PHOSPHOLIPID COMPOSITION AND ENZYME ACTIVITY OF THE SARCOPLASMIC RETICULUM FROM PATIENTS WITH CUSHING'S DISEASE WITH A MYOPATHIC SYNDROME

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The total content and fractional composition of the phospholipids, together with acetylcholinesterase (ACE) and Mg^{++}-dependent ATPase (Mg-ATPase) activity, were studied in the sarcoplasmic reticulum of muscle tissue from patients with a secondary myopathic syndrome accompanying Cushing's disease. Phospholipid analysis of the membranes of the pathological reticulum revealed a decrease in the phosphatidylcholine content and a simultaneous increase in the sphingomyelin and lysolecithin content. The activity of Mg-ATPase and ACE remained within normal limits in the myopathic syndrome. Disturbance of the morphology of the striated muscles in the hereditary myopathies is accompanied by considerable changes in the structure of their sarcoplasmic reticulum (SPR) [11]. Similar degenerative changes in the muscle tissue are observed in patients with Itsenko–Cushing's disease, in which the myopathic changes are secondary in character [10].

This paper describes the results of an investigation of quantitative changes in the phospholipid fractions and in acetylcholinesterase (ACE) and Mg^{++}-dependent ATPase (Mg-ATPase) activity in the SPR of human muscle tissue under normal conditions and from patients with a secondary myopathic syndrome accompanying Cushing's disease.

EXPERIMENTAL METHOD

Pieces of muscle tissue were taken from patients at operation from the latissimus dorsi muscle. Pieces of tissue taken from the same muscle at cholescystotomy and operations on the kidneys from patients of the same age were used for the control investigations. The muscle tissue was cut up into small pieces with scissors and homogenized in four volumes of 0.1 M KCl solution, pH 7.4, in a Potter's homogenizer (glass–Teflon) for 1.5 min. All procedures with the tissue were carried out at 0–4°C. The homogenate was centrifuged for 20 min at 600 g. Mitochondria were sedimented from the supernatant fraction at 12,000 g for 20 min. The SPR fraction was obtained by centrifuging the postmitochondrial supernatant for 1 h at 105,000 g. Protein was determined by Lowry's method [7]. Activity of succinate: cytochrome c-reductase was determined by the usual method [5] modified into a micromethod, and ACE activity was estimated by Hestrin's method [4], using the medium and conditions of incubation specified by Lipskaya [2]. Mg-ATPase activity was measured from the increase in inorganic phosphate (P_i) in the incubation medium (volume 2 ml) containing 0.1 M KCl, 5 mM $MgCl_2$, 5 mM ATP, and 5 mM histidine buffer, pH 7.4. The reaction was started by adding the SPR preparation in an amount of 0.1–0.2 mg protein/ml. Samples were incubated for 10 min at 25°C. Rathbun's method [9] was used to determine P_i in TCA filtrates. Phospholipids were extracted with a chloroform: methanol (2:1) mixture with homogenization. Phospholipids were frac-

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TABLE 1. Phospholipid Composition of SPR and Mitochondria from Muscle Tissue of Normal Subjects and from Patients with a Myopathic Syndrome Accompanying Cushing's Disease

Fractions	Phospholipids (in % of total phosphorus)					
	phosphatidylcholine	phosphatidylethanolamine	phosphatidylserine + phosphatidylinositol	sphingomyelin	lysolecithin	cardiolipin + phosphatidic acid
SPR:						
normal (five expts.)	49.2±4.4	21.2±4.4	10.1±3.0	2.3±1	1.35±1.2	10.7±0.82
in myopathic syndrome (eight expts.)	39±5.6	18±2.8	14.8±3.3	9.1±1.5	9.9±4.6	11.7±3.2
P	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05
Mitochondria: normal	41	25	10	4	3	17
in myopathic syndrome	40	26	8	6	2	18

tionated by two-dimensional thin-layer chromatography in a reinforced layer of KSK silica gel in the following systems: 1) chloroform : methanol : water (65 : 25 : 4) and 2) chloroform : methanol : 25% ammonia (14 : 6 : 1). The phospholipid stains on the chromatogram were detected with ninhydrin and 50% sulfuric acid [1]. The quantity of identified phospholipids was measured from the P_i content determined by the method of Gerlach and Deuticke [3].

EXPERIMENTAL RESULTS

The analysis of the yield of SPR per gram muscle tissue showed that in the secondary myopathic syndrome associated with Cushing's disease less of the reticulum (1.38 ± 0.47 mg protein) was extracted from 1 gram of muscles than from the muscles of healthy subjects (2.3 ± 0.43 mg protein). The decrease in the SPR protein content could be due to fatty degeneration of the muscle tissue and to the sharp decrease in the content of true muscle fibers in Cushing's disease with a well-marked myopathic syndrome.

The results of investigation of the individual phospholipid fractions of the SPR of normal and pathological muscles did not differ in the qualitative composition of the phospholipids. Meanwhile, definite quantitative differences were observed in the individual fractions. In the pathological muscle, for instance, the SPR contained much more sphingomyelin and lysolecithin ($P < 0.05$) and significantly less (by 21%) phosphatidylcholine. A marked tendency was observed for the total phosphatidylserine and phosphatidylinositol fraction to increase in the disease, but this difference was not significant ($P > 0.05$). No significant changes were found in the phosphatidylethanolamine or acid phospholipid (cardiolipin + phosphatidic acid) fractions. An increase in the content of sphingomyelin and lysolecithin, accompanied by a decrease in the phosphatidylcholine content, was observed in an investigation of the phospholipid composition of muscle SPR from mice with a hereditary form of progressive muscular dystrophy, in which the primary lesion occurs in the skeletal muscle tissue [6].

Activity of the marker mitochondrial enzyme – succinate : cytochrome c-reductase – in the SPR of normal muscle tissue is very low, only 6% of the total activity of the mitochondrial fraction according to the present experiments. The SPR in the secondary myopathic syndrome accompanying Cushing's disease contained not more than 8% of mitochondrial fragments as impurities. The percentage of mitochondrial structures contaminating the sample was thus about the same for the normal and pathological SPR preparations and varied from 6 to 8. Incidentally, SPR isolated from human pectoral muscles normally contains 5% of mitochondrial fragments as impurities [12], in agreement with the present results.

Considering that a lesion of muscle tissue at certain stages can be accompanied by morphological changes in the mitochondria, the content of phospholipid components in the mitochondrial fraction was studied in normal subjects and in patients with secondary myopathic syndrome. The results in Table 1 show that the content of phospholipid components in the mitochondrial membranes was virtually unchanged in the disease. No significant changes were found in the content of the individual phospholipids in the mitochondrial membranes in investigations of hereditary dystrophies [8]. The phospholipid composition of intracellular structures such as myofibrils likewise is unchanged in the hereditary myopathies [6].

The ACE activity in the SPR isolated from normal muscle tissue was from 3.9 to 5.4 μmoles acetylcholine/mg protein/h, whereas the Mg-ATPase activity varied from 0.08 to 0.12 μmole P_i/mg protein/min.

The experiments showed that Mg-ATPase and ACE activity in the SPR of patients with the myopathic syndrome associated with Cushing's disease did not go outside the normal limits obtained in healthy subjects.

It can be postulated on the basis of data in the literature [6, 8] and the results of the present experiments that the lesion of muscle tissue in the hereditary myopathies and in Cushing's disease with a secondary myopathic syndrome is accompanied by considerable and similar changes in the content of the phospholipid components of the SPR membranes. The disturbance of the structural composition of the SPR membranes is evidently one aspect of the degradation of the muscle tissue as a whole both in the hereditary myopathies and in Cushing's disease, in which the lesion of the skeletal muscles is secondary in character.

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