T. I. Torkhovskaya, and L. D. Bergel'son, Biokhimiya, 34, 177 (1969).
2. T. Yu. Lipskaya, Byull. Éksperim. Biol. i Med., No. 4, 49 (1969).
3. E. Gerlach and B. Deuticke, Biochem. Z., 337, 477 (1963).
4. L. Heiner, J. Domonkos, and M. Vargha, Acta Physiol. Acad. Sci. Hung., 40, 243 (1971).

5. S. Hestrin, J. Biol. Chem., 180, 249 (1949).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. U. Muscatello and P. Patriarca, Am. J. Path., 52, 1169 (1968).
8. C. Pellegrino and C. Franzini, J. Cell. Biol., 17, 327 (1963).
9. W. Rathbun and V. Betlach, Analyt. Biochem., 28, 436 (1969).
10. F. A. Sreter, Exp. Neurol., 29, 52 (1970).
11. M. Zatti, P. Patriarca, V. Terribile, et al., Experientia, 25, 1242 (1969).