ATPases OF ERYTHROCYTES FROM RATS WITH 20, 25-DIAZACHOLESTEROL INDUCED MYOTONIA

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1. Introduction

20, 25-Diazacholesterol an inhibitor of the desmosterol reductase induces in humans and animals myotonia that is electrophysiologically indistinguishable from congenital myotonia in man and goat [1]. It is characterized by a prolonged relaxation of skeletal muscle following contraction. During the relaxation phase bursts of repetitive depolarizations reveal the hyperirritability of the muscle cell membrane. It is most likely that the reason therefore is an increased membrane resistance and a decreased chloride conductance demonstrated in myotonia congenita [2] and experimental myotonias as well [3, 4]. In 20, 25-diazacholesterol induced myotonia these electrophysiological changes might be due to the replacement of cholesterol by its precursor desmosterol that is accumulated in the membranes of the skeletal muscle [5]. This theory is supported by observations of Peter [6] who found the chloride conductivity decreased in mixed lipid bilayers in which cholesterol was replaced by desmosterol. We have found an increase in ion transport ATPase activities in sarcolemma isolated from skeletal muscle of myotonic rats ([5], but see [7]). Our intention was to search for similar changes in the erythrocytes of these animals.

2. Experimental

Erythrocyte ghosts were prepared according to Post et al. [8] modified in the following way. The blood was collected into heparinized tubes by aortic puncture of the ether anesthesized rat; after 4 washings with 0.9% NaCl the erythrocytes were hemolyzed by adding 5 mM Tris, 0.05 mM EDTA pH 7.0, after centrifugation at 20 000 *g* the ghosts were washed 3 times in 10 mM Tris pH 7.0, once with 10 mM Tris pH 8.1 and finally with 10 mM Tris pH 7.0 again. The pellet of very light pink colour was homogenized with a Teflon pestle, protein determination was done by the Lowry method [9], ATPases were assayed in a medium as described in table 1. Sterol determination was performed as described earlier [5, 10].

3. Results and discussion

In accordance with previously published results [5] more than 90% of the cholesterol was replaced by desmosterol in the serum as well as in the erythrocytes of rats with myotonia induced by 20, 25-diazacholesterol. Table 1 shows the results of the study of transport ATPases of the erythrocyte ghosts. The activity of the basic Mg^{2+}-dependent ATPase is very much the same for ghosts from control and myotonic animals and is only slightly higher than that reported from other authors [11], while the stimulation by Na^+ and K^+ is much higher in our preparation. In all the experiments done the $(Na^+ + K^+)$-ATPase activity of ghosts from myotonic animals was significantly higher than that of the control animals (0.01, P 0.005). The effect of ouabain on the $(Na^+ + K^+)$-stimulation was qualitatively the same for ghosts of myotonic and control rats and the small quantiative difference in the inhibition is not significant. Ca^{2+}-stimulated ATPase on an average was also found to be higher in the ghosts of myotonic rats, though this observation was made only in 4 out of 6 preparations and was less marked (0.2, P 0.15).

Table 1
ATPase activities of erythrocytes from 6 control and 6 myotonic rats; experiments were done in doublicates.

		Control rat	Myotonic rats
A)	Mg^{2+}-ATPase	1.19 ± 0.12	1.27 ± 0.15
	$Na^{+} + K^{+}$, Mg^{2+}-ATPase	2.50 ± 0.24	3.61 ± 0.38
	$Na^{+} + K^{+}$-ATPase	1.30 ± 0.13	2.34 ± 0.20
	Stimulation (%)	112.60 ± 6.50	196.00 ± 9.40
	Ouabain inhibition (0.5 mM) (%)	74.30 ± 8.60	66.80 ± 7.20
B)	Mg^{2+}-ATPase	1.28 ± 0.14	1.22 ± 0.17
	Ca^{2+}, Mg^{2+}-ATPase	3.24 ± 0.32	3.60 ± 0.32
	Ca^{2+}-ATPase	1.96 ± 0.21	2.38 ± 0.18
	Stimulation (%)	153.00 ± 13.3	195.00 ± 20.1

Enzyme activities are expressed as μmoles P_i/mg protein per hr. Assay media contained 30 mM Tris pH 7.0, 3 mM $MgCl_2$, 3 mM Tris-ATP and 0.2 mg protein/ml. Temperature 36°C. Medium A for the study of the $(Na^{+} + K^{+})$-ATPase activity additionally contained 60 mM NaCl and 6 mM KCl or 66 mM choline chloride and 0.5 mM EGTA. Medium B for assay of the Ca^{2+}-ATPase additionally contained 100 mM KCl, 0.5 mM $CaCl_2$ or EGTA.

Our data demonstrate that the enzymatic changes found by us in the sarcolemma of 20, 25-diazacholesterol treated rats are also present in the erythrocytes. It is of course not known why the transport ATP-ases have higher activities in these animals and what the relation to the myotonic reaction of their muscles is. Further studies will have to show whether these changes are found only in 20, 25-diazacholesterol induced myotonia or also in other myotonic diseases as myotonia congenita and myotinia dystrophica, where we, in contrast to Wakamutsu et al. [12], did not find an increased desmosterol level in the serum or erythrocytes, though every now and then small amounts of sterols other than cholesterol and desmosterol were present in myotonic dystrophy.

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