RELAXANT ACTION OF SARCOPLASMIC RETICULUM UNDER NORMAL CONDITIONS AND IN EXPERIMENTAL LOCAL TETANUS

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Preparations of sarcoplasmic reticulum isolated from muscle of normal rats and rats with tetanus inhibit ATPase of the myofibrils equally. Inhibition of ATPase of the sarcoplasmic reticulum by potassium oxalate takes place equally under normal conditions and in tetanus.

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The sarcoplasmic reticulum plays an important role in the contractile activity of muscles [3, 5, 6, 12].

We have investigated the effect of prolonged tetanization on the relaxing power of the sarcoplasmic reticulum.

EXPERIMENTAL METHOD

Experiments were carried out on adult male rats weighing 280-320 g. Local tetanus of the hind limb of the rat was used as model of prolonged tetanus and was produced by injection of a minimal pathogenic dose of tetanus toxin, equal to 1/25-1/30 MLD, into one hind limb of the rat. This dose of toxin was injected into various groups of leg and thigh muscles (4 injections), as a result of which the local action of the toxin was reduced to a minimum. The muscles were investigated 3 days after the injections, i.e., after tetanus had lasted for more than two days. The leg and thigh muscles into which the toxin was injected were studied. Analogous muscles of the corresponding limb of a healthy rat served as control.

Myofibrils were isolated from the muscles by a modification of the method of Perry and Grey [11]. The experiments were performed only on myofibrils of healthy rats, because the ATPase activity of the myofibrils of healthy animals and animals with tetanus is the same. The extraction medium consisted of a 0.1 M solution of tris-HCl (pH 7.5). The myofibrils were kept in 50% glycerol in a refrigerator at 4° for at least one week. Before the experiment the glycerol was removed by centrifugation and by washing the myofibrils three times in 0.1 M tris-HCl (pH 7.5).

The sarcoplasmic reticulum (granules) was isolated by the method described by Martonosi and Feretos [8]. Experiments were carried out on the day after isolation of the reticulum. Experimental samples, 2 ml in volume, had the following composition: KCl 0.0625 M, histidine 0.005 M, ATP 0.005 M, $MgCl_2$ 0.005 M, potassium oxalate 0.005 M, tris-HCl 0.08 M, pH 7.4. The protein content of the myofibrils was 4 mg per sample for the first preparation and 3 mg for the second. The ratio of reticulum protein to myofibril protein was 5:100. The experiment was carried out in the following order. A suspension of reticulum was poured into test tubes containing the salt mixture. After 1 min the suspension of myofibrils was added and the samples were incubated for 5 min in a Warburg apparatus at 24°. The reaction was stopped by addition of 2 ml cold 10% TCA; inorganic phosphate was determined by the method of Fiske and Subbarow [4]. Other samples were tested at the same time to allow for the ATPase activity of the reticulum itself and the ATPase activity of the myofibrils without addition of reticulum.

Protein was determined by the method of Lowry and co-workers [7].

Samples of the following composition were used to investigate the effect of potassium oxalate on the ATPase activity of the reticulum: 0.1 M KCl, 0.004 M $MgCl_2$, 0.004 M ATP, 0.005 M histidine, pH 7.4,

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TABLE 1. Inhibition of ATPase Activity of Myofibrils by Granule Preparations under Normal Conditions and in Tetanus (ATPase activity expressed in μmoles P_i/mg protein/min)

No. of myofibril preparation	ATPase of myofibrils	ATPase of myofibrils in presence of granules	Inhibition of ATPase of myofibrils (in %)	ATPase of myofibrils in presence of granules	Inhibition of ATPase of myofibrils (in %)
		Normal		Tetanus	
1 (3)	0.105 ± 0.005	0.059 ± 0.004	43.8 ± 2.7	0.059 ± 0.004	43.8 ± 3.9
2 (4)	0.380 ± 0.009	0.101 ± 0.011	73.4 ± 2.4	0.107 ± 0.017	71.8 ± 3.8

Note. Number of experiments shown in parentheses.

0.005 M potassium oxalate (in the samples with oxalate), volume of experimental samples 2 ml, protein of granules 0.15-0.20 mg, temperature 24°, incubation time 2 min. The reaction was stopped by addition of 0.5 ml cold 10% TCA.

EXPERIMENTAL RESULTS

The decrease in ATPase activity of the myofibrils in the presence of sarcoplasmic reticulum can be used to estimate the relaxant action of the latter [2]. When the reticulum has its maximal action, the ATPase of the myofibrils in its presence amounts to only 10% of its initial value, i.e., it is all accounted for by the myosin ATPase [10, 12].

The results of the experiments to determine the relaxant action of sarcoplasmic reticulum preparations on myofibrils are given in Table 1.

It is clear from Table 1 that the degree of inhibition of ATPase of the first myofibril preparation was much lower, presumably because of its lower activity. However, in both cases the "normal" and "tetanus" granules possessed equal ability to inhibit ATPase activity of the myofibrils.

Martonosi and Feretos [9] showed that Ca^{++}-dependent ATPase of the sarcoplasmic reticulum is located on the outer side of the vesicles and that its activity is determined by the Ca^{++} concentration in the surrounding solution and is independent of its concentration inside the granules. Addition of oxalate, lowering the rate of departure of Ca^{++} from the vesicles of the reticulum as a result of the formation of insoluble calcium oxalate in them leads to a rapid decrease in Ca^{++} concentration in the incubation medium and, consequently, to a decrease in ATPase activity of the granules. The ability of the reticulum to take up Ca^{++} can be judged from the degree of inhibition of ATPase in the presence of K-oxalate compared with the ATPase activity of the reticulum in samples without oxalate.

During investigation of the effect of oxalate on ATPase of the reticulum, during the first 2 minutes of incubation inhibition of ATPase activity of the sarcoplasmic reticulum under normal condition was 0.18 ± 0.03, and in tetanus 0.16 ± 0.04 μmoles P_i/mg protein/min.

These experiments thus also showed that the ability of the reticulum to take up Ca^{++} was unchanged after tetanus lasting two days. The calcium "pump" is evidently relatively insensitive to changes in the working conditions of the muscle within quite wide limits, but as Aloisi [1] showed, its activity is very quickly disturbed after interruption of the trophic influence of the nervous system.

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