ACETYLCHOLINESTERASE ACTIVITY OF THE SARCOPLASMIC RETICULUM UNDER NORMAL CONDITIONS AND IN EXPERIMENTAL LOCAL TETANUS

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The effect of prolonged tetanization on acetylcholinesterase activity of the sarcoplasmic reticulum was investigated. Prolonged tetanus was simulated by local tetanus of the hind limb in rats. Acetylcholinesterase activity per milligram protein was increased at pH 6.2 and 7.0 but unchanged at pH 8.0 and 9.0. The increase in activity at pH 7.0 was 44%. The yield of protein from the sarcoplasmic reticulum per gram of tissue was reduced by 14% during tetanus. It is concluded that prolonged tetanization in vivo leads to an increase in the acetylcholinesterase activity of the sarcoplasmic reticulum.

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The membrane fraction obtained by differential centrifugation between 12,000 and 40,000 g, consisting mainly of cisterns and undestroyed triads of sarcoplasmic reticulum (SPR) [8], according to the findings of Ulbrecht and Kruckenberg [11], possesses high specific cholinesterase activity. These workers suggest that the activity of this cholinesterase is associated with the conduction of excitation from the surface of the sarcolemma into the depths of the muscle fiber.

We studied the acetylcholinesterase activity of the SPR during prolonged, continuous tetanus produced by injection of small doses of tetanus toxin.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 280-320 g. Tetanus toxin was injected in a dose of 1/20-1/30 MLD into the muscles of one hind limb as described previously [6]. Tissue was taken for investigation at the end of the 3rd day, i.e., after tetanus lasting more than 48 h. The corresponding muscles of healthy rats acted as the control.

The SPR was isolated in 0.1M KCl solution by differential centrifugation. The excised muscles, from which fat was removed, were passed through a "Latapie" mincer and homogenized in a microblender with four volumes of isolated medium for 2 min. Myofibrils and cell fragments were removed by centrifugation at 1000 g for 25 min. The supernatant was filtered through four layers of gauze, the mitochondria were sedimented by centrifugation for 20 min at 12,000 g, and the SPR was sedimented by centrifugation at 40,000 g for 1 h. The SPR residue was suspended in isolation medium to give a final concentration of 3 mg protein/ml. The acetylcholinesterase activity was determined by Hestrin's method [9]. Protein was determined by the method of Lowry et al. [10].

Experimental samples measuring 2.5 ml consisted of 0.06M KCl, 0.09M NaCl, 0.04M $MgCl_2$, 0.01M phosphate buffer (pH 6.2, 7.0, or 8.0) or 0.01M KCl, borate buffer (pH 9.0), 0.003M acetylcholine, and 1.5 mg SPR protein. The incubation time was 1 h and the temperature 37°. Control samples to allow for spontaneous hydrolysis of acetylcholine were tested at each pH value.

EXPERIMENTAL RESULTS AND DISCUSSION

Since the acetylcholinesterase activity depends on the state of ionization of acid and basic groups contained in its active center, it might be expected that, if changes take place during local tetanus in the

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TABLE 1. Acetylcholinesterase Activity (μmoles acetylcholine/mg protein/h) Under Normal Conditions and in Experimental Local Tetanus

Series of expt.	pH			
	6,2	7,0	8,0	9,0
Normal	0.10±0,02 (17)	0.54±0.02 (18)	1.46±0.06 (17)	1.55±0.008 (17)
Tetanus . . .	0.22±0.03 (17)	0.78±0,03 (18)	1,59±0,05 (17)	1.70±0,08 (18)
% of normal activity . . .	220	144	109	110

Note. Number of experiments in parentheses.

enzyme molecule, they could be detected by studying the relationship between acetylcholinesterase activity and pH.

As Table 1 shows, the acetylcholinesterase activity of the SPR was increased during tetanus in acid and neutral medium but unchanged at pH 8.0-9.0, i.e., in the region of the pH optimum.

A study of the SPR protein yield per gram tissue showed that during local tetanus less protein (1.67 ± 0.1) was isolated from 1 g muscles than from muscles of healthy animals (1.94 ± 0.08), i.e., 86% of normal (15 experiments).

The decrease observed in the SPR protein content per gram muscle tissue was evidently due to the fact that prolonged continuous tetanization of muscles causes changes in the relative intensities of SPR protein synthesis and breakdown in favor of the latter. No such changes were discovered in the mitochondria. It may be that protein synthesis in the cell structures most closely connected with the trophic influences of the nervous system [7] are modified first. The changes discovered were opposite to those observed after denervation [3, 7]. In contrast to denervation, when acetylcholinesterase synthesis takes place more rapidly than synthesis of other enzymes [5], the increased electrical activity did not affect the relative content of acetylcholinesterase among the other SPR proteins. Evidence of this was given by the identical activity of the enzyme in the region of the pH optimum under normal conditions and during experimental local tetanus. The increase is acetylcholinesterase activity at acid and neutral pH values apparently indicates that the enzyme molecule underwent certain changes as a result of which the pH of the imidazole group of histidine in the active center was displaced toward the acid side. Since the decrease in content of SPR protein in tetanus, i.e., 14%, was at least 3 times smaller than the increase in acetylcholinesterase activity at physiological pH values (44%), it may be considered that in vivo prolonged tetanization must lead to an increase in acetylcholinesterase activity, especially if it is remembered that the pH of muscle tissue during tetanus is displaced slightly toward the acid side [1]. The increase in acetylcholinesterase activity of SPR which we discovered is evidently due to the fact that the muscles remained for a long time in an active state, because training leads to an increase in the cholinesterase activity of muscles [2]. The increased acetylcholinesterase activity of the SPR may be connected with the phenomenon demonstrated by Kryzhanovskii [4] and which can be interpreted as increased lability of the muscles of tetanized animals.

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